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Endodontics: Where biology and technology merge

Abstracts

Wladimir Adlivankine Research Prize,
Education Prize
and
Original Scientific



International Endodontic Journal

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Aims and Scope

The *International Endodontic Journal* is a leading international forum for publications in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health.

The *International Endodontic Journal* is published monthly and strives to publish original articles of the highest quality to disseminate scientific and clinical knowledge; all manuscripts are subjected to peer review. Original

scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

The *International Endodontic Journal* is essential reading for general dental practitioners, specialist endodontists, research scientists and dental teachers.

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ESE EDUCATION PRIZE

EP1

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Innovations in teaching biostatistics to residents in endodontic fellowship: a pilot study

Aim The aim of this study was to develop, implement and evaluate an innovative transformational curriculum in biostatistics in response to the needs of fostering critical thinking in graduate health care education for a successful evidence-based practice and life-long learning education.

Methodology The curriculum was designed for first-year residents in a postgraduate endodontic program using a six-step approach to curriculum development, following the current Accreditation Standards for Advanced Specialty Education Programs in Endodontics. It applies a learner-centred approach, to provide sufficient understanding in research and biostatistics to allow residents to evaluate biomedical publications critically, knowledgeably and confidently, to design the best research strategy to address a specific problem and to analyse data by selecting the appropriate statistical test. Multiple instructional methods (lectures, targeted readings, small group discussions, videos, role plays and polls using an audience response system), formative and summative assessments (written tasks, simulation exercises, portfolios and pre-post knowledge tests) were used to accomplish the learning outcomes. The analysis of the achievement of the group of students and a satisfaction survey for further feedback provided to the residents at the end of the curriculum were used for curriculum evaluation.

Results All residents demonstrated competency at the end of the curriculum. From the 28-item selected-response extending matching and multiple-choice knowledge test, the total number of questions answered correctly at the end of the curriculum (22.33 ± 3.22) was higher than prior to implementation (10.33 ± 3.22). Correct answer rate changed from 36.9% in the pre-test to 79.8% in the post-test. No common errors were detected in the rest of the assessment activities. All participants completed the questionnaire demonstrating high satisfaction for each independent category and with the overall educational program, instruction and course in general.

Conclusions The curriculum was validated by the assessment of students' performance and a satisfaction survey completed by the participants at the end of the course, offering an example of a tremendously practical approach to the teaching of statistics to prepare postgraduate endodontic students for an evidence-based endodontic practice and life-long learning education as practicing clinicians.

EP2

A. Baaij* & A.R. Özok

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Effect of curricular changes in endodontic education on root canal treatments performed by undergraduate students

Aim To assess whether the curricular changes in undergraduate endodontic education at ACTA influenced the performance of students carrying out root canal treatments.

Methodology Conventionally, both teaching and assessment comprised performing root canal treatments on patients by students, supervised by general dental practitioners. The contemporary education comprises initial extensive clinical training and assessment (not involving human subjects), supervised by endodontists, followed by treatment on patients. In this study, the quality of the first root canal treatment performed on a patient by students, assessed as competent, was evaluated according to pre-determined criteria. Data was analyzed by Chi-square, Mann-Whitney and Kruskal-Wallis tests.

Results Sixty two percent of root canal treatments performed by students who took the contemporary assessment, were evaluated as having good quality, in comparison to 47% of treatments performed by students who took the conventional assessment ($P > 0.05$). When supervised by endodontists, 71% of the root canal treatments were evaluated as having good quality, whereas when supervised by general dental practitioners, 54% of the root canal treatments were evaluated as having good quality ($P > 0.05$).

Conclusions Contemporary teaching and assessment in performing root canal treatments seems to be a valid alternative to the conventional endodontic education for undergraduate students.

ESE WLADIMIR ADLIVANKINE RESEARCH PRIZE

RP1

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Evidence for nitric oxide and prostaglandin signalling in the regulation of odontoblast function in identified regions of the rodent mandibular incisor

Aim A deep understanding of tissue structure, function and control systems within the dental pulp is essential to support therapies aimed at tissue preservation and regeneration. The major objective of this study was to investigate the presence and localization of immunoreactivity to cyclo-oxygenase 1 (COX-1 IR), EP2 and nitric oxide synthase (NOS IR) in an effort to explore the possibility of complex nitric oxide/prostaglandin signalling pathways in the rat mandibular incisor utilizing contemporary immunohistochemical and confocal microscopy techniques.

Methodology Pulp tissues, both fresh and contained within demineralised sections of rat mandibular incisors were prepared for standard immunohistochemistry. Sections were first labelled with antibodies to structural protein vimentin, COX-1, NOS-1, and prostaglandin receptors EP2, before staining with fluorescent markers alexafluor 488 and 594, and then examination by fluorescent-confocal microscopy.

Results COX-1-IR was found in only discreet regions of the odontoblast cytoplasm. Some cells within the subodontoblast cell layer also showed COX-1 IR, suggesting cellular diversity. COX-1 IR was also observed in the walls of blood vessels of the central region of the pulp.

EP2-IR was observed in the distal part of the odontoblast cell body, more weakly in the proximal region of odontoblast process and in cells of the subodontoblast cell layer.

NOS1-IR was observed primarily in the distal part of the odontoblast cell body, weakly in the proximal part of odontoblast process and in arterial walls but not in the veins within the bulk of the pulp.

Conclusions Variable IR to COX-1, NOS and EP2 has been identified in specific regions within the dental pulp, suggesting hitherto unrecognised and complex cell-signalling within tissue compartments.

RP2

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Guided endodontics: accuracy of a novel method for guided access cavity preparation and root canal location

Aim To present a novel method utilizing 3D printed templates to gain guided access to root canals and to evaluate its accuracy *in vitro*.

Methodology Sixty extracted human teeth were placed into six maxillary jaw models. Preoperative CBCT scans were matched with intraoral scans using the coDiagnostix™ software. Access cavities, sleeves and templates for guidance were virtually planned. Templates were produced by a 3D-printer. After access

cavity preparation by two operators a postoperative CBCT scan was superimposed on the virtual planning. Accuracy was measured by calculating the deviation of planned and prepared cavities in three dimensions and angle. 95% confidence interval was calculated for both operators.

Results All root canals were accessible after access cavity preparation with 'Guided Endodontics'. Deviations of planned and prepared access cavities were low with means ranging from 0.16 to 0.21 mm for different aspects at the base of the drill and 0.17–0.47 mm at the tip of the drill. Mean of angle deviation was 1.81°. Overlapping 95% confidence intervals revealed no significant difference between operators.

Conclusions 'Guided Endodontics' allowed accurate access cavity preparation to be performed up to the apical third of the root utilizing printed templates for guidance. All root canals were accessible after preparation.

RP3

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Comparative evaluation of the shaping ability of ProTaper Next and ProTaper Universal rotary NiTi files in curved root canals of extracted human molar teeth

Aim The aim of the present study was to compare the shaping ability of ProTaper Next™ and ProTaper® Universal instruments in curved root canals of extracted human molar teeth.

Methodology A total of 17 extracted human molars were collected, to give a total of 30 canals. Teeth were randomly assigned to two experimental groups ($n = 15$). The specimens were shortened coronally to a length of 15 mm and standard access cavities were prepared. Standardized digital radiographs were acquired before instrumentation in both Clinical View and Proximal View with a size 10 K-file inserted into the canal. Each tooth was placed in a special platform (Pro-Train; Simit Dental) to maintain a fixed film-object-source position. One group was assigned to preparation with ProTaper® Universal rotary files system, the other group was instrumented with ProTaper Next™. The final size of all apical foramina was 0.25 mm in diameter. After instrumentation, the master apical rotary file was inserted into the canals at WL, and radiographs were taken. The angle of curvature of each canal was measured before and after canal preparation according to the method described by Schneider (1971). The determination of canal curvature and apical transportation was performed by a second blind examiner. Preparation time and fractured or deformed instruments were also recorded. The unpaired Student's *t* test was used to compare results.

Results No instrument fractured or deformed during root canal preparation. There was no significant difference between the two instruments with respect to canal straightening and apical transportation before and after instrumentation ($P > 0.05$). The use of both instruments resulted in significant reduction of the angle of curvature after instrumentation ($P < 0.05$). Instrumentation time was significantly greater for ProTaper® Universal ($P < 0.05$).

Conclusions ProTaper® Universal and ProTaper Next™ systems performed similarly regarding straightening of curved root canals and apical transportation in an extracted tooth model. ProTaper Next™ was significantly faster than ProTaper® Universal.

RP4

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The effect of different ferrule and reattachment designs on strengthening of vertically fractured teeth using fibre and adhesive materials

Aim The aim of this *ex vivo* study was to evaluate the effect of different fibre applications and ferrule designs on compressive strength of reattached fragments of vertically fractured roots using a resin based cement.

Methodology Eighty extracted single-rooted premolars were instrumented and divided into eight groups. The negative control group ($n = 10$) consisted of intact teeth, whereas the positive control group ($n = 10$) included endodontically treated teeth. The roots of the remaining sixty teeth were vertically fractured into two equal fragments. Separated fragments were reattached using Superbond C&B, quartz fibre posts (D.T. Light-Post) and/or glass fibre bundles (TESCERA, Bisco) in root canals and/or cervical area with 1 or 2 mm ferrule design to constitute six experimental groups ($n = 10$). The core build-ups were created with composite resin. Compressive loading was performed under a constant speed of 0.5 mm min^{-1} to the specimens until fracture. Mean load to fracture was recorded (Newton) for each sample and analyzed statistically using One-way ANOVA and Duncan's Multiple Range tests. Fracture types were recorded and classified.

Results The highest mean initial fracture load was obtained in the negative control group (1036.7 N), which was significantly higher than that of positive control group (989.7 N) and test groups ($P < 0.05$). The roots reattached with resin cement and quartz fibre posts (871.9 N) had significantly less fracture strength than all other groups ($P < 0.05$). The roots reattached with resin cement and glass fibre bundles with 2 mm ferrule design (981.6 N) had the highest fracture strength among all test groups, which was not significantly different from that of the control groups. Four types of fractures were observed following loadings.

Conclusions Reattachment with fibre bundles in root canals and 2 mm ferrule in the cervical area, and Superbond C&B cement can be a good treatment option for vertically fractured teeth.

Acknowledgements This study was performed in Suleyman Demirel University Faculty of Dentistry and supported by the Scientific Research Projects Coordination Center of Suleyman Demirel University (BAP Project Number: 3978-DU2-14).

RP5

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Canal and isthmus cleanliness of mesial roots of mandibular molars after laser activated irrigation: a micro-computed tomography study

Aim To investigate the effectiveness of different irrigant agitation techniques on removal of accumulated hard tissue debris (AHTD) in mesial roots of human mandibular molars.

Methodology Thirty extracted human mandibular molars with joining mesial root canals and an isthmus between them were selected based on micro-CT (μ CT) scans. The canals were instrumented to an apical diameter of size 30 using the ProTaper rotary system. NaOCl (2.5%) irrigation was used throughout instrumentation, followed by a final flush with 2 mL EDTA (17%) and 2 mL NaOCl. Teeth were then randomly assigned to three irrigant activation groups ($n = 10$): (i) ultrasonically activated irrigation (UAI) using the size 20 Irrisafe (Satelec Acteon Group, Merignac, France) for $3 \times 20 \text{ s}$, (ii) laser activated irrigation (LAI) with an Er:YAG-laser and plain $300 \mu\text{m}$ fibre tip inside the canal (20 mJ, 20 Hz, $3 \times 20 \text{ s}$) and (iii) LAI with the same laser and parameters but with the $400 \mu\text{m}$ PIPS tip held at the canal entrance. All teeth were scanned in a μ CT system (Triumph-II, TriFoil Imaging, Paris, France) at $30 \mu\text{m}$ resolution before and after instrumentation and after irrigant activation. After reconstruction and image processing the percent of total canal system volume filled with hard tissue debris before and after irrigant activation was calculated. Data were compared using one-way ANOVA and a paired samples *t*-test.

Results The volume% AHTD after irrigant activation was significantly lower than before activation in all groups. Although the lowest AHTD values were observed in the laser groups, no significant differences in volume% AHTD after activation were observed between groups.

Conclusions All activation groups were associated with a significant reduction of AHTD; UAI, and both LAI protocols performed equally well in this respect. No method was capable of removing all AHTD.

ORIGINAL SCIENTIFIC ABSTRACTS

SESSION 1: THURSDAY 17TH SEPTEMBER

ANATOMY

R1

N. Sathienvuttivong & O. Suratanasurang*

*Department of Endodontology, Mahidol University, Bangkok, Thailand***Root and canal morphology of Thai premolars****Aim** To investigate the root and canal morphology of 348 permanent premolars collected from an indigenous Thai population.**Methodology** The cleaned teeth were identified as maxillary and mandibular first and second premolars. The teeth were accessed, the pulp dissolved by sodium hypochlorite under ultrasonication and the pulp system injected with Indian ink. The teeth were rendered clear by demineralization and immersion in methyl salicylate. The following observations were made: (i) root canal length; (ii) number of root canals per tooth; (iii) root canal configuration in each root using Vertucci's classification, with additional modifications; and (iv) presence and location of lateral canals and intercanal communications.**Results** The average root canal length of Thai premolars was 19–21 mm. Of 73 maxillary first premolars, 54% had type IV canal systems and 28% had type V canal systems. A variety of canal types were found in the maxillary second premolars. Of 186 maxillary second premolars, 30% had one root and one canal (type I). 20%, 15%, 13%, 10% had type IV, type V, type III, and type VII, respectively. Of 39 mandibular first premolars, the most common (56%) was type I and 25% had type V canal. The majority of 50 mandibular second premolars (76%) had type I canal. There was an increase in the prevalence of lateral canals towards the apical part of the roots and intercanal communications were present in 9% of second Thai maxillary premolars.**Conclusions** This study suggests that Thai premolars exhibit features of both Caucasian and Chinese teeth. The majority of maxillary first premolars had type IV canals and the common maxillary second and mandibular premolars had mainly type I canals. Maxillary second premolar were associated with the greatest diversity of root canal morphology. Only a small proportion of the roots had intercommunicating canals and lateral canals, which were most common in the middle and apical thirds.**Acknowledgements** Faculty of Dentistry, Mahidol University, Bangkok, Thailand.

R2

D. Helvacioğlu-Yigit¹, S. Cora¹, A. Sinanoglu² & C. Gür^{1,*}¹Department of Endodontics & ²Department of Oral and Maxillofacial Radiology, Faculty of Dentistry, Kocaeli University, Kocaeli, Turkey**Analysis of root canal morphology and symmetry of mandibular anterior teeth using cone-beam computed tomography: a retrospective study****Aim** The purpose of this study was to analyse the root canal configurations and symmetry of mandibular anterior teeth in a Turkish subpopulation using cone-beam computed tomography (CBCT).**Methodology** CBCT images of 200 patients who were referred to the Faculty of Dentistry, Kocaeli University and required CBCT examination for diagnostic purposes were enrolled in the study. A total of1200 mandibular anterior teeth were examined in axial, coronal and sagittal planes. The number of roots, the number of canals and root canal symmetry were assessed. The root canal configurations were evaluated according to Vertucci's classification. Kruskal Wallis and Mann Whitney *U* test were used to analyse the results.**Results** Vertucci Type I (78%) was the most common detected canal configuration. The prevalence of two canals was 27.8% in lateral incisors and 23% in central incisors ($P > 0.05$). Overall, 14% of mandibular canines had two canals which was significantly less than lateral and central incisors ($P < 0.01$); 2% of mandibular canines had two separate roots. Symmetry was observed in 93.5% of lateral incisors, 91% of central incisors and 91% mandibular canines ($P > 0.05$). Correlation of root canal configuration with gender and position of teeth (left or right) was also evaluated and there was no significant difference ($P > 0.05$).**Conclusions** Mandibular anterior teeth revealed a high symmetry rate in the Turkish subpopulation. Use of CBCT facilitated assessment of the root and canal configurations.

R3

A.M. Mavridou^{1,*}, G. Pyka², M. Wevers² & P. Lambrechts¹¹Department of Oral Health Sciences, BIOMAT Research Cluster, KU Leuven & University Hospital Leuven & ²Department of Materials Engineering (MTM), KU Leuven, Leuven, Belgium**Applying nano-CT technology in endodontology: understanding external cervical root resorption****Aim** The introduction of a rapid, non-destructive, high resolution technique to efficiently analyse and understand complex endodontic phenomena, such as external cervical resorption (ECR).**Methodology** In this study ten permanent teeth with fatal ECR were extracted. Diagnosis of ECR was based on clinical and radiographic (CBCT) examination. After extraction the teeth were assessed with a high performance NanoTom scanner (GE Measurement and Control Solutions, Wunstorf, Germany), which provided accurate images with a resolution of 7 µm. Structural analysis and 3D modelling was achieved using the CTAn, CTvol CTvox and DataViewer software (Bruker micro-CT, Kontich, Belgium), in order to study the internal tooth structure and ECR architecture.**Results** The use of a Nano-CT system, for the selected experimental settings, allowed accurate observation of the ECR tissues. In particular, cementum defects, resorption cavity and resorption channels, reparative mineralized tissue, pericanalar resorption resistant sheet (PRRS) and pulp reaction with pulp stones formation were investigated. The reparative tissue-dentine interface was always visible due to the difference in X-ray absorption leading to a good contrast between these two structures, providing vital information on the growth mechanism of ECR. In particular, Nano-CT gave clear evidence that repair takes place by ingrowth of bone-like tissue into the resorption cavity. Furthermore, Nano-CT revealed that the PRRS tissue consisted of dentine and eventually reparative bone-like apposition. The thickness of this layer was measured and subsequently correlated to the resorption resistant characteristic of this layer.

Conclusions Nano-CT is a rapid and minimally invasive technique for *in vitro* analysis and understanding of ECR, which can provide an ideal platform for future imaging and exploration studies in Endodontology.

R4

D.E. Böttcher*, L.R. Del Vico, J.A.P. Figueiredo, F.V.V. Pelisser & R.K. Scarparo

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Clinically relevant dimensions of C-shaped molars obtained via high-resolution computed tomography

Aim This study aimed to characterize the dimensions of a selection of C-shaped molars through high-resolution computed tomographic analysis considering measures of clinical interest for root canal treatment, such as anatomy, root wall thickness, canal diameters, and distance between the root apex and foramen.

Methodology Eight C-shaped human mandibular molars extracted for therapeutic reasons were individually scanned using a desktop high-resolution computed tomographic system. Starting from the apical foramen, the selected cross-sectional images corresponding to each millimetre of the roots were evaluated. Measures of clinical interest were determined using OsiriX Imaging Software (version 5.6 32-bit; Pixmeo SARL, Bernex, Geneva).

Results Great variability was noticed regarding canal anatomy: 3 teeth were classified as C1 (Fan *et al.* 2004) along the whole root; the others presented C1, C2 and C3 classifications in different parts from the same root. The lingual wall had the lower thickness mean measures (0.585–1.798 mm), in all thirds, compared to buccal (1.281–2.234 mm), mesial (1.212–2.179 mm) and distal (1.047–2.009 mm) walls. The thinnest lingual wall thickness (0.063 mm) was detected in the apical portion. According to diameter, canals classified as C1 had the largest longitudinal measures in the 1 mm distance (0.757–3.180 mm). At the same point, transversal measures ranged from 0.106 to 0.374 mm. When present, mesiolingual canal always had circular shape and the greater distances between the root apex and the foramen in comparison with the others showing a tendency for foramina to be eccentric.

Conclusions C-shaped molars are a clinical challenge not just because of their low frequency and difficulties regarding diagnosis but also because of their anatomical variability. These phenomena are critical in terms of the efficacy of root canal cleaning and shaping as well as for filling, specially considering canals with the C1 anatomy in the apical third.

R5

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Prevalence of three-rooted mandibular molars in a Brazilian subpopulation and micro-CT analysis of extracted mandibular first molars with distolingual root

Aim To determine the prevalence of three-rooted mandibular molars in a Brazilian population using CBCT images, and to analyse

the anatomy of root canal systems of mandibular first molars with three roots by micro-CT quantitative and qualitative assessments.

Methodology Cone beam computed tomography images of 116 patients were used to determine the prevalence of three-rooted first and second mandibular molars. Fifty-five three-rooted first mandibular molars were scanned in a SkyScan 1174 micro-CT system and reconstructed to analyse the length of mesial, distobuccal and distolingual roots, distance between canal orifices, apical diameter of the root canals, Vertucci's classification, and angle of canal curvature of additional distolingual roots.

Results The prevalence of three-rooted mandibular molars was 6.89%. Differences in the distribution of the internal anatomy according to Vertucci's classification were found between mesial and distal roots. The most prevalent Vertucci's classification of the additional distolingual root was type I. The median of major diameters of mesiobuccal, mesiolingual and single mesial canals were 0.34, 0.41 and 0.60 mm, respectively. The largest major diameters were found in distobuccal canals (0.56 mm), and the smallest ones were in the distolingual canals (0.29 mm). The shortest distances between canal orifices were found between mesial canals (MB-ML), whilst the largest distances were between the distal root canals (DB-DL). Almost all distal roots had one root canal and one apical foramen, and no significant differences were observed in the number of lateral canals, accessory canals, or apical deltas in the three roots. The additional distolingual root was smaller compared to the other roots. All distolingual roots had severe curvature.

Conclusions The prevalence of three-rooted mandibular molars in a Brazilian population was 6.89%. Mesial roots had complex distribution of the root canal system in comparison to the distal roots. Distolingual roots in mandibular molars generally had short lengths, severe curvature and a single root canal with a small apical diameter.

R6

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Evaluation of isthmus incidence and types in mesial roots of mandibular first molars: a histological study

Aim To investigate *ex vivo* the incidence and type of root canal isthmuses in the coronal, middle and apical part of the mesial root of mandibular first molars by histological sections.

Methodology One hundred extracted mesial roots of human mandibular molars that had fully formed roots were selected. The mesial roots were sectioned from the distal roots and were kept in 10% formalin until use. After fixation, the teeth were demineralized in 10% formic acid for 28 days. After rinsing for 24 h in tap water, the specimens were dehydrated and processed for routine histological examination. The coronal, middle and apical thirds of the decalcified roots were cut and embedded in paraffin. A total of thirty semiserial sections of each tooth were mounted on glass slabs, stained with haematoxylin–eosin and examined under an optical microscope (Olympus STM, Tokyo, Japan) at $\times 40$ magnification. Incidence and types of isthmus were evaluated by two examiners based on the classifications by Hsu & Kim. All data were statistically analysed by the Kruskal–Wallis test. The statistical significance level was established at 0.05.

Results The incidence of isthmuses in mesial roots of mandibular molars were 86% in coronal, 72% in middle and 84% in apical regions ($P < 0.05$). The most prevalent isthmus in coronal (70%), middle (56%) and apical (62%) parts was type V ($P < 0.05$).

Conclusions The incidence of root canal isthmus in the mesial root of mandibular first molars was high. Therefore, cleaning and filling of these isthmuses are a major challenge during root canal treatment.

R7A. Iturralde^{1,*}, E. Ambu², L. Forner¹, C. Llena¹ & S. Ortolani¹¹Department of Stomatology, Universitat de València, Valencia, Spain& ²Department of Stomatological Sciences, Università di Siena, Siena, Italy**Evaluation of root canal systems and C-shaped canal prevalence in mandibular molars and premolars in an Italian population using cone beam computed tomography: a retrospective study****Aim** To describe root, canal configuration and C-Shaped canal prevalence in permanent mandibular molars and premolars in an Italian population using Cone Beam Computed Tomography (CBCT) images.**Methodology** Tomographic images of patients were retrieved from the database of a private practice in Italy. All CBCT scans were required as part of patients' treatment planning. Scans were produced using a Kodak 9000 3D unit (Carestream/Trophy, Marne-la-Vallée, France), operated at 70 kVp, 10 mA, exposure time of 1.8 s and a voxel size of 76 µm, following the manufacturer's recommendation and the 'as low as reasonably achievable' (ALARA) protocol.

The inclusion criteria were: sound mandibular permanent molars and premolars; no periapical lesions; fully developed roots. Images were evaluated using the Osirix software in a darkroom and the following characteristics were recorded: C-shaped canals; number of roots and canals per root; variations in root canal systems. For the purpose of this study the type and configuration of each canal system was not classified. Descriptive statistic analysis was used.

Results The study group was formed by 600 scans, 62% were women and 38% men, with a mean age of 47 years. A total of 742 mandibular first and second molars and premolars (first premolars 186–25.06%; second premolars 197–26.54%; first molars 166–22.37; second molars 193–26.01%) were analyzed. The most prevalent root and root canal configuration among molars was two separate roots with two canals in the mesial root and one canal in the distal root (first molars 87.95%; second molars 66.83%) and in premolars was one root and one canal (first premolars 92.47%; second premolars 97.46%). C-shaped canals were observed in 17.09% of second molars, 3.76% of first premolars and 2.03% of second premolars.**Conclusions** Although C-shaped canals are not common in Caucasian populations, clinicians must consider the possibility of its presence as well of several other anatomical variations. CBCT has proven to be an excellent tool for diagnosis and treatment planning of clinical root canal treatment.**R8**E.S. Tolentino^{1,*}, P.A. Amoroso-Silva², R. Ordinola-Zapata²,A.G.A. Maliza², M.A. Hungaro-Duarte², L.C.V. Iwaki³, M.Chicarelli-Silva³ & I.G. Moraes²¹Department of Dentistry, Unicesumar, Maringá, ²Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru & ³Department of Dentistry, Maringá State University, Maringá, Brazil**Anatomical study of fused mandibular second molars with C-shaped root canals and shallow radicular grooves****Aim** To describe the morphological and morphometric aspects of fused mandibular second molars with radicular shallow grooves using micro computed tomography (Micro-CT).**Methodology** Eighty (80) extracted mandibular second molars with fused roots were scanned in a Micro-CT at a voxel size of 19.6 µm. After reconstruction using Dataviewer software, only teeth with a maximum radicular groove depth of 2 mm were selected. For this, a tangent line on the side of the lingual groove was traced, and from the middle point a line was drawn to the vertex of the groove to obtain its depth. Thirty molars were chosen for further analysis. The canal cross-sections were classified as C1, C2, C3, and C4 canal configurations according to the modified Melton classification. Morphometric parameters such as the major and minor diameters of the canals, aspect ratio, roundness, and 3-D configuration (merging, symmetric, and asymmetric) were evaluated. The median, minimum and maximum ranges were processed using Prism 5.0 software.**Results** 3-D reconstructions indicated a higher prevalence of Merging ($n = 25$), followed by Symmetric ($n = 3$) and Asymmetric ($n = 2$) types. The prevalence of the different cross-sectional configurations of the C-shaped molars revealed predominant oval and round shaped canals corresponding to the C4 classification within the first 3 mm from the apex. In the middle and cervical thirds, the C3 configuration was frequently observed followed by the C4 and C1 configurations. Considering the morphometric parameters, the roundness values that ranges from (0 to 1), where 1 indicates a perfect circle, revealed more rounded shaped canals at 3 (0.72), 2 (0.64) and 1 (0.63) mm from the apex in the C4 configuration. From all the cross-sections types, the C1 configuration had the lowest roundness, highest area, and major diameter values at all sections, while the mesial canal in the C3 configuration had the smaller diameters.**Conclusions** When fused second mandibular molars had shallow radicular grooves a higher prevalence of the Merging type classification was found. Also a major occurrence of the C4 cross-sectional configuration was observed in the first 3 mm apical segment. Furthermore, the apical portion of the C4 configuration had higher roundness values and major apical diameters.**Acknowledgements** This study was supported by the Brazilian agency FAPESP Process 2014/03009-1. The authors deny any conflicts of interest related to this study.**R9**E. Sariyilmaz^{1,*} & A.C. Yucel²¹Department of Endodontics, Ordu University, Ordu & ²Dentistanbul, Istanbul, Turkey**Morphological investigation of the physiological foramen of mandibular first and second molars****Aim** To investigate the size and count of physiological foramina of permanent mandibular first and second molars.**Methodology** Mandibular first ($n = 460$) and second (460) molars were investigated. Residual tissue in the anatomical foramen area was removed. Roots were dyed with Indian ink, washed under running water and then dried using pressurized air before examination. The root morphology of the apical area was examined at 80× magnification using a computer-aided stereomicroscope. Each root was directly illuminated and oriented until the physiological foramen was located in the middle of and parallel to the objective lens. The statistical data were arranged as mean, maximum, minimum and standard deviation.**Results** Twenty-six percent of mandibular first molars had only one mesial (M) foramen, while 73.91% had two mesial (MB and ML) foramina in the mesial root. In distal roots, 65.22% had only one foramen (D) and 34.78% had two apical foramina (DB, DL). The mean size of the narrow and wide physiological foramen diameters were: 283 and 382 µm in M; 211 and 257 µm in MB; 178 and 210 µm in ML; 309 and 420 µm in D; 200 and

244 µm in DB; and 182 and 245 µm in DL, respectively. For mandibular second molars 69.57% had a single foramen, 30.43% had two foramina in the mesial root, while for distal roots 60.87% had a single apical foramen and 39.13% had two apical foramina. The mean size of the narrow width and physiological foramen diameters of second molars were 198 and 364 µm in M; 218 and 285 µm in MB; 218 and 284 µm in ML; 255 and 380 µm in D; 179 and 251 µm in DB; and 247 and 341 µm in DL, respectively.

Conclusions Termination of the apical root canal preparation at around a size 25 file is usually insufficient, especially for roots in mandibular molar teeth that have one apical foramen.

R10

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CBCT evaluation of the internal and external morphology of mandibular molars from a Peruvian population

Aim To describe using CBCT analysis the internal and external morphology of first and second mandibular molars from a Peruvian population.

Methodology One hundred and eighteen patients who required a cone beam computed tomography examinations as a part of their diagnosis were included. A total of 147 first and 160 mandibular second molars were analyzed using the following criteria: (i) number of roots, (ii) number of canals per root, (iii) internal configuration (Vertucci 1984), (iv) presence of distolingual roots in first mandibular molars and (v) presence of C-Shaped canals.

Results The prevalence of distolingual roots in first mandibular molars occurred in 11.6% and in 1.3% of mandibular second molars. Second mandibular molars with C-shaped canals were present in 26.2% of cases. The presence of three canals was the common finding in the first and second mandibular molar (70% and 40%). Vertucci type II was the more frequent anatomy in mesial roots whereas Vertucci type I was the more prevalent anatomy in distal roots.

Conclusions The presence of three root canals and C-shaped canal anatomy were the more common anatomical variation of mandibular molars from a Peruvian population.

R11

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Root and canal morphology of maxillary molars in a Turkish population using cone beam computed tomography

Aim The aim of this retrospective study was to analyze and characterize root canal morphology using CBCT in a group of Turkish Cypriot population.

Methodology The sample for this cross-sectional study consisted of the retrospective evaluation of CBCT (Newtom 3G, QR Verona, Italy) scans of 290 adult patients (age range 16–80). The number of roots and their morphology; the number of canals per root; the

canal configuration were also classified according to the method of Vertucci. Pearson Chi-square tests were performed among canal configurations, sides and gender ($P \leq 0.05$).

Results Of the 373 first molars, there was no single-rooted specimen. Two (0.53%) teeth had two roots, 365 (97.8%) teeth had three roots and six (1.6%) had four roots. The total number of canals per tooth in maxillary first molars (female + male) was: two canals 0.5%, three canals 48.2%, four canals 50.7% and five canals 0.5%. Of the 438 second molars, 14 (3.1%) were single-rooted. 26 (5.9%) teeth had two roots, 392 (89.4%) teeth had three roots and six (1.3%) had four roots. The total number of canals per tooth in maxillary second molars (female + male) was: one canal 1.3%, two canals 7.7%, three canals 67.8%, four canals 22.8% and five canals 0.2%. In the first molar group with three and four separate roots, the mesiobuccal roots with a Vertucci's Type I anatomy was: 104 (52.7%) for females and 81 (46.5%) for males. No gender difference was found in the frequency of additional canals in the both maxillary first and second molars. The occurrence of additional canals did not differ with age.

Conclusions These results provide detailed knowledge of the root canal anatomy in this particular population and emphasise the clinical importance of the maxillary molar tooth anatomy which are of utmost importance for dental professions when performing root canal treatments.

R12

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An ex vivo cone-beam computed tomography study of the internal and external morphology of two-rooted mandibular canines

Aim To investigate the anatomy of the roots and root canals of extracted two-rooted permanent human mandibular canines using cone-beam computed tomography (CBCT).

Methodology A total of 77 extracted two-rooted permanent mandibular canines were selected and scanned using a high-resolution CBCT system (NewTom, VGI, IT). The axial sections and the three-dimensional reconstructions of the images were processed to evaluate both the external anatomy, regarding the length and size of the roots, the furcation region, the course of the roots and the direction of root curvatures, as well as the internal anatomy, regarding the number of the root canals, the bifurcation level and the presence of lateral canals, isthmi and apical delta.

Results In a total of 77 two-rooted mandibular canines, 4 (5.2%) had fused roots, 10 (12.9%) bifurcated in the cervical third, 35 (45.4%) in the middle third and 28 (36.3%) in the apical third. The size of the buccal and lingual roots was similar in 27 (35.1%) canines, whereas in 32 (41.5%) the size of the buccal root was bigger and in 14 (18.1%) the lingual was bigger. 31 canines (40.2%) had a longer buccal root, whereas 19 (25.6%) a longer lingual root. The average length of the buccal root was 22.8 mm (min. 17.63 mm, max. 28.06 mm) and for the lingual root 21.9 mm (min. 17.45 mm, max. 26.58 mm). 17 of the specimens (22.9%), had lingual and buccal roots of the same length. Most of the lingual (33.7%) and buccal (25.9%) roots curved buccally, whereas only 5% of the roots had a lingual curve. S-shaped roots were found in 11 (14.2%) buccal and 2 (2.6%) lingual roots. The axial sections revealed that all teeth had two main root canals. The distance between the root canal bifurcation and the root apex varied widely between specimens. Lateral canals were found in 59% of the specimens and mostly in the middle third of the root. A small percent presented with

isthmuses, mainly in the cervical third. Apical deltas were observed in 3% of the specimens.

Conclusions The evaluation of two-rooted mandibular canines revealed that bifurcations occurred mainly in the cervical third. In most cases, the buccal root was bigger in size and longer than the lingual. The majority of the buccal and lingual roots curved buccally in the apical third. Each root had a single root canal and the incidence of lateral canals was high.

R13

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Age-related modifications to root canal anatomy in human teeth: a micro-CT study

Aim Root canal treatment presents a particular technical challenge in older patients, due to root canal 'scleroses' and calcific-occlusions. Presently, no high resolution micro-CT studies have been performed to support this traditional clinical view. Therefore, the aim of this study was to analyse age-related root canal morphology changes in the root canal system of human teeth using micro-CT.

Methodology Sixty-four consecutively-extracted human teeth (37 male) were scanned using micro-CT (spatial resolution 13.68 µm per pixel). Teeth that had previous root canal treatment were excluded. After extraction the teeth were cleaned in a 5.25% sodium hypochlorite solution for 24 h. Dedicated commercial software (SkyScan™) was used to create virtual reconstructions and perform 3D-analysis (CTVox, CTAnalyser, CTVol [SkyScan]). Reconstruction parameters were applied to optimize contrast between enamel, dentine/cementum and root canal space. The non-parametric Mann-Whitney *U*-test or the chi-square test were used to assess differences between groups and Spearman's rank correlation co-efficient was also calculated for selected variables. Statistical significance was set at ($P < 0.05$).

Results The mean age of the donors was 68.7 years ($SD \pm 25.1$) with a range of 18–80 years. The mean root canal diameter 1 mm short of apex in patients <55 years was 0.42 mm, and ≥ 55 years was 0.21 mm. Root canal diameter 1 mm short of apex negatively correlated with the patient's age ($r = 0.52$; $P < 0.0001$), while positive correlations were found between the patient's age and root wall thickness at the coronal third, as well as the thickness of the pulp chamber floor in its thinnest part ($r = 0.57$; $P < 0.0001$ and $r = 0.41$; $P < 0.001$ respectively). Root canal system calcifications were sporadic findings.

Conclusions This micro-CT study confirms the hypothesis that root canals of human teeth narrow with increasing age. This is as a result of an age-related increase in canal wall diameter and pulp chamber floor thickness. These results using high resolution modern imaging techniques confirm the traditional views regarding the difficulties of root canal treatment in a geriatric population.

R14

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External morphology and root canal system of permanent maxillary molars with two palatal roots: an *in vitro* study using cone-beam computed tomography imaging

Aim To assess the root canal systems of permanent maxillary molars (PMM) with two palatal roots and to determine the frequency of anatomical landmarks, which may serve as diagnostic indicators of two palatal roots.

Methodology A sample of 27 second and third PMM with two palatal roots was taken from the collection of extracted teeth at the Faculty of Medicine in Ljubljana. The external morphology of these teeth was examined under stereomicroscope. Then they were scanned using a Veraviewepocs 3D R100 CBCT machine (J. Morita, Kyoto, Japan) (voxel size 125 µm) and analysed with i-Dixel One Volume Viewer 2.0.0 imaging software to determine the inter-orifice distances, divergence of the palatal canals, type of the root canal system, and root canal curvatures according to the modified method of Schneider (1971).

Results Anatomical landmarks had the following frequencies: crown wider over the palatal cusps 87% (20/23), palatal enamel extension 92% (24/26), palato-radicular groove 19% (5/26), enamel pearl 7% (2/27), and double Carabelli's cusp 27% (6/22). The average distance between the palatal orifices (3.8 mm) was significantly greater than between the buccal orifices (2.5 mm) ($P < 0.0001$). The average angle of divergence between the palatal root canals was 38 degrees (range 4 to 85 degrees). The second mesiobuccal canal was observed in 52% (12/23) of the mesiobuccal roots. The distobuccal, mesiopalatal, and distopalatal root harboured a single canal in 93% (25/27), 83% 19/23, and 89% (24/27) of cases, respectively. In four teeth with fusion of mesiobuccal and mesiopalatal root a more complex canal system was observed. Severe primary and secondary curves were frequently seen in a proximal view for the mesiopalatal canals (57.7% and 44.0%) and for the distopalatal canals (38.5% and 26.9%).

Conclusions The results indicate that there are two major diagnostic clues for the existence of two palatal roots: a pronounced palatal part of the crown and a palatal enamel extension. Other less frequent clues include a palato-radicular groove, an enamel pearl and a double Carabelli's cusp. When treating such PMM, the clinician should anticipate severe curvature of the palatal canals in the radiographically unseen buccopalatal direction.

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MICROBIOLOGY

R15

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Microbiological evaluation of reprocessed endodontic files collected from general dental practices in Istanbul

Aim To examine the presence of microbial contamination on reprocessed endodontic instruments those were subjected to different cleaning methods prior to sterilization.

Methodology A questionnaire was administered to 20 general dental practitioners to obtain information on the reprocessing of used endodontic files. A Hedström file, a rotary instrument and a lentulo spiral which had been used and reprocessed were collected from each practice. A total of sixty endodontic instruments were analysed. Each file was transferred aseptically to tubes containing brain heart infusion (BHI) broth culture medium for bacteriological analysis. Statistical analysis was carried out using chi-square test.

Results Of the twenty questionnaires distributed, seventeen were deemed usable. None of the practitioners used endodontic files as a disposable instrument. In addition to the use of an autoclave or a dry-heat sterilizer for the sterilization of instruments, various cleaning methods before sterilization, which ranged from manual brushing to chemical immersion and the use of a washer-disinfector were reported. The most frequently employed combination for decontamination was manual cleaning followed by autoclaving. Of the sixty endodontic instruments, twelve instruments (20%; six Hedström files, five rotary instruments and one lentulo spiral) produced growth on BHI agar. The number of infected H-files were significantly higher than the number of infected lentulo spirals ($P = 0.037$).

Conclusions There have been variations in decontamination methods reported and applied. The methods used to clean endodontic instruments appear to be generally ineffective for complete sterility. As a result, potentially infective material could be transmitted from an infected individual to other patients. These instruments should be viewed as single-use devices, unless significantly more efficient cleaning processes can be validated for use in general dental practice.

R16

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Expression of cell membrane proteins in response to alkaline stress in *Enterococcus faecalis* V583

Aim To compare membrane protein expression of *Enterococcus faecalis* (Ef) ATCC grown at a limited growth rate (1/10th maximum) in pH8 and pH11 conditions.

Methodology The maximum growth rate of *E. faecalis* was determined for both pH 8 and pH 11 conditions. *E. faecalis* was then grown in continuous culture at 1/10th the maximum growth rate at pH 8 and pH 11 with the controlled addition of sodium hydroxide and Todd Hewitt Broth. After ten generations at both conditions, samples were recovered from the Chemostat.

Cells were lysed using a French Press and membrane proteins fractionated with ultracentrifugation and membrane shaving. After chymotrypsin digest of the membrane fractions, Isotope-coding protein labels, light (L) & heavy (H) were added to the pH8 (L) and pH 11 (H) samples respectively. Samples were combined and the membrane proteins identified with LC-ESI mass spectrometry and MaxQuant software. The H/L ratios were log2 transformed and the mean determined. The proteins that had a Heavy/Light ratio greater than, or less than, one standard deviation of the mean were considered to be up- or downregulated.

Results The maximum growth rate of *E. faecalis* at pH 8 was 1.0 h and 7.7 h at pH 11. Six proteins were up regulated in pH 11 conditions including: a phage tail protein; polysaccharide biosynthesis family protein; glycerol uptake facilitator protein; and glycosyl hydrolase protein. Five were down regulated including: a C4-dicarboxylate transporter protein; phosphotransferase system (PTS) mannose-specific II C and IID component proteins.

Conclusions Adaptive responses of *E. faecalis* to a high pH environment include a reduced maximum growth rate and when combined with a limited nutrient environment there is shift in expression of selective membrane proteins associated with virulence. Survival of *E. faecalis* in an extremely alkaline environment is not solely due to the activation of cell membrane proton pumps.

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R17

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Chemical characterization of extraradicular biofilm by vibrational spectroscopy Raman

Aim To characterize the chemical components of the extraradicular biofilm of teeth with persistent apical periodontitis by vibrational spectroscopy Raman.

Methodology Fifteen apical samples obtained by surgical resection were sectioned into two parts, one half were placed in a tube with thioglycollate broth and incubated in an anaerobic chamber for 24–72 h at 35°C. A serial dilution technique was performed, microscopic and macroscopic characteristics were observed. The pure cultures were identified by analysis of biochemical profile API 20 A and API 20 Strep. In the other half of the sample, measurements were made using a 50× objective of an optical microscope Leica integrated to a micro-Raman system incident on the sample, using a wave length laser with 830 nm and 12.5 mW for 30 sec. An average of 15 spectra were collected per sample in a spectral range of 100–2000 cm⁻¹. The biofilm spectra were compared in their spectral characteristics with different spectra of the database provided by Professor Joke De Galder.

Results After the chemical characterization of the single species and multi species of extraradicular biofilm present in the samples, elements related to their formation were identified: polysaccharides, triglycerides and extracellular material of bacteria. The main component found corresponded to cellulose, which was identified in 11 of 15 samples, corresponding to 73%.

Conclusions Cellulose plays an important role as a protective agent embedding bacteria within biofilms.

R18

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Molecular analysis of microorganisms and their antibiotic resistance profiles in severe endodontic infections

Aim It is not known whether periapical abscess cases with severe life-threatening infections have different microbiota from routine outpatient cases. So the aim of this study was to describe differences in bacterial communities and associated antibiotic resistance between hospital inpatients and outpatients diagnosed with severe endodontic infections.

Methodology Aspiration samples from abscesses were collected from 34 subjects diagnosed with acute apical abscesses. 15 subjects were hospitalized patients at the University of Maryland Medical Center and 19 patients were treated as outpatients. Bacterial community profiling was performed using 16S rRNA gene sequencing using Illumina MiSeq. Molecular analysis of antibiotic resistance genes was performed using the QIAGEN Antibiotic Resistance Genes Microbial DNA qPCR Array, as well as gene-specific PCRs tested for five common antibiotic resistance genes, pertaining to β -lactams and tetracyclines. Statistical analysis was performed using LEfSe (LDA [Linear discriminant analysis] Effect Size) to identify bacterial taxa associations with each patient group. Fisher's exact test was performed to compare antibiotic resistance genes profiles between groups.

Results No significant differences in bacterial community diversity, richness or the four most prevalent bacterial genera were observed when comparing 16S community profiles from inpatients and outpatients. Outpatient specimens had 363 different genera, versus 269 for inpatient specimens. LEfSe analysis identified bacterial taxa significantly associated with each patient group. Comparison of antibiotic resistance gene profiles using Fisher's exact test revealed no significant differences between groups. TetM was the most prevalent antibiotic resistance gene in both groups (about 87% for both). Blatm1 was numerically more prevalent in the inpatient than outpatient specimens (67% versus 44%) ($P > 0.05$).

Conclusions Significant inter-individual variability in antibiotic profiles and bacterial community composition was observed. Bioinformatic analyses revealed some difference in microbial communities between the two groups of patients.

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R19

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Highlighting the weakest link in endodontic microbiology: the opportunistic *Propionibacterium acnes* and nosocomial endodontic infections

Aim The opportunistic *Propionibacterium acnes* contributes to endodontic patholosis and might be the result of nosocomial infec-

tions occurring at the time of root canal treatment (RCT). The aim of the study was to identify the *P. acnes* phylotypes predominating within primary endodontic infections and to investigate if gloves can be the potential source of these nosocomial infections.

Methodology The cultivable microbiota of 15 primary endodontic infections [without ($n = 7$) and with ($n = 8$) open communication with the oral cavity] was identified using partial 16S rRNA gene sequencing and by interrogating the Human Oral Microbiome Database (<http://www.homd.org/>). Phylogenetic analysis by recA gene sequencing (using PAR1 and PAR2 primers) of 47 *P. acnes* isolates was done. The cultivable microbiota of gloves ($n = 5$) worn during RCT at 4 time points (T1-start of treatment, T2-before x-ray, T3-after x-ray, T4-end of session) were identified using partial 16S rRNA gene sequencing. *P. acnes* isolates ($n = 46$) were typed by recA gene sequence comparison. The phylogenetic relationship were determined using MEGA 4.1 (http://www.megasoftware.net/fixed_bugs.html). Data distributions were compared using χ^2 -tests, means were compared using the Mann-Whitney U test in SPSSPC (Version 21. IBM, USA).

Results *P. acnes* was the most prevalent isolate recovered from primary endodontic infections with oral communication. No contamination of samples was identified, as control cultures were sterile. The richness of the bacterial taxa distributed in different phyla identified from primary endodontic infections with and without communications were significantly different ($P < 0.05$). 36 cultivable bacterial taxa were identified from the gloves. The quantitative viable counts at T4 [(aerobically (2.97 ± 0.68), anaerobically (3.43 ± 0.52))] were significantly greater ($P < 0.05$) than at T1 [(aerobically (0.4 ± 0.59), anaerobically (0.14 ± 0.31))] and T2 [(aerobically (1.88 ± 0.70), anaerobically (2.47 ± 0.70))]. *P. acnes* was the predominant taxa identified at T2, T3 and T4. recA gene sequencing revealed 2 phylogenetic lineages of *P. acnes* with type I (further split into type IA and type IB) associated with primary endodontic infections and type II and IB prevalent on gloves.

Conclusions *P. acnes* in primary endodontic infections with communications is an opportunistic pathogen. The microbial load of gloves can lead to the nosocomial infections at the time of root canal treatment.

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R20

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Presence of antibiotic resistance genes in the oral cavity from patients with primary endodontic infections

Aim To evaluate the presence of genes associated with bacterial resistance to three classes of antibiotics in patients with primary endodontic infections.

Methodology This study was approved by the Ethics Committee of Research (Federal University of Rio Grande do Sul, Porto Alegre, Brazil). Saliva (S), supragingival biofilm (SB), and root canal (RC) samples were collected from patients with primary endodontic infection, and divided into two groups: Group I – acute endodontic infection ($n = 12$) and Group II – chronic endodontic infection

($n = 12$). The non-stimulated saliva was collected in a sterile plastic tube. The SB was collected randomly at 15 sites using sterile microbrushes. To collect RCs the involved tooth was isolated. The operative field disinfection was performed. The paper points were introduced near the total length of the RC according to the radiographs, and kept in position for 1 min. Ultrapure water served as negative control. The presence of resistance genes blaTEM and ampC (betalactamics); ermB and mefA (macrolides), and lnuB and lsaB (lincosamides) were assessed by PCR. Descriptive analysis was conducted.

Results All samples were positive for the presence of bacteria as determined by universal 16S rRNA gene. Betalactam blaTEM gene was detected in 8.3% of S and RC samples from Group I, and 16.6% in SB samples from Group II. ampC gene was not detected only in SB of Group I; it was detected in 25%, and 8.3% of S samples from Groups I and II respectively. RC samples of both groups presented 16.6% of ampC gene. Macrolide resistance genes were frequently detected. ermB gene detection taxa varied from 8.3% in RC from Group II to 75% in S from Group I. All SB samples (Groups I and II) and S (Group II) were positive for the presence of mefA gene. Five samples of RC from Group I and two from Group II were positive for mefA gene. There were no positive results in any of the samples for the lincosamide lnuB gene. lsaB gene was positive in one sample of S from both Groups.

Conclusions Different oral sites can harbour high rates of genes of bacterial resistance to different groups of antimicrobial agents.

R21

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Development of endodontic biofilm: an experimental model for the evaluation of microbial viability, structure and topography

Aim Experimental models that reproduce the endodontic microenvironment contributes to the accuracy of studies that evaluate strategies for root canal disinfection. Intraoral devices applied to verify features of biofilm formation, caries development and durability of dental materials have already been developed. In the field of endodontic microbiology no device has been described. This study aimed to develop an intraoral device that allows endodontic biofilm formation in coupled teeth. Moreover, the present investigation points out several aspects in terms of spatial analysis of biofilm viability, topography and structure.

Methodology Three acrylic custom-made mandibular devices were employed. Four prepared roots were attached on the lingual surface of each, in such a way that only the cervical opening of the root canal would be exposed to the oral environment. The devices were used by a volunteer over 14 days. After the experimental period, all roots were removed from the device and evaluated under scanning electron microscopy (SEM) and Confocal Laser Scanning Microscope (CLSM). Microbiological features were described and differences regarding biofilm viability and density were compared among the root thirds using the Kruskal–Wallis nonparametric analysis of variance, and Dunn post hoc. To compare the differences between viable and nonviable microorganisms in each one of the thirds the Mann-Whitney test was used.

Results No significant differences of biofilm viability were observed comparing root canal thirds. LIVE and DEAD markers

were detected in similar proportions along the root canal. Microorganisms were observed in all thirds of the root canals lumen, and frequently in dentinal tubules. The predominant bacteria morphotype were cocci and rods. Filamentous bacteria and spirochetes were observed in lower quantities. In many samples, human cells were identified, mainly in the cervical and middle portions of root canals. Biofilm density was higher in the cervical portion of the canal ($P < 0.05$).

Conclusions The experimental model was effective in reproducing the development of the biofilm of *in vivo* intracanal primary infections. The use of human roots coupled with intraoral devices should be explored in other studies for testing endodontic disinfection protocols.

R22

Withdrawn

IMAGING

R23

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Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration: a CBCT study

Aim To evaluate the relationship of the quality of the coronal restoration and root canal filling on the periapical status of endodontically treated teeth using Cone-Beam Computed Tomography (CBCT) analysis.

Methodology CBCT images of 1010 teeth, with root canal treatment performed at least 3 years before, were randomly selected from a database of patients at the University. Technical quality of the root fillings was scored as either good (GE) when all canals were filled, no voids were visible and distance to the apex was approximately 0.5 mm, or poor (PE) when one or more criteria were not met. The quality of the coronal restoration was similarly evaluated as good (GR) with a perfect radiographic marginal seal, or poor (PR) in case of open margins and/or over- or under-filled restorations. The periapical status was categorized as absence of periapical lesion (APL) or presence of periapical lesion (PPL). Data was recorded and statistically analysed using Chi-square test at 0.001 significance level.

Results In 54.9% of the 1010 endodontically treated teeth, periapical status was analysed as APL. GE resulted in significantly more APL cases than PE and GR resulted in significantly more APL than PR ($\chi^2 = 24.68$ resp. 22.64; $p\chi^2 < 0.001$). Combination of GE and GR had the highest APL rate of 70.1%.

Conclusions CBCT revealed a high incidence of periapical lesions. However, performing a high quality root filling and coronal restoration significantly increased long-term radiographic success of root filled teeth.

R24

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The use of CBCT for the detection of vertical root fractures of different widths in root filled teeth restored with post-core: *in vivo* and *ex vivo*

Aim To assess the accuracy of CBCT *ex vivo* and *in vivo* for the detection of artificially induced vertical root fractures (VRFs) of various widths in extracted post-core restored teeth.

Methodology The root canals of 50 extracted intact single-rooted human teeth were enlarged and post spaces were prepared. Metal cast post-cores for each tooth were fabricated out of cobalt-chrome alloy. VRFs were induced in 30 teeth by gently tapping posts with a hammer. All roots were autoclaved, covered with a thin layer of wax, stabilized in plastic cylinders filled with acrylic resin and embedded into biteplates made of silicon putty. CBCT scans (3D Accutomo 170) were taken *ex vivo* and *in vivo*. For the *in vivo* scanning teeth in bite plates were put into 2 sterile plastic bags and placed in the mouth of study volunteers, scheduled to undergo CBCT scanning as part of treatment planning. Then teeth with VRFs were sectioned into axial slices and the widths of the VRFs were measured with the aid of a light microscope. Teeth were divided in 2 groups: with large (wider than 150 µm) and small (ranging from 50 to 150 µm) widths of VRFs. Five observers assessed the presence of VRFs using axial CBCT. Values for sensitivity, specificity, accuracy and inter-examiner agreement were calculated.

Results CBCT diagnostic accuracy for the detection of large and small VRFs *in vitro* was 0.56 and 0.40 respectively ($P = 0.043$). The sensitivity of CBCT was 0.27 and 0.53 for small and large VRFs respectively ($P = 0.043$). It was impossible to assess the presence or absence of 90% VRFs *in vivo* because of artifacts. Only large VRFs could be visualized *in vivo* in 10% of cases. Inter-examiner reliability analysis showed Kappa values 0.02–0.54 for different pairs of observers.

Conclusions The detectability of VRFs by CBCT *in vitro* and *in vivo* depended on its width and was affected by the presence of metal post and core, which caused strong artefacts, obscuring real fractures and creating false positive results.

R25

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Functional assessment of dental pulp response to caries by T2 and ADC mapping: an *in vivo* magnetic resonance feasibility study

Aim Magnetic resonance imaging (MRI) enables non-invasive visualization of both hard and soft dental tissues at the same time, however *in vivo* studies are still lacking. The aim of present study was to evaluate the advanced MRI methods of T2 and apparent diffusion coefficient (ADC) mapping in a functional assessment of dental pulp response to caries.

Methodology The study was performed with a 3T MRI scanner (Philips, Achieva) using a 32-channel SENSE head coil. All exami-

nations were performed unilaterally on each premolar and molar mandibular tooth of 6 volunteers with carious and non-carious teeth. Each examination included high-resolution fat-suppressed proton density and T1 weighted imaging as well as T2 mapping and diffusion weighted imaging (DWI). The T2 mapping was performed by a 6-echo SE sequence of one-time scan (TR/TE = 2000 ms/15, 30, 45, 60, 75, 90 ms, slice thickness = 2 mm, FOV = 150 mm), while DWI was conducted by using echo-planar imaging (TR/TE = 3273/83 ms, with identical slice thickness and FOV) and four b-values = 0, 200, 400 600 s mm⁻². Corresponding T2 and the ADC maps of pulps of all teeth were obtained by post-processing software (ImageJ, NIH). The statistically significant differences were tested by Student's t-test.

Results The T2 and ADC maps of non-carious teeth revealed that their pulps were continuously uniform, with average values of 133 ± 12 ms and $1.41 \pm 0.32 \cdot 10^{-9}$ m² s⁻¹, respectively. The T2 and ADC maps of carious teeth were non-uniform, with accumulations of free fluid in the pulp chamber along with pulp remnants. The average T2 and ADC values of pulp remnants were significantly lower, the average values being 103 ± 10 ms and $1.06 \pm 0.25 \cdot 10^{-9}$ m² s⁻¹, respectively. The differences were statistically significant (t-test, $P < 0.01$).

Conclusions The results of this *in vivo* feasibility study confirmed the relevance of T2 and ADC mapping for the discrimination and localization of intact and affected regions in dental pulps of carious teeth.

R26

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Dose reduction and cone-beam computed tomography accuracy

Aim To assess in an *ex vivo* model the accuracy of detection of simulated periapical lesions using digital intraoral periapical radiography and two CBCT rotations and dose modes.

Methodology Small and large artificial apical bone lesions were created with burs at the base of the socket of eleven mandibular molars, in 7 human dry mandibles. Digital periapical radiographs, CBCT scans with 360° and 180° of rotations in standard dose and dose reduction (DR) modes were taken prior to and after each periapical lesion were prepared. Six blinded examiners independently scored the presence/absence of bone lesions. Inter- and Intra-examiner reliability were determined. Receiver operating characteristic (ROC) area under the curve, sensitivity, specificity, positive predictive values and negative predictive values were determined for each imaging technique.

Results The sensitivity and specificity of 360° and 180° CBCT scans with dose reduction mode were higher compared to that of standard dose CBCT scans. The overall area under the curve (AUC) value was 0.616 for intraoral radiography, 0.935 and 0.946 for 360° scan with standard dose and dose reduction, respectively and 0.945 and 0.966 for 180° scans with standard dose and dose reduction, respectively. There was no difference in the diagnostic accuracy of both CBCT rotations obtained using standard dose mode and dose reduction mode at detecting small and large artificially created periapical lesions, while their accuracy was significantly higher than intraoral radiography.

Conclusions Dose reduction protocol applied to 360° and 180° CBCT scans is a promising technique which demonstrated a good diagnostic accuracy in the detection of artificially created bone lesions while reducing the radiation dose by 54% and 51% during the full-arc rotation (360°) and the half arc rotation (180°), respec-

tively compared to conventional settings. Therefore, the radiation dose reduced by up to 74% between 360° standard dose and 180° DR mode which is advisable and in line with the ICRP guidance.

R27

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Prevalence of hypercementosis in a sample of the Turkish population

Aim The purpose of this study was to assess the prevalence of hypercementosis and to classify the alteration types of hypercementosis in a sample of the Turkish Central Anatolian population.

Methodology A retrospective study was performed using the cone beam computed tomography (CBCT) records of patients examined in the Department of Oral and Maxillofacial Radiology, University of Erciyes in Kayseri, Turkey. A total of 2477 patients who had complete records with satisfactory images were selected. These images were measured in three dimensions and a total of 49 198 teeth (including third molars) were evaluated and analysed for hypercementosis and its alteration types. The relative incidence of hypercementosis and the correlations regarding the location, as well as between female and male, were recorded and statistically analysed using the chi-square test.

Results Hypercementosis was found in 2.18% of the patients and in 0.12% of the teeth. An incidence of hypercementosis was significantly higher for the mandible when compared to the maxilla ($P < 0.05$). Hypercementosis was significantly more often associated with molars than premolars, or incisors, respectively ($P < 0.05$). The most commonly type of hypercementosis was moderate club shape (49.15%), followed by focal hypercementosis (25.42%) and mild club shape (20.35%), whereas 5.08% of teeth had severe club shape. There was a significant difference between all alteration types ($P < 0.001$), except between focal hypercementosis with mild club shape ($P > 0.05$).

Conclusions This study is one of the first studies which used CBCT to evaluate such a phenomenon. Hypercementosis was found rarely in this sample of the Turkish population. Moderate club shape hypercementosis was the most common alteration type.

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IRRIGANTS/DISINFECTION: DENTINE INFECTIONS

R28

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Characterization of an artificial root canal polybacterial biofilm using a combination of FISH technique and SEM observation

Aim Management of an infected root canal with a polybacterial biofilm is a real challenge in endodontics. The aim of this pilot

study was to characterize an *in vitro* polybacterial biofilm produced in a root canal and to visualize distribution and position of each species on the root canal walls and inside dentinal tubules.

Methodology Ten single-rooted teeth were prepared manually and irrigated with NaOCl and EDTA before autoclaving. Sterile teeth were incubated for 21 days with a mixture of *Streptococcus salivarius* ATCC13419, *Enterococcus faecalis* ATCC29212, *Porphyromonas gingivalis* ATCC33277 and *Fusobacterium nucleatum* ATCC10953. Teeth were fixed, sectioned longitudinally and then treated with different 16S rRNA probe matched to each bacterial species. Fluorescent signals were visualized and recorded using Confocal Laser Scanning Microscopy. Canals were observed using Scanning Electron Microscope, to identify different aspect of this biofilm and to locate bacteria inside dentinal tubules.

Results During FISH observation, Dy-405, ATTO488, Cy3 and Cy5 were identified respectively for *P. gingivalis*, *E. faecalis*, *F. nucleatum* and *S. salivarius*. Except Cy3 which was spotted on dentinal wall only, all markers were detected in both main canal and dentinal tubules. Subsequently SEM observations confirmed the organization of all bacterial species in an adherent biofilm to root canal walls. Bacterial packs were present inside dentinal tubules as far as 500 µm from the root canal.

Conclusions A 21 day-old biofilm seems to be enough to reproduce *in vitro* endodontic infection. Combination of FISH technique and SEM microscopy appears to be a reliable protocol to monitor endodontic biofilm state especially inside dentinal tubules.

R29

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Effect of additional laser and sonic activation devices during root canal disinfection

Aim To evaluate the effect of sodium hypochlorite (NaOCl), Chlorhexidine (CHX), and Octenidine dihydrochloride (OCT) with or without the additional use of Nd:YAG laser and two sonic devices on the disinfection efficacy of *E. faecalis* from the root canals.

Methodology One hundred-fifty-six single-rooted teeth were decoronated, randomly divided into 13 test groups ($n = 12$) and instrumented with ProTaper Universal (Dentsply Maillefer) files to size F3. Smear layer was removed with sequential use of 5.25% NaOCl, 17% EDTA and distilled water for 3 min in an ultrasonic bath. The roots were sterilized (121°C and 20 min), infected for 21 days with *E. faecalis* (A197A) and treated as follows; Group 1: control (5 mL saline irrigation for 1 min); Group 2: 2.5% NaOCl (with double side vent endodontic irrigation needle, 5 mL-1 min); Group 3: CHX (5 mL-1 min); Group 4: OCT (5 mL-1 min); Group 5: 2.5% NaOCl (5 mL-30s)+Nd:YAG (Fotona, Slovenia, 1.5 W, 100 mJ, 15 Hz, 4 × 10s); Group 6: CHX (5 mL-30s)+Nd:YAG; Group 7: OCT (5 mL-30s)+Nd:YAG; Group 8: 2.5% NaOCl (5 mL-30s)+EndoActivator (Dentsply)(30s); Group 9: 2% CHX (5 mL-30s)+EndoActivator; Group 10: OCT (5 mL-30s)+EndoActivator; Group 11: 2.5% NaOCl (5 mL)+Vibringe (Cavex)(1 min); Group 12: CHX (5 mL)+Vibringe (1 min); Group 13: OCT (5 mL)+Vibringe (1 min). Dentine chips were collected from inner root canal walls with Gates-Glidden burs (sizes 2–6) into vials containing 2 mL of phosphate buffered saline, vortexed for 30 s, serially diluted to 10–4 and seeded on tryptic soy agar plates and incubated (37°C, 48 h). Total colony-forming units (CFU) were calculated for each sample and statistically analyzed with the Mann-Whitney and Kruskal-Wallis tests ($\alpha = 0.05$).

Results Complete sterilization was achieved in the all 2.5% NaOCl groups ($P > 0.05$). Mean colony forming units (CFU) values obtained after CHX-Vibringe and CHX-EndoActivator were log10 CFU (2.20) and (2.36) respectively while CHX-Nd:YAG and

CHX-conventional irrigation groups showed no bacterial growth. All OCT groups were completely sterilized except OCT-Nd:YAG group (\log_{10} CFU 2.2).

Conclusions NaOCl was effective in killing all the bacteria with all the irrigation techniques. Use of EndoActivator and Vibringe sonic devices was not beneficial with chlorhexidine irrigation. Use of Nd:YAG laser together with Octenisept was detrimental to the disinfection efficacy of this irrigant.

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R30

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Influence of calcium hydroxide intra-canal medication on the elasticity of dentine. An ultrasonic assessment

Aim To determine the change of ultrasonic velocity of dentine after the application of intra-canal medication of calcium hydroxide over time.

Methodology Single rooted premolars were microCT scanned to disclose the canal topographies and to determine the optimal placement location of the ultrasonic transducer for the best signal navigation. Eight premolars were finally selected and fixed in individual mounting platforms to assure exact replacement of teeth without changing the position and angulations of the transducer in relation to dentine. Ultrasonic longitudinal velocity was measured according to the principle of go-and-return path, which included run times of waves propagating to a) the tooth root surface b) the inner dentinal wall surface. The repeatability, reproducibility and accuracy of the measurements were checked by repeating each measurement three times. Teeth were endodontically accessed and dentine ultrasonic velocity was measured before and after irrigation of the canal as well as eight times after the application of calcium hydroxide (1 hr, 2 hr, 1d, 3d, and each week until the 4th week). This resulted in ten measurements per tooth. For each time the US measurement was repeated three times and the mean of the three measurements was recorded. The change of ultrasonic velocity through dentine was calculated and compared using matched paired analysis for repeated measurements per tooth and the Wilcoxon signed rank test.

Results The longitudinal ultrasonic velocity significantly decreased 2 h after the application of calcium hydroxide (mean=20 ns, $P = 0.0078$). In all other times no significant change in dentine ultrasonic velocity was recorded (mean = 0–4 ns, $P = 0.3711$ to $P = 0.6797$).

Conclusions In a whole tooth model, intra-canal medication of calcium hydroxide reduced the rigidity of dentine up to 2 h. Longer applications did not reduce dentine ultrasonic velocity.

IRRIGANTS/DISINFECTION: ANTIMICROBIAL ACTIVITY

R31

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Effect of different endodontic irrigants on a simulated biofilm matrix

Aim To compare the effect of different endodontic irrigants on agarose, a polysaccharide polymer linked by α 1–3 and β 1–4 glycosidic bonds. Agarose has been used to simulate the biofilm matrix in previous investigations, yet never in the current context.

Methodology A 5% (w/v) agarose gel was used for this study. Standard cylinders were punched from the gel with a diameter of 14 mm and a height of 12 mm. The cylinders were weighed in a precision balance. Subsequently, they were immersed in 15 mL of the following solutions: 2% (w/v) CHX, 17% EDTA, 3% H₂O₂, 5% NaOCl, or 0.9% NaCl (saline) as the control treatment. Agarose cylinders were immersed for 20 h at room temperature (25°C). Their remnants were then washed over a pre-weighed plastic mesh with a pore size of 0.5 mm, and their final weight was determined (% of initial weight). Data were compared between groups using one-way ANOVA/Tukey HSD, $\alpha = 0.05$. The data are presented as means \pm standard deviation.

Results The saline control treatment resulted in an end weight of $102 \pm 1\%$. The end weight of these specimens did not differ significantly ($P > 0.05$) from those immersed in CHX ($101 \pm 3\%$) or H₂O₂ ($102 \pm 1\%$). EDTA caused a significant weight increase with an end weight of $113 \pm 5\%$ ($P < 0.05$ compared to all other groups). In the NaOCl solution, the agarose gel blocks were almost entirely dissolved, with an end weight of $2 \pm 2\%$.

Conclusions NaOCl appears to be the only irrigant under investigation capable of breaking glycosidic bonds. It thus has the potential to dissolve polysaccharides, which are a core component of the biofilm matrix.

R32

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The effects of traditional endodontic irrigants on the ability of *Candida albicans* and *Enterococcus faecalis* biofilms to induce Toll-like receptor activation on THP-1 monocytes: a pilot *in vitro* study

Aim To test the effects of traditional endodontic irrigants on the ability of *Candida albicans* and *Enterococcus faecalis* biofilms to stimulate the innate immune response of monocytes.

Methodology Planktonic *C. albicans* and *E. faecalis* were prepared at different multiplicities of infection, and biofilms were grown on coverslips for 24 h in artificial saliva with Log 7 enterococci per mL, Log 6 *Candida* cells per mL or both. These were treated for 1 h with 2% sodium hypochlorite or 2% chlorhexidine. *Listeria monocytogenes* and untreated biofilms served as positive controls; phosphate buffered saline served as a negative control. The viability and biomass of biofilms was verified using qPCR, resazurin

and crystal violet assays. To assess THP-1 monocyte stimulation, THP1-XBlue cells (Invivogen) were exposed to the planktonic microorganisms or biofilms. THP1-XBlue cells release secreted embryonic alkaline phosphatase (SEAP) following Toll-like receptor (TLR) stimulation and activation of nuclear factor kappa B and AP-1 pathways. Monocyte activation was also quantified by assessing TNF levels by enzyme-linked immunosorbent assay. Damage to monocytes during inflammatory challenge was determined by assessing lactate dehydrogenase levels in culture media. Enzymatic substrate conversion in all assays was quantified in a spectrophotometer. Statistical analysis involved log transformation where necessary, ANOVA with Bonferroni *post hoc* tests and Spearman correlation. The level of significance was set to $P < 0.05$.

Results Planktonic microorganisms stimulated SEAP release by monocytes in a dose response manner ($rs=0.615$, $P < 0.001$). Only untreated *E. faecalis* (planktonic $P < 0.001$, biofilms $P < 0.001$) and NaOCl-treated planktonic *E. faecalis* ($P < 0.001$) elicited a significant inflammatory response. SEAP levels were significantly correlated to TNF levels in all three experiments ($rs=0.920$, $P = 0.004$; $rs=0.470$, $P = 0.006$; $rs=0.508$, $P < 0.001$). The proportion of dead monocytes could not be correlated to SEAP levels ($rs=-0.112$, $P = 0.511$), and only significantly increased after exposure to CHX-treated and NaOCl-treated dual-species biofilms ($P=0.009$; $P=0.007$).

Conclusions Within the limitations of this *in vitro* study, *C. albicans* was not immunostimulatory. *E. faecalis* effects on TLR activation were nullified when the bacteria were treated with sodium hypochlorite or chlorhexidine or combined with *C. albicans*.

R33

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The effect of herbal medicine against *Enterococcus faecalis* on infected root canal substrate: an *ex vivo* study

Aim To evaluate the antimicrobial effects of ginger, cinnamon and their combination against *Enterococcus faecalis* located in infected root canals.

Methodology A quasi experimental pre-test post-test design was applied through microbiological testing upon prepared root canals of 50 extracted single-rooted teeth. To standardize the length; all roots were cut to the length of 12 mm and their canals were prepared up to size 50. To ensure no leakage the roots were sealed using clear acid-resistant nail varnish. The roots were divided into a total of 5 groups, each group consisted of 10 root canals; three groups contained extracts of ginger, cinnamon, and their combination in a gel form. In addition to the above mentioned groups a positive control group of calcium hydroxide gel and a negative control group of solely infected root canals were used for comparison. All root canals in each group were infected by *Enterococcus faecalis*. The colony forming units in each root (CFU) were recorded before and after the intervention, and the minimum inhibitory concentration for each test solution was also obtained. Comparisons between the different variables were conducted through the Kruskal Wallis, Wilcoxon and Mann Whitney U tests with a level of significance set at $P \leq 0.05$.

Results Extracts of ginger, cinnamon and their combination showed a reduction in (CFU) from an initial count of 83 colonies to a mean (CFU) of 26.5, 77.8 and 49.7, respectively. Ginger had the greatest antibacterial efficacy in comparison with the others. Significant differences were observed between the negative control group and the test solutions of ginger, cinnamon and their combination ($P < 0.05$).

Conclusions An inhibitory effect against *Enterococcus faecalis* occurred upon testing ginger, cinnamon and their combination. Ginger was found to have the highest effect in comparison to other test groups.

R34

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Cytotoxic effects of human- β -defensin-3 and their variant molecular forms on human periodontal ligament cell and human dental pulp cells

Aim To evaluate the cytotoxicity of human- β -defensin-3 (HBD3) and their variant molecular forms on human periodontal ligament cells (hPDL) and human dental pulp cells (hDPC), establishing the scientific bases of their use in regenerative endodontics as potential intracanal medicaments.

Methodology hPDL and hDPC were cultured up to 6 passages and seeded to a 96 well plate. HBD3 and their variant molecular forms with deformed functional group (C3 and C3O, improved mineralization activity; M2, improved anti-inflammatory peptide) diluted at 200–20 $\mu\text{g mL}^{-1}$ were used to assess the cytotoxicity on the cells by using cytotox-XTT (AKXTRS 96.300, Aniaara) at 1, 3, 6, 12, 24, 48, and 72 h. A mismatched peptide (MP) was used as a control. All experiments were performed in triplicate and the results were statistically analysed by t-test ($\alpha=0.05$).

Results All peptide groups except MP showed cellular cytotoxicity that was concentration dependent reaching 50.7% in hPDL and 54.9% in hDPC at 20 $\mu\text{g mL}^{-1}$ after 72 h of exposure. C3 exhibited a greater cytotoxic effect than HBD3 on both cell lines, whereas C3O and M2 revealed different pattern according to the cell lines ($P < 0.05$). The hDPC was more resistant to the peptides than the hPDL ($P < 0.05$).

Conclusions C3 peptide with improved mineralization activity was most cytotoxic, whereas M2 peptide with improved anti-inflammatory function had the least effect on hPDL and hDPC viability. Within the limitation of this study, HBD3 with improved anti-inflammatory functional group (M2) might have a potential benefit as an intracanal medicament in regenerative endodontics.

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R35

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A comparative analysis of two irrigants and two activation techniques in endodontics. Clinical and microbiological study

Aim The goals of this randomized double-blind trial were to assess the antimicrobial activity *in vivo* of sodium hypochlorite (NaOCl) versus chlorhexidine gluconate (CHX) used in combination either with sonic activation (EndoActivator[®]) or ultrasonic activation (IRRI S[®] files) in patients with apical periodontitis.

Methodology A total of 120 patients with apical periodontitis (in single or multiple root canals) were randomly assigned to the four irrigation protocols outlined below: Group A: 5.25% NaOCl +

EndoActivator®; Group B: 5.25% NaOCl + IRRIS® files; Group C: 2% CHX + EndoActivator®; Group D: 2% CHX + IRRIS® files. Paper points were used to collect microbiological samples before (1A samples) and after (1B samples) irrigation. Viable colony-forming units (CFU) were quantified twice: (1) without speciation, and (2) only for *Enterococcus faecalis*. Statistical analysis was performed using SPSS 22.0 for Windows. Comparative analysis was performed by comparing the mean colony-forming unit count for each group before and after the intervention using Mann-Whitney U test, since variables did not have a normal distribution. A *P* less or equal to 0.05 was considered significant.

Results No significant differences were observed between NaOCl and CHX in the reduction of CFU; in fact, reduction was >93% for the two irrigants. Conversely, significant differences were found between the two activation techniques (sonic and ultrasonic) in the reduction of *Enterococcus faecalis*. Thus, the effectiveness of ultrasonic activation was significantly higher (>93%; *P* = 0.012) as compared to sonic activation. Following the combination of the two irrigants with the two activation techniques (groups A, B, C and D), significant differences were observed between group A and B (*P* = 0.025) in the reduction of *E. faecalis* populations, reaching up to 94%.

Conclusions NaOCl and CHX were effective in reducing intracanal bacterial load and that ultrasonic activation was better than sonic activation in reducing *Enterococcus faecalis* populations.

R36

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Antimicrobial efficacy of QMix irrigant and sodium hypochlorite activated by sonic energy and photon-initiated photoacoustic streaming (PIPS): an *ex vivo* study

Aim To assess the antibacterial effectiveness of sonic activated irrigation and photon-initiated photoacoustic streaming (PIPS) using an Er:YAG laser with QMix irrigant or sodium hypochlorite against *Enterococcus faecalis* intracanal biofilm.

Methodology Root canals of 91 human extracted teeth with single straight canals were prepared with ProTaper files, sterilized in plasma, contaminated with *Enterococcus faecalis* and incubated at 37°C for 15 days. The infected teeth were then randomly distributed into six experimental groups (*n* = 13/each): sonic activated irrigation (EndoActivator system) for 60 s with the QMix irrigant (G1) or 2.5% NaOCl (G2); PIPS/Er:YAG laser (pulse energy: 20 mJ, 15 Hz, pulse duration: 50 µs, 3 × 20 s) with the QMix irrigant (G3) or 2.5% NaOCl (G4); 30-gauge syringe irrigation with the QMix irrigant (G5) or 2.5% NaOCl (G6, positive control group). The positive control group received no treatment in infected teeth (*n* = 10). The root canals were sampled by flushing with saline solution at baseline and after the treatments, serially diluted and cultured. The number of bacteria in each canal was determined by plate count. The presence and the absence of *Enterococcus faecalis* in root canals were also demonstrated by polymerase chain reaction (PCR) and the pattern of *Enterococcus faecalis* colonization after 15 days was visualized by scanning electron microscopy on three samples. The results were analysed by the Wilcoxon test (intragroup analysis) and the Mann-Whitney U test (intergroup analysis).

Results There was significant reduction in the bacterial population for all groups (*p* < 0.001). The best antibacterial efficacy was

recorded after sonic activated irrigation with both NaOCl (99.97%) and QMix (99.99%) and after PIPS with QMix (99.97%), which were more effective than conventional irrigation with NaOCl (88%) and PIPS with NaOCl (97%). Also, the PIPS with QMix solution provided the highest number of sterile samples (five).

Conclusions The PIPS with QMix and the sonic activated irrigation showed equal antimicrobial efficacy. However, the QMix solution activated by the PIPS showed the complete eradication of *E. faecalis* biofilm in the highest number of root canals.

R37

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Antibacterial effect of silver nanoparticles as an alternative irrigation solution on *E. faecalis*

Aim To evaluate the antibacterial effect of 5.25% sodium hypochlorite (NaOCl), 2% chlorhexidine (CHX) gluconate, 0.0023% and 0.0047% concentrations of silver nanoparticles (AgNPs) solutions in human root canals infected by *E. faecalis*.

Methodology Forty six extracted maxillary central incisor teeth were decoronated, and their canals chemomechanically prepared with the ProTaper System (Dentsply Maillefer, Switzerland) up to a master apical file size of F3. The specimens were sterilized. The three sterilized root canals were divided as a negative control. Forty three root canals were inoculated with *E. faecalis* (ATCC 29212) for 1 week. The three root canals, inoculated with *E. faecalis*, were used as positive control. Microbial samples were obtained before irrigation procedures and colony forming units were counted. Forty root canals were divided into four (*n* = 10) groups according to the irrigation solutions used as follows: Group 1: 5.25% NaOCl; Group 2: 2% CHX; Group 3: 0.0023% AgNPs solution, Group 4: 0.0047% AgNPs solution. After irrigation procedures, the percentage reduction in colony counts (% RCC) was determined. The data were statistically analyzed using one-way analysis of variance and Tukey's T2 post hoc tests.

Results NaOCl (100%) and CHX (98.1%) solutions were equally effective (*P* > 0.05) on *E. faecalis* and the RCC of them were significantly higher than 0.0047% AgNPs (64%) and 0.0023% AgNPs (60%) solutions. (*P* < 0.05).

Conclusions The antibacterial effect of AgNPs solutions as a root canal irrigant was inadequate on *E. faecalis*.

R38

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Photodynamic therapy (PDT) for root canal system asepsis – a systematic literature review

Aim To review the literature on the efficacy of PDT efficacy to improve root canal disinfection protocols.

Methodology A systematic literature review was undertaken to answer the PICO question 'Does PDT improve the root canal asepsis revealing significant bacterial reduction in the root canal system?'. Articles of scientific relevance were searched on the databases of the Cochrane Collaboration, Evidence Based Dentistry (EBD), Journal of Evidence-Based Dental Practice (JEBDP), NHS Evidence PubMed from 2000 to August 2014 using the key-expressions:

endodontic photodynamic therapy; antimicrobial photodynamic therapy; photo-activated disinfection; light-activated disinfection; laser-assisted photosensitization and root canal disinfection.

Results The review identified 58 studies: 41 were performed with *in vitro* conditions, 8 were *in vivo* studies and nine *ex vivo*. Considering all publications, 85% showed that PDT was more efficient in root canal disinfection than NaOCl (0.5–6% concentrations). *Enterococcus faecalis* (strain ATCC29212) was the most tested microorganism in experimental models, followed by *Fusobacterium nucleatum* and *Candida albicans*. It is worthy of note in the disparity in methodologies: the most commonly LASER has 660 nm wavelength (446–805 nm range) with a 400 nm (400–500 nm range) diameter of intracanal fibre. Irradiation time varied from 60–240s delivered with spiral movements from apical to cervical. Best results for different photosensitizers (PS) were achieved with methylene blue (MB) at an incubation period of 300s, closely followed by toluidine blue (TBO) and rose bengal (RB). In clinical studies, root canal instrumentation was accomplished with nickel-titanium rotary instruments, outcomes combined with classical irrigant solutions as NaOCl and EDTA, and outcomes compared with PTD additional disinfection. The two most used assessment parameters for PDT outcomes are quantitative analysis, expressed in CFU's per volume, and qualitative study using image techniques such as SEM and CLSM.

Conclusions PDT has been used without a well-defined protocol and still remains at an experimental stage waiting for further optimization. Additional studies using consistent parameters are warranted to assess the PDT efficacy. Level of evidence of clinical studies available to answer this question is low and with high risk of bias.

R39

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Antimicrobial efficacy of different ultrasonic irrigation regimens: a microbiological and microscopic evaluation

Aim The study evaluated the bactericidal effect of sodium hypochlorite (NaOCl) irrigation agitated with a new NiTi ultrasonic device with increased oscillation frequency. The secondary objective was to compare the incidence of canal walls aberrations by SEM analysis.

Methodology Root canals of 128 extracted human single-root teeth were preflared using K-Flexofiles up to size 20 and shaped using ProTaper Universal until F3 (Dentsply Maillefer) up to working length. Irrigation was performed with 33 mL 5% NaOCl, alternating with 10 mL 10% EDTA. After ethylene oxide sterilization, the canals were infected with 30 µL of *Enterococcus faecalis* culture for 3 weeks, and randomly assigned to three groups ($n = 40$) of different irrigation regimens plus positive ($n = 4$) and negative ($n = 4$) controls. Irrigation was performed with 2 mL of 5% NaOCl. In NaOCl-30 group, irrigant was left in place for 30 s; in EC-30 group, NaOCl was ultrasonically-agitated with Endo-Chuck EMS stainless-steel tip size 15, 0.02 taper for 30 s; in EU-30 group, NaOCl was ultrasonically-agitated with EndoUltraTM activator NiTi tip #size 5, 0.02 taper for 30 s. Post-irrigation bacterial count was evaluated with agar plating technique and bacterial load reduction was calculated. The roots were then sectioned longitudinally and analyzed with SEM at 1000 and 5000× magnification for evaluation of debris at coronal, middle and apical level. Differences among post irrigation microbe counts were analyzed with one-way ANOVA and post-hoc Bonferroni's test ($P < 0.05$) and canal walls aberrations were analysed with Kruskal Wallis and post-hoc Dunn's test ($P < 0.05$).

Results Post-irrigation microbe counts (10^5 CFUs) and bacterial load reduction were respectively: EC-30 (mean = $1.90 \pm 1.20 \times 10^2$, 99.88%); NaOCl-30 (mean = $3.80 \pm 2.17 \times 10^2$, 99.62%); EU-30 (mean = $1.21 \pm 0.84 \times 10^2$, 99.81%). A statistically significant difference was evidenced among groups ($P < 0.05$). SEM analysis showed significant differences between standard and ultrasonic irrigation techniques but no differences between ultrasonic groups. ($P < 0.0025$ for coronal measurement; $P < 0.0023$ for middle measurement and $P < 0.0154$ for apical measurement). Kruskal Wallis Statistic KW was 11.986 for coronal debris, 12.116 for middle debris and 8.343 for apical debris.

Conclusions Thirty seconds of NaOCl ultrasonic agitation appeared to be slightly more effective in reducing root canal bacterial load compared to NaOCl irrigation alone, however, neither the oscillation frequency nor the tip alloy seemed to significantly influence the bactericidal efficacy or the traumatic effects against canal walls.

R40

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Confocal scanner laser evaluation of bactericidal effect of different antibiotic mixtures used for dental pulp regeneration

Aim The objective was to evaluate the antibacterial efficacy and depth of action into dentinal tubules of different antibiotic mixtures used for dental pulp regeneration procedures.

Methodology Seventy-two human single-root teeth with fully formed apex were used. Cylindrical root dentine blocks were longitudinally sectioned and enlarged to a size 110 with a Gates Glidden drill size 4. After ethylene oxide sterilization, root canals were infected with a culture of *E. faecalis* to match the turbidity of 3×10^8 CFU mL⁻¹ and further incubated for 3 weeks to allow penetration into dentinal tubules. Specimens were randomly assigned to three experimental groups ($n = 20$) plus positive ($n = 6$) and negative ($n = 6$) controls. In group A specimens were exposed to TRIMIX (ciprofloxacin, metronidazole and minocycline), in group B to BIMIX (ciprofloxacin and metronidazole) and in group C to TRICLARITRO (ciprofloxacin, metronidazole and clarithromycin) antibiotic pastes. Exposure was prolonged for 3 weeks and fresh broth was replaced every 4 days. Each experimental group was divided in two subgroups of different antibiotic paste formulations added to macrogol or ialuronic acid. In group D (positive control group) infected specimens were not exposed to antibiotic pastes while in group E (negative control group) specimens were not infected after sterilization. After 3 weeks all specimens were rinsed and vertically fractured. Confocal laser scanning microscopy (CLSM) and viability staining (Live/Dead BacLight Viability Stain – Molecular Probes, Eugene, OR) were used to quantitatively analyze the ratio of dead/live bacteria into dentine tubules. Volume ratio of red fluorescence (Dead) was calculated in three-dimensional reconstructions. Differences among groups were analyzed with Kruskal-Wallis and post-hoc Dunn's test ($P < 0.05$). Mean penetration depth of action was recorded and differences were analyzed with one-way ANOVA and post-hoc Bonferroni's test ($P < 0.05$).

Results Ratio of red fluorescence over total green/red signal In TRICLARITRO, TRIMIX and BIMIX groups was 87.1%, 84.2% and 76.4% respectively. The mean depth of action was: TRICLARITRO 520 ± 30 µm, TRIMIX 500 ± 60 µm and BIMIX 320 ± 60 µm. A statistically significant difference was evidenced only for BIMIX group ($P < 0.05$).

Conclusions TRICLARITRO antibiotic mixture was associated with an effective antibacterial action deep into dentinal tubules.

R41

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Short term antimicrobial activity of a novel silicate-based sealer in comparison with contemporary sealers

Aim To investigate the antimicrobial activity of a novel silicate-based root canal sealer on *Enterococcus faecalis* and *Candida albicans* in comparison with five contemporary sealers.

Methodology Filter paper discs were either immersed in optically standardized *E. faecalis* (A197A) or *C. albicans* (ATCC 90028) suspensions (OD₆₀₀ = 0.65) and exposed to freshly mixed six sealers; AH Plus Jet, RealSeal SE, EndoRez, MTA Fillapex, Septodont, and RoekoSeal Automix in teflon wells for 30 min. A teflon disc, instead of a sealer, was used for controls. After exposure, the filter paper discs were transferred to vials containing phosphate-buffered saline (PBS) and glass beads, and vigorously vortexed. PBS with resuspended cells was serially diluted to 10⁻⁴ and droplets of 25 microL from each dilution were seeded on Brain Heart Agar plates. The plates were incubated in air at 37 degrees C for 24 h and colony-forming units were counted. Using alpha = 0.05 as level for statistical significance, the data obtained were analysed using One-Way ANOVA and Tukey HSD tests.

Results When sealers came in direct contact with *E. faecalis* cells, none of the sealers killed this bacteria to a level below the detection limit and the sealers ranked: Septodont, AH Plus Jet, EndoREZ, RealSeal SE, and RoekoSeal in descending order of antibacterial potency. Antifungal activity of sealers ranked as follows; RoekoSeal Automix, EndoRez, RealSeal SE, MTA Fillapex, Septodont and AH Plus Jet in ascending order of antifungal property. AH Plus Jet killed the yeast cells to a level below the detection limit. Silicone-based RoekoSeal sealer were not statistically different from *E. faecalis* (Log 10 6.75 ± 0.25) and *C. albicans* (Log 10 6.05 ± 0.43) control groups ($P > 0.05$).

Conclusions AH Plus Jet and the new calcium silicate-based Septodont sealer were effective in reducing the number of cultivable cells of *E. faecalis* and *C. albicans* and could be better alternatives for the clinicians to refill the root canals of retreatment cases as these microorganisms could persist frequently. Further studies are warranted to validate these results.

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R42

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pH of calcium hydroxide dressings mixed with sodium hypochlorite, anaesthetic or saline solution

Aim Determinate the influence of sodium hypochlorite, 2% mepivacaine/epinephrine and saline solution on the pH of calcium hydroxide pastes after 24, 72 h and 7 days.

Methodology Forty extracted human teeth instrumented until the diameter of size 50, 0.04 taper and irrigated with 5.25% sodium hypochlorite (NaOCl) and a final rinse with EDTA were used. Three groups were determined according to the vehicle used to mix the calcium hydroxide (CH) using a proportion of 4 g of CH with 10 mL of the vehicle solutions ($N = 12$): group A: 5.25% NaOCl; group B: 2% mepivacaine; group C: saline solution. Two teeth without a paste were used as positive controls. The calcium hydroxide pastes were placed using ultrasonic activation and the coronal access cavities were sealed. Then, all the specimens were introduced into test tubes filled with 10 mL of distilled water to measure the pH released through the foramen. A pH meter (Checker, Hanna) was calibrated at 7.0 and 10.01, measurements of the tubes with distilled water were taken at 24 h, 72 h and 7 days, calibrating the pH device for each group. The pH was measured before the insertion of the teeth in the water and in three different times after including the teeth with the Ca (OH)₂ dressings at 24 h, 48 h and 7 days. The statistical analysis was performed using Kruskal-Wallis test.

Results Significant differences were found in the pH values of group A ($P < 0.05$) being the pH higher after 7 days. As for group B and C no significant differences were found between groups B and C.

Conclusions NaOCl mixed with calcium hydroxide was the vehicle with the higher alkalinity. The values of pH increased significantly after 7 days.

IRRIGANTS/DISINFECTION: CANAL CLEANING

R43

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Effect of diode laser activation of EDTA with two different output powers on removal of smear layer and dentine erosion

Aim The aim of this study was to evaluate the activation of EDTA by 810 nm diode laser with two different output powers on removal of smear layer and dentine erosion.

Methodology Forty-eight extracted mandibular premolar teeth were selected and decoronated. The root canals prepared using the ProTaper system to a size F4. Samples were divided into three experimental groups and one positive control group with no treatment ($n = 12$). In group 1, root canals were irrigated by 17% EDTA for 2 min without laser irradiation; in group 2, EDTA was activated by 810 nm diode laser with 2W output power for 20 s, and in group 3, EDTA was activated by 810 nm diode laser with 3W output power for 20 s. The samples were split longitudinally and scanning electron microscope images were taken from coronal, middle and apical thirds of the root canals. Three-grade scoring systems were used for evaluation of smear layer removal (score 1: no smear layer, score 2: moderate smear layer, score 3: heavy smear layer) and dentin erosion (score 1: no erosion, score 2: moderate erosion, score 3: severe erosion). The data were analyzed using Kruskal-Wallis test.

Results According to the smear layer removal scores, the three experimental groups had significantly lower scores than the positive control group in all three regions of the root canal ($P < 0.05$). Additionally, no significant difference was recorded among experimental groups ($P > 0.05$). Apical thirds of the samples had significantly higher smear layer scores than the coronal and middle thirds in experimental groups. No significant differ-

ence was found among the experimental groups in terms of dentine erosion ($P > 0.05$).

Conclusions The activation of EDTA with a diode laser did not increase the efficacy of smear layer removal and showed no dentine erosion on the root canal surface. Additionally, the output power did not influence the removal of smear layer.

R44

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Removal of calcium hydroxide from the root canal: influence of volume of irrigant and activation

Aim The aim of the present study was to evaluate the amount of calcium hydroxide suspension (CaOH_2) that is removed by several irrigation procedures with different volumes and activations.

Methodology One hundred and thirty extracted straight single-rooted human teeth were cut to equal lengths (15 mm). Root canals were instrumented to size 45, .04 taper. CaOH_2 , radioactively marked with ^{14}C -glucose, was placed in the root canals for 7 days at 35°C together with a gutta-percha point size 40, .02 taper (negative control: no medication). The gutta-percha point was taken out of the root canal. Within the first part of the study intracanal medication was removed by an alternating syringe irrigation with citric acid 40% and sodium hypochlorite 3% with total volumes of 0.5, 1, 2, 4 or 8 mL, carried out in 0.5 mL-increments for each irrigation solution (0.25 mL increments for the 0.5 mL group; positive control: medication left in place). For the second part of the study, additional mechanical activation following each of the first two 0.5 mL increments of irrigation was carried out with a FlexMaster instrument size 45, .04 taper (Instr), a CanalBrush size M (CB), or passive ultrasonic irrigation with a smooth wire (PUI), each for two times 20 s. This was carried out for the 2 mL and 4 mL volume groups only. All groups had $n = 10$. Residual CaOH_2 was measured using a scintillation counter and expressed as percentage of the original medication.

Results Increasing the irrigation volume led to a decrease of residual CaOH_2 (mean/SD in %): 0.5 mL (21.7/4.8); 1 mL (16.5/2.5); 2 mL (12.9/1.9); 4 mL (8.7/2.4); 8 mL (5.0/2.3); posCtrl (98.5/0.8); negCtrl (0.0001/0.00003) (all groups different from each other; ANOVA/SNK, $P < 0.05$). Activation led to less residual CaOH_2 : 2 mL Instr (12.0/2.1); 2 mL CB (11.7/2.3); 2 mL PUI (9.1/2.2); 4 mL Instr (8.5/2.0); 4 mL CB (7.4/1.9); 4 mL PUI (6.2/1.8), with significant differences according PUI (ANOVA/SNK, $P < 0.05$).

Conclusions No irrigation procedure was able to totally remove CaOH_2 . Passive ultrasonic irrigation was the most effective activation method tested. However, it was more effective to double the amount of irrigation solution than to activate it.

R45

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Evaluation of various instrumentation techniques on the removal of calcium hydroxide paste from canals in single-rooted teeth

Aim To evaluate the efficacy of various instrumentation techniques on the removal of calcium hydroxide paste from single-rooted extracted teeth.

Methodology Thirty single-rooted teeth were used. The root canals were shaped with ProTaper Universal F4 files. Calcium hydroxide paste was placed into each root canals, and packed to the working length. Then cotton pellets were placed over the canal orifices and coronal parts of the roots were sealed with Cavit. Specimens were stored in distilled water for 7 days at 37°C . After 7 days the temporary coronal seal was removed and the samples were randomly divided into four experimental groups (ProTaper Universal F4, ProTaper Next X4, Wave One Large, Resiproc 40) ($n = 12$) and a control group (only irrigated with NaOCl) ($n = 12$) according to the method used for calcium hydroxide paste removal. The roots were split longitudinally into halves (60 specimens) and the both canals examined using a stereomicroscope. The amount of remaining calcium hydroxide paste in the canal walls were measured under a stereomicroscope at $\times 15$ magnification. The data were statistically analysed using with Cronbach alpha method.

Results It was not possible completely to remove CaOH paste from root canals. Reciproc files removed significantly more CaOH paste than the Wave One, ProTaper Universal, ProTaper Next and control groups ($P < 0.05$). Control group removed less CaOH than the other experimental groups but this was not significant ($P > 0.05$).

Conclusions Reciproc instrumentation technique with NaOCl were more effective in removing CaOH from canals than only NaOCl irrigation and Wave One, ProTaper Universal, ProTaper Next instrumentation techniques.

R46

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In vitro investigation of the influence of various lasing parameters on the cleaning efficacy of laser-activated irrigation

Aim The purpose of this *in vitro* study was to investigate the influence of different lasing parameters (pulse energy, length and frequency and fibre tip position, shape and diameter) on the cleaning efficacy of Laser-Activated Irrigation.

Methodology Resin blocks containing standardized root canals (apical diameter of 0.4 mm, 6% taper) were the test model. A standardized groove in the apical part of each canal wall was packed with stained dentine debris. The canals were filled with irrigant, activated by an Er:YAG laser (2940 nm). The influence of pulse energy (10, 20, 40 mJ), length (50, 100, 300, 600, 1000 μs) and frequency (5, 10, 15, 20, 30 Hz), irradiation time (5, 10, 20, 30, 40s), fibre tip shape (flat or conical), position (pulp chamber, canal entrance, next to groove) and diameter (300, 400, 600 μm) was determined by treating 20 canals per parameter. The amount of debris remaining in the groove was scored and compared among the different treatments by means of the Kruskal–Wallis and the Mann–Whitney U test.

A comparison with the cleaning efficacy of Passive Ultrasonic Irrigation was also made. An Irrisafe needle (size 20) driven by an ultrasonic device (30 kHz) at 35% power was placed in the root canal 1 mm from working length.

Results The parameters significantly ($P < 0.05$) effecting debris removal from the groove were pulse energy, length and frequency and irradiation time and fibre tip position. Fibre tip shape and diameter had no significant influence on cleaning efficacy. The difference between the debris scores after PUI and the best results after LAI was not significant.

Conclusions The highest cleaning efficacy of LAI with the Er:YAG laser was achieved with the following laser settings: pulse energy of 40 mJ, very short pulse length (50 μs), pulse frequency

of 20 Hz, irradiation time of 4×5 s and position of the fibre tip at the beginning of the groove.

R47

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Comparison of different techniques for the removal of calcium hydroxide paste from root canals

Aim The aim of this study is to evaluate the removal of different calcium hydroxide (Ca(OH)₂) pastes from an artificial standardized groove in the apical root canal using three irrigation methods.

Methodology The root canals of 34 extracted single-rooted human mandibular premolars were prepared with ProTaper Universal rotary instruments of size F3. The teeth were sectioned longitudinally and standardized groove was prepared in the apical part of both segments. In Groups 1, 2, and 3 grooves were filled with Ca(OH)₂ + iodoform paste, whereas in Groups 4, 5, and 6 with Ca(OH)₂ paste. After the roots were reassembled, they were completely filled with the aforementioned medicaments. The negative control did not receive any medicament placement and the positive control received the intracanal dressing, but no subsequent removal. After 7 days, Canal Brush with 5.25% NaOCl in Groups 1 and 4, Endoactivator in Groups 2 and 5, Passive Ultrasonic Irrigation for 30 s with 5.25% NaOCl in Groups 3 and 6 were used in removal of the medicament from the root-canals. Subsequently, the roots were disassembled and digital photographs were acquired. The quantity of the remaining medicament was evaluated using a four-grade scoring by two calibrated observers. The results were statistically analysed using Kruskal-Wallis and Mann-Whitney U tests.

Results Ca(OH)₂ paste removal was significantly more effective compared to the removal of Ca(OH)₂ + iodoform paste ($P < 0.01$). There were no significant differences between irrigation methodologies.

Conclusions None of the techniques removed the medicaments completely. Regardless of the irrigation technique, removing Ca(OH)₂ + iodoform paste from irregular canal walls was more difficult in comparison to Ca(OH)₂ paste.

R48

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Ex vivo assessment of smear layer removal with a negative pressure irrigation system

Aim Currently, there is no irrigation technique that fulfils the objectives of root canal irrigation. Debris or smear layer persists, particularly in the apical third of the root canal. The purpose of this study was to evaluate smear layer removal when using a negative pressure irrigation system (Endovac®, SybronEndo, USA) after root canal shaping with Ni-Ti rotary files.

Methodology Twenty single-rooted teeth were selected. All the root canals were prepared by the same Ni-Ti rotary instruments (BioRace® sequence (FKG, La Chaud de Fond, Switzerland), last instrument size 35, 0.04 taper). For group A, the Endovac system

was used as the irrigant distribution device during shaping. For the group B, an irrigation syringe with an endo-needle (side-exit) of 30 gauge was used. For both groups, the same quantity of NaOCl was used: 2 mL between each instrument with a total of 12 mL for each canal, and a final rinse using 3 mL of EDTA agitated by the EndoActivator® (DentsplyMaillefer, Switzerland) and 3 mL of NaOCl, then 3 mL of physiologic serum as final flush flow. SEM pictures ($\times 500$) were obtained from each sample at the apical third with the SEM JEOL (JSM-5310LV Tokyo, Japan), used in low vacuum mode, without metallisation. Three independent examiners evaluated smear-layer removal on SEM pictures, according to the ROME scoring method. An exact test of Fisher (Chi2 for small samples) was achieved to compare the scores between the two groups.

Results There was no significant difference between the two groups ($P > 0.05$). The mean value for the score for the Endovac® group: 1.057 ± 1 , and for syringe group: 1.403 ± 0.713 .

Conclusions In the conditions of this experiment, the use of a negative pressure irrigation system did not improve the removal of smear layer in the apical-third of straight canals. Curved canal studies are needed.

Acknowledgements Thanks to Sybron-Kerr France for supplying the Endovac system.

R49

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Analysis of hypochlorite extrusion based on several final irrigation systems

Aim To compare the extrusion of sodium hypochlorite when using several final irrigation systems used in a semi-closed environment, simulating the periodontal ligament.

Methodology Eighty four extracted human single-rooted teeth were selected. They were cut at the cement-enamel junction and the root portions were embedded in an agarose 0.3% colloidal gel placed in individual transparent methacrylate boxes. All the canals were instrumented using PathFile®, ProTaper® (Until F2) and Profile® (size 35, 0.04 taper) following the same procedure of irrigation. A mixture of sodium hypochlorite 5.25% and methylene blue (96% of sodium hypochlorite and 4% of methylene blue) was used as irrigant. After instrumentation, six randomized experimental groups (of 14 samples each) were established based on the final irrigation method used: needle –1 mm of the working length (N-1), needle –4 mm of the working length (N-4), EndoActivator® (EA), EndoVac® (EV), WaterPik® power flosser used as sonic activation (WP) and ultrasonic activation (US).

During all the process, the blue irrigant mixture was extruding through the apical foramen and created blue periradicular areas of varying sizes. To make sure that the shape of the periradicular area was homogeneous, a pilot study was completed with 12 samples taking a frontal picture and a lateral one, resulting in no differences between projections. Because of that, only two frontal pictures of each sample were taken, the first one at the end of the instrumentation phase and the other one after the final irrigation. The size of these areas was quantified by ImageTool® 3.0 analyzer. The results were statistically analysed using Kruskal Wallis test and multiple comparisons between groups and Wilcoxon test for paired samples (IBM SPSS Statistics 22).

Results The final irrigation increased the extrusion level in all experimental groups, except in the EV group. Comparing the final extrusion area, this was greater in N-1 and US than in the

EV group. In the other groups there were no significant differences.

Conclusions Although there were differences in the degree of extrusion of different final irrigation systems used, most of the extrusion of the irrigant was produced during the instrumentation procedures within the root canals.

R50

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Influence of the irrigants used during biomechanical cleaning and shaping on tooth discoloration with photodynamic therapy

Aim To assess if discoloration of the tooth structures occurs after photodynamic therapy (PDT) using toluidine blue (TB) as a photosensitizer and the influence of the irrigant used during the biomechanical shaping.

Methodology Thirty incisors with perfect crown were randomly divided into 3 groups according to the irrigant used during the biomechanical cleaning and shaping: (i) saline 0.9%, (ii) sodium hypochlorite 5.25% and (iii) chlorhexidine 2%. 0.02 mL of TB were placed into the root canal and irradiated by a LED light 630 nm for 30 s. Then PDT photosensitizer was removed first with 5 mL saline 0.9% and later with 5 mL sodium hypochlorite 5.25%. Via a split the shade of the buccal surface of the teeth was measured by a Vita Easyshade Compact spectrophotometer at four different experimental times: before biomechanical cleaning and shaping (T0), after biomechanical cleaning and shaping (T1), after removing the photosensitizer with saline 0.9% (T2) and finally after final irrigation with sodium hypochlorite 5.25% (T3). Statistical analysis: repeated-measures analysis of variance was used to compare colour changes at different time-points, post-hoc comparisons between groups were based on the Bonferroni test. All data were deemed to fulfil the criteria for parametric tests.

Results After using PDT and removing the photosensitizer with saline, a reduction of brightness in groups 2 and 3 was noted, with no significant differences ($\Delta L = -1.46$ y -1.39). An upward trend through green was also noted with a significantly higher value in group 3 ($\Delta a = -2.23$) as well as an upward trend through blue with no significant differences between groups. No statistical differences were found in ΔE .

Conclusions The irrigant used during biomechanical shaping and cleaning did not cause discoloration after the use of PDT.

R51

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Uncontrolled removal of dentine during *ex vivo* ultrasonic activation of sodium hypochlorite

Aim To evaluate uncontrolled removal of dentine during *ex vivo* ultrasonic activation of sodium hypochlorite in prepared root canals using two different ultrasonic files.

Methodology Fifty-four human single-rooted teeth with straight canals were prepared to size 35, 0.06 taper using rotary Ni-Ti instruments. The specimens were randomly allocated to three groups ($n = 18$). Two millilitres of 2% sodium hypochlorite were delivered 3 times to each root canal by a syringe and an open-ended needle. After each delivery, the irrigant was ultrasonically activated at 35% power (manufacturer's recommendation), either by a size 20 Irrisafe file (Acteon Satelec, Merignac, France) or a

size 15 ultrasonic K-file (Acteon Satelec) placed 2 mm short of working length (Groups A and B). The irrigant in the control specimens (Group C) was not activated. All groups received a final irrigation of 2 mL sodium hypochlorite and 5 mL distilled water. Specimens were scanned by micro-computed tomography at 10- μ m voxel size before preparation, after preparation and after the final irrigation/activation sequence. Scans were co-aligned, segmented, filtered and the amount of dentin removed from each specimen during the final activation sequence was quantified by arithmetic operations. Results were analyzed by non-parametric statistical tests. The level of significance was set at $P < 0.05$.

Results Both ultrasonic files removed significantly more dentine from the apical third of the root canal than irrigant delivery in the control group ($P < 0.004$). K-files removed significantly more dentine than Irrisafe files ($P = 0.001$).

Conclusions Ultrasonic activation of sodium hypochlorite by widely-used files may result in uncontrolled removal of dentine even in straight root canals and at manufacturer-recommended power settings.

R52

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The use of micro-computed tomography to investigate the effect of ultrasonic irrigation on the removal of debris from lateral canals

Aim To determine the amount of debris removed from lateral canals using passive ultrasonic irrigation in canals with varying tapers.

Methodology Polydimethylsiloxane (Sylgard, Dow Corning, UK) root canal models with branching lateral canals were fabricated using a customised model maker. Each main canal was made with either a 3% or 6% taper with lateral canal branches (200 μ m in diameter) aligned 90 degrees to the main canal at either 3 or 6 mm from the apex ($n = 5$ per group). To simulate debris accumulation in lateral canals, human extracted teeth (NRES ethical approval 09/H0405/33) were decoronated and sectioned longitudinally. Dentine debris was created with a round stainless steel bur (size 3) and then packed into lateral canals to a consistent radio density based on previous micro-computed tomography (μ CT) studies in our laboratory. An ultrasonic file [Sonofiles K15 25 mm (Satelec, France)] positioned 3 mm from the apex in a flooded canal was activated for 60 s on power setting 5 [P5 ultrasonic unit (Satelec, France)]. The models were scanned using μ CT (Skyscan 1172, Belgium) at a resolution of 13.6 μ m before and after passive ultrasonic irrigation. The scans were then reconstructed using NRecon Software (Skyscan, Belgium), and debris was segmented and quantitatively evaluated using Fiji-Image J software.

Results More debris was removed from canals with a 6% taper and where the lateral canal was placed 3 mm from the apex ($P < 0.05$). Less debris was removed from lateral canals positioned at 6 mm compared to 3 mm from the apex for both tapers.

Conclusions This study demonstrates a reproducible working model for analysing debris removal. It appears that passive ultrasonic irrigation is more effective at removing debris from lateral canals when a larger tapered preparation is used for the main canal and when the ultrasonic instrument is positioned closer to the lateral canal itself. Further research is necessary to determine if this improves clinical techniques.

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R53I.F. Ertugrul^{1,*}, M. Maden¹ & E.O. Orhan²¹Department of Endodontics, Graduate School of Health Sciences, Isparta & ²Eskisehir, Turkey**The effect of micro electric current and other conventional activation techniques on the solvent action of sodium hypochlorite on bovine tissues****Aim** To evaluate sodium hypochlorite (NaOCl) use with the micro-electric current method and compare for tissue dissolution with other conventional activation methods such as sonic, ultrasonic, pipetting, temperature.**Methodology** A total of 154 pieces of bovine muscle tissues were prepared to a standard size and weight. Tissue samples were divided into seven groups for room temperature experiments and seven groups for 45 centigrade degrees temperature experiments as following: 'D', Distilled water (- control); 'NaOCl', 5.25% Passive NaOCl (+ control); 'P', 5.25% NaOCl with pipetting; 'SA', 5.25% NaOCl with sonic motion device; 'UA', 5.25% NaOCl with ultrasonic motion device; 'E-NaOCl', 5.25% NaOCl with micro-electricity; 'E-NaOCl+P', 5% NaOCl with micro-electricity+pipetting. Specimens were weight before and after, treated with 5.25% NaOCl. The averages, standard deviation, minimum, maximum and median were calculated for each group. The resulting data were analyzed statistically using the multi-way ANOVA and Tukey HSD tests. The level of the alpha-type error was set at <0.05.**Results** The weight loss of the tissue mostly increased in E-NaOCl+P group compared with all other groups ($P < 0.05$). There were no significant difference between UA, SA, P groups at the same temperatures ($P < 0.05$).**Conclusions** Use of NaOCl with microelectric current improved the tissue dissolution ability of the solution. Electric current can be used with additional methods (temperature, pipetting) for synergic effect on the NaOCl solution.**Acknowledgements** This project labeled 3691-D1-13 has been supported by Süleyman Demirel University, Scientific Research and Coordination Center.**R54**

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The effect of micro bubble irrigation on removal of canal wall debris and smear layer**Aim** Canal irrigation is essential for successful debridement of root canals after mechanical debridement procedures because endodontic instruments cannot contact canal walls completely. NaOCl is known as a standard irrigant with powerful debris removal and antimicrobial ability; however, there are complications due to its toxicity. Microbubbled water, which is effective in cleaning and removal of bacteria, has been utilized in various fields such as decomposition of organic chemicals in waste-water treatment and cleaning of vegetables, etc. The purpose of this study is to evaluate the effect of microbubbled water irrigation on removal of canal wall debris and smear layers.**Methodology** Fifty extracted single-rooted human mandibular premolars were divided into five groups according to the irrigation solution pairs (5 mL) used: group 1: Distilled water + Distilled water (DD); group 2: NaOCl + NaOCl (NN); group 3: Microbubble water + Microbubble water (MM); group 4: EDTA + NaOCl (EN); group 5: EDTA + Microbubble water (EM). Debris and smear scores were evaluated in the coronal, middle, and apical

third of longitudinally fractured canal spaces using scanning electron microscopy followed by Cochran-Mantel-Haenszel statistical analysis.

Results In debris scores, group 1 (DD) was significantly different from the other 4 groups (group 2,3,4,5), while there was no significant difference among groups 2,3,4,5. Considering the canal level, debris scores were significantly higher in group 1 (DD) in middle and apical thirds, but there was no significant difference in the coronal third with different irrigation protocols. Also, group 2 (NN) and 3 (MM) had significantly lower smear scores than group 1 (DD), but had higher smear scores than group 4 (EN) and 5 (EM). ($P < 0.05$)**Conclusions** Microbubbled water was as effective as NaOCl in removal of canal wall debris. Combined use of EDTA with NaOCl or microbubbled water was more effective in removal of smear layer than using NaOCl and microbubble alone.**R55**A. Beaugendre^{1,*}, M. Dedieu¹, W. Nehme², J.P. Mallet¹ & F. Diemer¹¹Department of Endodontology, Toulouse University, Toulouse, France & ²Saint Joseph University, Beyrouth, Lebanon**Low vacuum SEM assessment of root canal surface: CleanJetEndo versus conventional irrigation****Aim** Endodontic success depends on a thorough cleaning and shaping of the root canal system. For this purpose sodium hypochlorite and chelating agents are usually combined with an activation system. The aim of this study was to assess the effectiveness of a new irrigation spray device (CleanJet Endo).**Methodology** Thirty-six freshly extracted human single root canals were selected. Crowns were removed and root canals were prepared using continuous rotation. Teeth were divided into three groups ($n = 12$) for irrigation. The new device CleanJet Endo was used for irrigation in the first group, a conventional syringe associated with the CleanJet Endo needle (comprised of three lateral exits) in the second group and a conventional syringe and needle (EndoNeedle) in the third. The irrigant used for all groups was sodium hypochlorite. Four more teeth were prepared for control groups: two were shaped without irrigation and two irrigated without mechanical preparation.All roots were fractured into 4 fragments, each which was analyzed in SEM Low Vacuum without preparation. For each fragment two pictures at $\times 100$ magnification and 4 at $\times 750$ were taken. Pictures were scored independently by two observers for the presence of debris and smear layer. Equality of variance between observers was calculated. Amount of debris and smear layer were analyzed with an ANOVA and a *posteriori* Fischer's test with an alpha risk fixed at 5%.**Results** More than 600 pictures were scored for the smear layer and root canal debris. The scores were significantly different for each level of observation and each group ($P < 0.0001$). The best results were obtained with the CleanJet Endo device. The conventional syringe associated with the CleanJet Endo device was more effective for root canal cleaning than a conventional EndoNeedle.**Conclusions** Cleaning quality seemed to be improved by the multiple lateral exit of the needle and the best results were observed with the spray.**Acknowledgements** The author may thanks the Produit Dentaire company for providing the CleanJet Endo spray.

R56

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An *ex vivo* analysis of apical irrigant pressure developed by different irrigation needle types at various irrigant flow rates

Aim To analyze apical irrigant pressure developed by four needles, each at different flow rates, using an *ex vivo* model for endodontic irrigation.

Methodology A human maxillary incisor root canal was shaped to the working length (WL) utilising rotary files up to size 40, 0.06 taper, patency size 10. The apical foramen of the root canal was sealed in closed system connected to digital manometer (PM-9100HA, Omega Engineering, USA). Two notched open-end irrigation needles (Needle 1: Endo-EZE; Ultradent Products, USA, and Needle 2: Appli-Vac; Vista Dental, USA), one single side-vented (Needle 3: Appli-Vac, Vista Dental, USA) and one double side-vented (Needle 4: Calasept, Nordiska Dental, Sweden), all 27G, were used. Each needle was mounted 1 mm short of WL and constant flow rates of 0.05, 0.10, 0.15 and 0.20 mL s⁻¹ of irrigant (3% sodium-hypochlorite) were delivered by Precision Syringe Pump (Aladdin, World Precision Instruments, USA). Apical irrigant pressure was measured (N = 20) for each flow rate. Two-way ANOVA without repetition analysis was used to determine statistical difference between needles types and the rise of irrigant flow rate.

Results The highest value of apical irrigant pressure was developed by Needle 2 at 0.20 mL s⁻¹ (68 ± 2.66 mmHg), while the lowest by Needle 4 at 0.05 mL s⁻¹ (2.25 ± 0.55 mmHg). Results showed a significant difference between the notched open-end (Needle 1 and 2) and side-vented irrigation needle (Needle 3 and 4) type groups (F = 6.53, P < 0.05). Differences in needle type were not significant. The increase of irrigant flow rate resulted in a significant increase of apical pressure for all irrigation needles.

Conclusions Apical irrigant pressure was influenced by both needle type and irrigant flow rate. Increase of irrigant flow rate increased apical pressure while the application of open-end needles was associated with increased apical pressure compared to side-vented.

R57

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Ex vivo study of two final passive ultrasonic irrigation protocols in root canals shaped by two different techniques

Aim To compare the efficiency of two passive ultrasonic irrigation (PUI) protocols, consecutive rotary or reciprocating adaptive motion canal instrumentation.

Methodology Forty extracted human teeth were selected with moderate curvature canals (20°–30°) and randomized into 2 groups (n = 20) according to the root canal shaping: group 1 ProTaper Next (Dentsply Maillefer) up to size X3 and group 2 TF Adaptive

(SybronEndo) up to size MS3. Each main group was randomly subdivided into 2 subgroups (n = 10) and treated with different irrigation protocols: 1 mL of 17% EDTA, 2 mL of 3% NaOCl, 1 mL saline (subgroup A) and 3 mL SmearClear (SybronEndo), 1 mL saline (subgroup B). In all four subgroups 1A, 1B, 2A, 2B Irrisafe (PUI)(Satelec) was performed for 10s. The roots were split longitudinally in a bucco-lingual direction and dried. Representative areas were selected for apical third by screening at low magnification; the selected areas were then analyzed at higher magnification. SEM images of the root canals were scored for the presence or absence of debris (×100) and smear layer (×1000) according to a numerical evaluation scale. The evaluations were performed by two independent examiners. Comparisons were done using one-way ANOVA test followed by non-parametric tests.

Results The analysis of the results with the Kruskal-Wallis test (P < 0.05) showed significant differences, in terms of debris (P = 0.0009) and smear layer (P = 0.0002). The Mann-Whitney-U test (P < 0.05) demonstrated that in the case of debris, the difference (P = 0.019) was due to the distinct motion of the ProTaper files compared to that of the TF-Adaptive files (P = 0.01). In contrast, the irrigation protocols did not generate significant differences (P = 0.12) in terms of debris. The smear layer differences were significant and are a consequence of the irrigation protocol, in both the ProTaper (P = 0.003) and the TF-Adaptive techniques (P = 0.008). Smear-Clear had the least score for the smear layer (20%).

Conclusions The irrigation protocols using PUI were effective in removing debris from the apical third, but not as effective in removing the smear layer entirely. Differences observed between root canal preparation techniques would require a separate study.

R58

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Radiopaque dye clearance beyond the needle tip in canals prepared to size 25, 0.08 taper

Aim To evaluate *ex vivo* radiopaque dye displacement beyond the needle tip in canals shaped to size 25, 0.08 taper using two syringe irrigating needles and a negative pressure needle.

Methodology The canals of eighteen mandibular premolars with slightly curved canals were enlarged to an apical size of 25, 0.08 taper and filled with a radiopaque dye. Irrigation with 1% NaOCl was performed at 4 mL min⁻¹ with syringe irrigating needles (30G flat, 27G side-vented) and a negative pressure needle (28G NP) placed 3 mm short of working length. Each tooth was evaluated three times, once for each needle. A standardized digital radiograph was taken before irrigation (negative control), at 10 s and at 60 s. Dye displacements beyond the needle tip were measured in mm. The independent variables were needle type (nominal) and time point (nominal), whereas the dependent variable was radiopaque dye displacement (continuous). Therefore, the data was analyzed using the mixed ANOVA procedure.

Results No displacement was observed in the negative controls. Complete displacement was observed in 8/54 samples, all corresponding to NP. Mean (standard deviation) displacements for flat, side-vented and NP needles were 2.42 (0.24) mm, 1.57 (0.14) mm and 2.58 (0.18) mm at 10 s, respectively and 2.67 (0.17) mm, 1.9 (0.13) mm and 2.90 (0.1) mm at 60s, respectively. A significant interaction was found between needles and time point (P = 0.025). There was a statistically significant difference of dye displacement between needles at 10 s (P < 0.001) and at 60 s (P < 0.001). All needle showed significantly greater displacement at 60 s than at 10 s; flat (P < 0.001), side-vented (P < 0.001) and NP (P < 0.001).

Conclusions Greater dye displacement was observed with NP needles compared with both syringe needles. A prolonged irrigation produced greater displacement with all needles.

R59

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Removal of calcium hydroxide medication from oval and round root canals using ultrasonics or the self-adjusting file (SAF). A micro-CT-study

Aim To evaluate the ability of ultrasonics or the Self-Adjusting File to remove calcium hydroxide from round and oval root canals.

Methodology The present study used 80 extracted teeth: 20 with oval and 60 with round root canals (ratio long to small diameter: >2.1 and <4 : 1). All teeth were shortened to a standardized length of 18 mm and the root canals enlarged to size 40, .04 taper, filled with calcium hydroxide paste (Ultracal XS, Ultradent, USA), stored at 37°C temperature and 100% humidity for 7 days and assigned to one of the experimental groups – Ultrasonics: 10 oval root canals, 30 round root canals; Self-Adjusting File: 10 oval root canals, 30 round root canals. Ultrasonics and the SAF both were used for 4 min (8 cycles of 30 s) with a total of 20 mL sodium hypochlorite (3%) as irrigant. Micro-CT scans (29 µm resolution) were taken after application of the medication and following removal. Volume and percentage of remaining calcium hydroxide were calculated. Statistical analysis was performed with ANOVA ($P = 0.05$).

Results Removal of the medication with the ultrasonic system left significantly ($P = 0.002$) more material (27%) than removal with the SAF (15%). Root canal diameter did not significantly influence the results ($P = 0.3$).

Conclusions Both techniques removed most of the medicament from round as well as from oval root canals but were unable to clean the root canal completely. The SAF performed significantly better than ultrasonics.

IRRIGANTS/DISINFECTION: IRRIGANT AGENTS

R60

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Effect of a potential chelating agent on root dentine microhardness

Aim To evaluate the effect of 25% copolymer of acrylic acid and maleic acid [Poly (AA-co-MA)] as a potential chelating agent.

Methodology Twenty-one human maxillary incisors were selected and the root canals instrumented. Thereafter the apical and coronal thirds of each root were discarded, leaving the 5 mm middle third section which was then separated into two pieces longitudinally. The specimens were divided into three groups according to the test materials used ($n = 7$). Group 1: 17%

ethylene diamine tetra acetic acid (EDTA) Group 2: 25% copolymer of acrylic acid and maleic acid (Poly(AA-co-MA)). Group 3: Saline (control). Dentine microhardness was measured with a Vickers microhardness tester with a load of with 200 g for 15 s before and after treatment. Obtained data were analyzed by Friedman and Wilcoxon Signed Rank Tests.

Results 17% EDTA reduced dentine microhardness more than Poly (AA-co-MA) ($P < 0.05$).

Conclusions Poly (AA-co-MA) has the potential to be used as chelating agent in endodontics. More studies are necessary for further investigation of that novel irrigant.

R61

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Detection of toxic chlorinated by-products from sodium hypochlorite interaction with dentine. Preliminary report

Aim To examine the formation and the identity of chlorinated by-products when sodium hypochlorite (NaOCl) reacts with dentine.

Methodology Freshly extracted intact human third molars were pooled, ground and dentine bulk was reduced into a powder state. Replicated dentine samples (3 × 50 mg) were treated as follows: Mineralised dentine powder was mixed with 25 mL distilled water (G1) and 25 mL NaOCl 5.25% (G2 + G3), respectively. Two blank samples containing 25 mL liquid suspensions of distilled water (DW)(B1) and NaOCl 5.25% (B2) were also prepared respectively. All solutions were inserted into glass beakers with their surface sealed with parafilm membrane. The glass beakers were placed on magnetic stirrers and the aliquots were stirred for 60 min at 250 rpm, at room temperature. Following this period, the samples were left for a resting period of 10 min to allow for powder sedimentation. Aliquots were collected with pipettes and samples were stored in amber glass containers with TFE-lined screw caps and in a deep refrigerator at –80°C until analysis. Samples were initially analysed with flow injection analysis and two types of mass spectrometry (MS) (ion trap/time of flight). Further analysis of the samples was performed with head space gas chromatography-mass spectrometry (GC-MS). Aliquots (0.5 mL) equilibrated in a 1.5 mL sealed glass vial at 80°C (in a GC oven) for 5 min. Head space injection (0.15 mL) was carried out.

Results The use of ion trap and time of flight mass spectrometry for the analysis of negatively charged molecules and positively charged molecules did not reveal any differences between the groups. The use of head space GC-MS revealed the existence of two organochlorine compounds, when NaOCl 5.25% interacted with dentine powder:CHCl₃ (chloroform) (G2: 5.4 mg L⁻¹, G3: 1.6 mg L⁻¹) and CCl₄ (carbon tetrachloride) (G2: 1.8 mg L⁻¹, G3: 1.4 mg L⁻¹).

Conclusions The emergence of toxic chlorinated by-products from the interaction of dentine with NaOCl should be critically appraised as a potential hazardous drawback during root canal irrigation. Further investigation is required with regard to the risk of inadvertent extrusion of toxic chlorinated compounds into the periapical space and to health effects for patients and dental staff.

R62

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Cytotoxicity and biocompatibility of calcium hypochlorite-based irrigating solutions: study *in vitro* and *in vivo*

Aim The aim was to evaluate cytotoxicity in mouse fibroblast cells and biocompatibility in subcutaneous tissue of rats, of calcium hypochlorite-based irrigating solutions.

Methodology The experimental groups were determined as follows: calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) and sodium hypochlorite (NaOCl), at different concentrations. For *in vitro* evaluation, 3T3 cell line (ATCC 2753) was used and the viability assay MTT was performed after 24 h. 1×10^5 cells per well were seeded in 96-well plate and exposed to the following concentrations of the evaluated solutions: 0.025%, 0.0125%, 0.0075%, 0.005% and 0.0025%. Culture medium was used as negative control, and 1 mM hydrogen peroxide as a positive control; statistical analysis was performed by ANOVA test followed by post-hoc Tukey, with 5% significance level. For *in vivo* evaluation, 18 Wistar rats were divided in 3 groups: 2 h, 24 h and 14 days. The solutions were evaluated at concentrations of 1% and 2.5% injected into the dorsum of rats under general anaesthesia. As controls, 0.9% saline solution and the needle were used. After euthanasia, tissue samples were obtained, histologically prepared and examined using a light microscope at $40\times$, $100\times$ and $400\times$ magnification. The results were described according to the presence of inflammatory cells. Statistical analysis was performed using the Kruskal-Wallis test followed by post-hoc Dunn, with 5% significance level.

Results The results of the *in vitro* assays revealed increased viability in lower concentrations for both evaluated solutions ($P > 0.05$). In the *in vivo* test, there were significant differences in the presence of neutrophils between the periods of 2 and 24 h ($P = 0.041$) and 2 h and 14 days ($P = 0.017$).

Conclusions $\text{Ca}(\text{ClO})_2$ demonstrated potential for use as irrigation solution of the root canal system, with biocompatibility similar to NaOCl solution.

R63

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Final irrigation protocols may affect dentine ultrastructure

Aim The aim of this study was to evaluate the dentine structure using transmission electron microscopy (TEM), after the application of different irrigation protocols.

Methodology Thirty-nine mandibular bovine incisors were randomly divided into 13 groups ($n = 3$), and were hemisected longitudinally. In the 36 roots belonging to the groups 2–13, the root canals were prepared in a crown-down direction, using Gates-Glidden drills (Dentsply Maillefer, Switzerland), sizes 6, 5, 4, 3, and 2, until reaching the working length limit of 15 mm. Irrigation and aspiration was performed with 2 mL of saline at each change of drill size; the specimens were held in a metal bench vice. Then, these roots were hemisected longitudinally and immersed in 17% EDTA for 5 min to remove the smear layer, and into distilled water for a further 5 min to remove residual EDTA. After the root halves

were reassembled, they received a specific irrigation protocol for each group: G1, distilled water (control); G2, 0.9% saline; G3, saline+17% EDTA; G4, saline+PUI (passive ultrasonic instrumentation); G5, saline+PUI+EDTA; G6, 2.5% NaOCl (sodium hypochlorite); G7, NaOCl +EDTA; G8, NaOCl +PUI; G9, NaOCl +PUI+EDTA; G10, 2% CHX (chlorhexidine); G11, CHX+EDTA; G12, CHX+PUI; G13, CHX+EDTA+PUI. One-half of each sample was prepared and evaluated using transmission electron microscopy.

Results The surface of the tubules presented smooth and intact collagen in G1 (distilled water), G2 (saline), G3 (saline + EDTA), G4 (saline + PUI), and G10 (CHX); dispersed, with characteristics of detachment from the surface in G5 (saline + PUI + EDTA), G11 (CHX + EDTA), G12 (CHX + PUI) and G13 (CHX+PUI+EDTA); and altered, demonstrating loss of substance and tapered ends of collagen fibre ribbons in groups G6 (NaOCl), G7 (NaOCl + EDTA), G8 (NaOCl + PUI) and G9 (NaOCl + PUI + EDTA).

Conclusions NaOCl caused alterations in the collagen ultrastructure. The synergistic action of PUI and/or EDTA enhanced the effects of NaOCl on dentine collagen. Thus, further research is needed to determine the depth of erosion that occurs in dentine walls when using the combination of NaOCl and PUI and/or EDTA, and how this erosion affects tooth structure and longevity.

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R64

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In vivo intracanal temperature evolution after heating sodium hypochlorite with a system B plugger

Aim To assess the intensity, persistence and the depth of heat transmission when heating sodium hypochlorite (NaOCl) inside root canals with a System B plugger *in vivo*, and to study the effect of repetition of heat activation.

Methodology The ethical committee of the institution approved this study and all patients signed an informed consent. In nine canals prepared to size 35, .04 taper, the irrigant was heated inside the canal using a System B plugger (Beefill, VDW, Germany). Two thermocouple microprobes (Testo, Belgium) were fixed to the plugger, exceeding respectively 1 mm and 3 mm beyond the point of the plugger. The plugger was activated four times consecutively. Temperatures were measured throughout the experiment at 1-s intervals. In between each heating stabilization of the temperature was confirmed. Student *t*-test was used to compare the temperature evolution after the first (t_1) and after the last activation (t_2).

Results At the time of activation temperatures, respectively at t_1 and t_2 were: 71.9°C (± 12.9) and 73.4°C (± 14.5) at 1 mm ($P = 0.49$), and 44.5°C (± 6.6), and 44.5°C (± 4) at 3 mm ($P = 0.43$) showing no significant difference. The temperature decreased under 45°C after 5 s at 1 mm and after 1 s at 3 mm. Temperature reached 37°C in less than 20 s and 30 s respectively at 3 mm and 1 mm in all groups. The temperature in t_2 decreased significantly less rapidly compared to t_1 at 1 mm ($P = 0.0004$) and at 3 mm ($P = 0.0006$).

Conclusions Heating NaOCl inside the canal using a System B plugger generated heat for a short period of time and heat diffusion beyond the plugger was limited. Repetition of heating did not increase heat intensity significantly although heat persisted longer. The question remains if heating is clinically relevant. A larger number of teeth is required to strengthen these results.

R65

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Quantitative evaluation of smear layer removal with two different EDTA solutions and irrigation periods

Aim To quantitatively compare the smear layer removal ability of 2 EDTA commercial solutions (REDTA™ or SmearClear™) at two time intervals.

Methodology Twenty human single root extracted teeth were prepared with a crown-down technique up to a size 50, .04 taper K3 instrument, 1% NaOCl was used as the irrigant between instruments. After canal enlargement, samples were randomly divided into four groups and their canals flooded according to the following protocols: Group 1: 0.5 mL of 1% NaOCl (control) 5 min, Group 2: 0.5 mL of REDTA™ for 5 min, Group 3: 0.5 mL of SmearClear™ for 1 min, and Group 4: 0.5 mL of SmearClear™ for 5 min. All the groups received a final irrigation with 5 mL distilled water. The roots were split in half and the middle and apical thirds were divided in 10 equivalent areas under a scanning electron microscope at 500X. One area of each specimen was randomly selected and photographed; this resulted in an area of 800 000 µm² approximately equivalent to 10% of the apical and middle root thirds respectively. Two calibrated blinded evaluators scored the absence or presence of smear layer as moderate or abundant. Measurements were quantitatively analyzed in each image by percentage area with an AutoCAD (2004 Autodesk) software tool. The results were statistically analyzed by Kruskal-Wallis and Mann-Whitney U-Test; significance was set at $P < 0.05$.

Results In the middle third, the three experimental groups showed no smear layer in more than 57% of the assessed area, and similar to the apical third, all groups showed significant difference in moderate and abundant smear layer when compared to the control group ($P < 0.05$). Unlike other treatment groups, the SmearClear™ 5 min group showed 20% absence of smear layer in the apical and 80% in the middle third versus 0% of the control group ($P < 0.05$) and abundant smear layer in 9% of the apical third and 0% in the middle third versus 100% in the control group ($P < 0.001$).

Conclusions With this quantitative evaluation, 5 min of SmearClear™ was more effective to remove smear layer than REDTA™ for 5 min or SmearClear™ for 1 min.

R66

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Final irrigation regimes effect fracture resistance of root filled teeth

Aim To compare the effect of different final irrigation solutions on fracture resistance of root filled teeth.

Methodology Eighty single-rooted decoronated mandibular incisors were used and randomly divided into 8 groups ($n = 10$). Twenty teeth were used as negative (only decoronated) and positive control (prepared/unobturated) groups. Sixty canals were prepared using the ProTaper System up to F2 and irrigated with 5 mL 2.5% NaOCl and 2 mL saline. Prepared teeth were divided into six groups according to final irrigation regime: 5 mL of saline, EDTA,

REDTA, Chlorhexidine, QMix, and MTAD. At the end, all specimens were irrigated with 2 mL saline. In all groups the final irrigation was performed for 1 min except for MTAD, which was applied for 5 min according to manufacturer's instructions. The specimens were filled with single cone gutta-percha technique and AH26 sealer. After being stored under 37°C and 100% humidity for a week, the specimens were loaded in vertical direction at 1 mm min⁻¹ speed until they were vertically fractured. The results were analyzed by Kruskal-Wallis and Siegel Castellan tests.

Results The negative control group had the highest fracture resistance value ($P < 0.05$). There were significant differences between the negative control group; and the positive control, Chlorhexidine and MTAD groups ($P < 0.05$). REDTA and QMix groups had higher fracture resistance values compared to the positive control ($P < 0.05$). Chlorhexidine and MTAD groups had lower fracture resistance compared to the negative control group ($P < 0.05$).

Conclusions Final irrigation regimes had an impact on fracture resistance values. Shorter time exposure to irrigation solutions (REDTA and QMix) with the effect of surfactants that they contain, probably contributed to higher fracture resistance values, when compared with lower fracture resistance of MTAD with longer time exposure.

R67

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Effect of different final irrigation solutions on dentinal tubule penetration of root canal sealer in root canals obturated with Herofill system

Aim The purpose of this study was to evaluate the effects of different solutions used for final irrigation on sealer penetration into dentinal tubules.

Methodology Thirty recently extracted human single-rooted teeth were used. All canals were instrumented with HERO Shaper rotary instruments. The teeth were divided into 3 groups according to the final irrigation used: group 1, 17% EDTA and then 2% chlorhexidine gluconate (CHX); group 2, QMix 2in1 and group 3, 2.5% NaOCl (control group). All teeth were dried and then obturated using the Herofill system with AH 26 sealer (Dentsply; DeTrey, Konstanz, Germany) labeled with fluorescent dye. After setting, the roots were sectioned horizontally at distances of 3, 5 and 8 mm from the root apex. Sealer penetration percentage into the dentinal tubules were measured by using confocal laser scanning microscopy (CLSM). For each section (coronal, middle and apical) statistical comparisons between final irrigation groups were made with Kruskal-Wallis Test. Mann-Whitney test with Bonferroni correction were used to identify the significant groups. Statistical significance was accepted at the alpha level of 5% ($P < 0.05$). The significant P -value was adjusted to < 0.017 when Bonferroni correction was undertaken.

Results In the coronal sections of the roots, no significant difference was detected in the percentage of sealer penetration between any of the final irrigation groups ($P > 0.05$). In the apical section the EDTA + CHX ($P: 0.008$) and QMix ($P: 0.014$) groups had significantly more sealer penetration than the control group, and no difference was found between QMix and EDTA + CHX groups ($P: 0.076$; $P > 0.017$). In the middle section, the control group had a significantly lower percentage of sealer penetration than the EDTA + CHX group ($P: 0.009$; $P < 0.017$). However, there was no significant difference between the control and QMix groups ($P: 0.917$; $P > 0.017$).

Conclusions Sealer penetration improved after final irrigation with EDTA + CHX or QMix when compared with NaOCl irrigation.

R68

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Temperature changes in 2% chlorhexidine gluconate using two activation methods with different inserts and ranges of intensity

Aim To assess the temperature rise in 2% chlorhexidine when activated by sonic and ultrasonic methods and to compare the use of different inserts and ranges of intensity.

Methodology Three groups were established: control group (without activation), ultrasonic activation (US) and sonic activation (S). A VDW Ultra device was used for the US group and the Endoactivator was used for the S group. Inserts used for the US group were size 15 and 35 NiTi K-files, IRRI safe 25 file and size 10 and 25 stainless steel K-files and for S group were: small, medium and large tips. Intensities used for the US group were 10%, 20% and 30% and for the S group, low, medium and high. 0.5 mL of chlorhexidine was placed in Eppendorf tubes, which were placed in a thermostatic bath at 37°C, and immediately ultrasonic activated for 3 min using cycles of 30 s of activation and 20 s stops and sonically for 1 min. A thermic couple registered the temperature. ANOVA and Tukey's test were used for the statistical analysis.

Results Significant differences were found between sonic and ultrasonic groups ($P = 0.000$) and between sonic and control group ($P = 0.000$). No differences were found between ultrasonic and control group ($P = 0.990$). There were no significant differences in ultrasonic activation groups using different inserts ($P > 0.05$), but with differences at higher temperatures and higher intensity ($P = 0.033$). For the sonic activation group no differences were found depending on the size of the insert or the intensity ($P > 0.05$).

Conclusions Sonic activation did not raise the temperature of chlorhexidine. Ultrasonic activation did not increase the temperature significantly higher when compared to the control group.

R69

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Evaluation of antifungal efficacy of novel root canal irrigant QMim 2in1 and 17% EDTA against *Candida albicans* – an *in vitro* study

Aim To evaluate the antifungal effect of final irrigation with a new endodontic irrigant, QMIX 2in1 (Dentsply Tulsa Dental, Tulsa, OK, USA) and 17% EDTA (Vista, Racine, WI, USA).

Methodology Thirty-three single-rooted extracted human teeth were used. The teeth were instrumented (size 40, 0.02 taper) using 1 mL of sodium hypochlorite (5.25%) between each file size with 1 mL 17% EDTA and 1 mL sterile saline rinse after final instrumentation. After root canal preparation, experimental teeth were inoculated with *Candida albicans* (ATCC 10231) and incubated for 72 h. Three groups of 11 roots were irrigated with 1 mL of QMix 2in1 for 60 s, 1 mL 17% EDTA for 60 s and 1 mL of sterile saline for 60 s, then rinsed with 30 mL sterile saline to prevent potential carry-over of the irrigants. The roots were irrigated using a 30 G side-vent needle with an apical-coronal motion to within 1 mm of the working length. Aliquots from the

experimental teeth were plated on Sabouraud 4% dextrose agar plates and colony-forming units were counted as a measure of antifungal activity.

Results QMix 2in1 was effective and significantly ($P < 0.05$) superior to 17% EDTA and sterile saline in antifungal activity (mean CFUs of *C. albicans*: 2.81 versus 14.10 versus 30.62). 17% EDTA had limited antifungal effects.

Conclusions Use QMix 2in1 during final irrigation was more effective than 17% EDTA and sterile saline against *Candida albicans*.

R70

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Determination of protein content in sodium hypochlorite used as an irrigation solution

Aim Sodium thiosulfate can neutralize NaOCl. Hence, the purpose of this study was to evaluate the quantity of 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, and 5% sodium thiosulfate required to neutralize 5.25% NaOCl in the presence of protein content of bovine serum as like the collagen in dentine.

Methodology The measurement of protein was carried out on a total volume of 20 $\mu\text{g mL}^{-1}$ containing bovine serum albumin, a variable amount of sodium thiosulfate (1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, and 5%) and sodium hypochlorite (5.25%) on different time intervals taken at 1, 5, 10, 30, 60, and 90 min. Total protein was determined using the Bradford method. Qualitative analysis of soluble protein was evaluated using a spectrophotometer. Raw bovine serum albumin was used as control group and different quotients of sodium thiosulfate + NaOCl were used for the experimental groups. Absorbance of mixtures was measured at 660 nm against a control containing raw protein extract. These absorbances were further converted to percentage of variation of the amount of protein against the absorbance of unaffected protein. The significances of differences between the control and experimental groups were analyzed statistically by one-way analysis of variance with the Duncan test in which $P \leq 0.05$ was considered statistically significant.

Results 1%, 1.5%, 2%, and 2.5% sodium thiosulfate were incapable of neutralising NaOCl and conversely, 3.5%, 4%, and 5% were accelerating concentrations for neutralization effects. In all concentrations except 3% sodium thiosulfate, protein was not detected properly due to the breakdown. Proteins were observed only in the 3% sodium thiosulfate group between 1 to 5 min after agitating the compounds ($P \leq 0.05$).

Conclusions In order to assign protein in NaOCl, 3% sodium thiosulfate should immediately be added to the mixture for neutralization and spectrophotometric evaluation had better be performed just after 1–5 min after agitating the compounds.

R71

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Effects of laser-activated irrigants in removing calcium hydroxide from the artificial grooves created in root canals: an *ex vivo* study

Aim The aim of this study was to compare the efficacy of Er:Cr:YSGG laser with different irrigation solutions [sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), QMix 2in1, and peracetic acid] in removing calcium hydroxide (CH) from artificial grooves created in root canals.

Methodology The root canals of 160 extracted single-rooted mandibular premolars were prepared with ProTaper Universal rotary instruments up to size F4. The roots were split longitudinally along the length of the instrumented canals and a standardized groove was prepared in the apical part of one segment. CH powder mixed with distilled water was placed into the grooves and the root halves were then reassembled. The roots were randomly divided into two main groups according to the selected irrigation techniques (needle and laser activation) and then each main group was divided into four subgroups according to the used final irrigation (NaOCl, EDTA, QMix 2in1, and peracetic acid) protocols. After irrigation, the roots were disassembled and digital images were taken using a stereomicroscope. Measurements of residual CH were performed as percentages of the overall groove surface area with image analysis software. The data obtained were analyzed by using one-way analysis of variance and Tukey honestly significant difference.

Results Significantly less residual CH was obtained with laser-activated groups than the needle irrigated groups ($P < 0.05$). According to the data from both main groups, NaOCl was associated with significantly more CH than the other groups ($P < 0.05$). However, there was no significant difference among the EDTA, QMix 2in1, and peracetic acid groups ($P > 0.05$).

Conclusions None of the techniques removed the CH dressing completely. Laser-activated irrigation was significantly more effective than needle irrigation.

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R72

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Comparison between HEBP and EDTA as chelating agent for root canal irrigation

Aim The aim of this study was to evaluate the influence of HEBP (H) on available chlorine content, tissue dissolving ability, antimicrobial effect and smear layer removing activity compared to EDTA(E) when mixed with NaOCl(N).

Methodology Available chlorine content in N + 18%H, N + 7%H and N+E were measured using a standard iodine/thiosulfate titration method. Tissue dissolving assay: bovine pulp tissues were weighed before and after immersion into the solutions [3%N, 1.5%N, N + 18%H, N + 7%H, N+E, N + 18%H (24 h), N + 7%H (24 h), N+E (24 h after mixing)]. The antibacterial effects of the

solutions (3%N, 18%H, 7%H, E, 1.5%N, N + 18%H, N + 7%H) on biofilm of *Enterococcus faecalis* were evaluated using BacTiter-Glo™ Microbial Cell Viability Assay Kit. Several groups were stained with LIVE/DEAD BacLight Bacterial Viability Kit and examined under a confocal laser microscope. Surfaces of human dentine disks with smear layer were exposed to specific solutions (18%H, 7%H, E, N + 18%H, N + 7%H, N+E). Each specimen was examined with a scanning electron microscope and the amount of the smear layer and the degree of erosion of dentinal tubules were scored.

Results While E caused N to lose its available chlorine rapidly, 7% and 18% H showed almost no short-term interference with N. The tissue dissolving capacity of 1.5%N, N + 7%H, N + 18%H were similar and greater than N+E. N + 7%H and N + 18%H had higher antibacterial effect than 1.5%N. N+E showed great smear layer removing ability and erosive effect. Smear layer and debris were efficiently removed without erosive effect when it was exposed to N + 18%H.

Conclusions N + 7%H showed large amounts of available chlorine for longer time and greater antibacterial effect than other solutions. However, it was less effective on smear layer removal while N + 18%H showed good ability to remove smear layer as well as efficient antibacterial effect. Therefore, 18% H can be used as a substitute for 17% E as a chelating agent during instrumentation process together with N.

R73

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The effect of phytic acid on the bond strength of three different adhesives to root dentine

Aim Recently, phytic acid (IP6) has been reported to be an effective alternative to phosphoric acid (PA) and EDTA as etchant and chelating agent, respectively. However, no study has been published about its effect on adhesion to root dentine. This study aimed to examine the effect of 1% IP6 on the adhesion to root dentine and compare it to 17% EDTA and 36% PA.

Methodology Flat dentine surfaces with smear layer were created from human single-rooted anterior teeth. Eight groups of 10 samples each were conditioned with one of the following protocols: (i) 1% IP6 for 60 s; (ii) 17% EDTA for 60 s or (iii) 36% PA for 15 s. After treatment, 3 different adhesives [Adper ScotchBond Multipurpose (ASM), Adper Single Bond Plus (ASB) and Clearfil SE Bond 2 (CSE)] were used to bond composite cylinders on each sample treated with IP6 and EDTA while samples treated with PA received only the firstly two mentioned adhesives. The control group of CSE received no conditioning step. Specimen were subjected to micro-shear test after 24 h of water storage at 37°C and failure modes were determined using scanning electron microscopy. Data were analyzed using two-way ANOVA and Tukey's post-hoc test ($\alpha = 0.05$).

Results IP6 resulted in higher bond strength values in all tested adhesives when compared to EDTA and PA. No significant difference was observed between EDTA and PA in both ASM ($P = 0.25$) and ASB ($P = 1.00$). EDTA decreased the bond strength of CSE ($P < 0.001$) while IP6 increased its bonding to root dentine ($P = 0.001$), when compared to the control.

Conclusions IP6 enhanced the bond strength of the adhesives to root dentine. The results of this study give insights on the potential use of IP6 with dental adhesives, a step that would optimize resin-root dentine bonding.

R74

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Comparison of smear layer removal ability of QMix with different activation techniques

Aim To evaluate the effectiveness of QMix solution on smear layer using with different irrigation activation techniques: EndoActivator® System (EA), photon-initiated photoacoustic streaming (PIPS), and Er:YAG laser with an endodontic fibre tip.

Methodology Sixty-four extracted single-rooted human teeth were decoronated and the canals instrumented with ProTaper up to size F4. The canals were irrigated with 5.25% NaOCl and saline solution for 1 min each. The specimens were then divided randomly into four experimental and control group (n=8) according to the final irrigation activation technique; Group 1: 2.5 mL QMix; Group 2: QMix+EA, Group 3: QMix+PIPS, Group 4: QMix+Er:YAG laser. Distilled water was used as control for each group. Teeth were split longitudinally and specimens were observed under a scanning electron microscope. Images were taken at coronal, middle and apical third of the teeth with a magnification of $\times 1000$ and were scored in terms of the presence of smear layer. The data were analyzed with Kruskal–Wallis and Mann–Whitney *U* tests.

Results The highest scores were found in the apical third of all groups ($P < 0.05$). QMix+EA group was the only group that removed the smear layer significantly in all third of teeth when compared with the non-activated QMix group ($P < 0.05$). QMix+Er:YAG group removed smear layer more effectively than non-activated QMix group in the apical third ($P < 0.05$). QMix+PIPS group showed significantly better effect than QMix group in the coronal third ($P < 0.05$).

Conclusions EA and Er:YAG laser enhanced the smear layer removal ability of QMix in apical thirds of the canals. QMix removed more smear layer in coronal thirds when activated with the PIPS technique.

R75

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Confocal scanner laser evaluation of bactericidal effect of chitosan nanodroplets loaded with benzalkonium chloride

Aim Experimental nanodroplets (NDs) with chitosan shell have been recently proposed. The objective of this study was to evaluate the antibacterial efficacy and depth of penetration into dental tubules of a solution of NDs loaded with Benzalkonium Chloride (BAK).

Methodology Seventy-two human single-root teeth with fully formed apex were used. Cylindrical root dentine blocks were sectioned longitudinally and enlarged to a size 110 with a Gates Glidden drill size 4. After ethylene oxide sterilization, root canals were infected with a culture of *Enterococcus faecalis* to match the turbidity of 3×10^7 colony forming units mL⁻¹ (CFU mL⁻¹) and further incubated for 3 weeks to allow penetration into dentinal

tubules. Specimens were randomly assigned to 3 experimental groups ($n = 20$) plus positive ($n = 6$) and negative ($n = 6$) controls. In group NDs-BAK-3 irrigation was performed with 2 mL of NDs solution loaded with BAK for 3 min, in groups NaOCl-3 with 2 mL of 5% NaOCl for 3 min, in group CHX-3 with 2 mL of 2% chlorhexidine (CHX) for 3 min. Specimens were rinsed and fractured vertically. Confocal laser scanning microscopy (CLSM) and viability staining (Live/Dead BacLight Viability Stain – Molecular Probes, USA) were used to quantitatively analyze the proportions of dead and live bacteria. The volume ratio of red fluorescence (dead) was calculated in three-dimensional reconstructions. Differences among groups were analyzed with Kruskal–Wallis and post-hoc Dunn's test ($P < 0.05$). Mean penetration depth was recorded and differences were analyzed with one-way ANOVA and post-hoc Bonferroni's test ($P < 0.05$).

Results Ratio of red fluorescence over the whole green/red fluorescence in NDs-BAK-3, NaClO-3 and CHX-3 groups, was 60.5%, 74.2% and 40.1% respectively. Statistically significance was noted for the CHX group ($P < 0.05$). The mean depth of efficacy was respectively: NDs-BAK 420 ± 80 μ m, NaOCl 360 ± 60 μ m and CHX 320 ± 60 μ m. Statistical significance was noted for the NDs-BAK-3 group ($P < 0.05$).

Conclusions NDs-BAK solution had an effective bactericidal action deep inside dentinal tubules.

TRAUMA/REGENERATION

R76

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Pattern of traumatic dental injuries in rugby players

Aim To describe the prevalence, injury pattern and the risk factors of traumatic dental injury (TDI) in rugby players.

Methodology A cross-sectional study was conducted in two rugby tournaments. Participants were asked to complete a self-administered questionnaire to enquire experience of TDI. After that, clinical examination was carried out by four calibrated examiners. Types of injury were charted according to the modified Ellis' classification.

Results All 456 participants who completed the questionnaires were male (mean age=22.73, SD=3.98). Median duration of playing was 6 years, median frequency was 6 h per week. These players mostly competed at state level (35.7%) and inter-varsity level (29.8%). Prevalence of self-reported TDI was 26.5% ($n = 121$). Sixteen percent ($n = 72$) of the players had experienced it only once. The percentages of players who reported two and three occurrences were 6.6% ($n = 30$) and 0.9% ($n = 4$). Injury experience was positively correlated with total playing time ($r = 0.247$, $P < 0.001$). Based on clinical findings, 25.9% ($n = 118$) of the players had signs of injured teeth. Eighty players (17.5%) had one injured tooth and 29 (6.4%) had two injured teeth. Teeth most frequently injured were maxillary incisors. The frequency decreased gradually towards posterior teeth, but a mild increase was noted for the first molars for both arches. The commonest injury was fracture of enamel (31.4%), followed by luxation (26.2%) and tooth loss (16.9%). Only 2.9% of the injured teeth were discoloured. Most of the injured teeth (84.3%) were not restored. Anterior teeth had greater odds of being restored than posterior teeth (Wald's Test P -value=0.002, Odds Ratio =10.0, 95% CI=2.3–42.6). When compared to enamel fracture

only, injuries involving dentine ($P = 0.005$, $OR = 7.8$, $95\% CI = 1.8-32.9$), pulp ($P = 0.009$, $OR = 10.4$, $95\% CI = 1.8-60.3$) and tooth loss ($P < 0.001$, $OR = 21.5$, $95\% CI = 4.5-103$) had greater odds of being restored.

Conclusions Prevalence of rugby-related dental trauma in this study was 26% which is a substantial number. The total playing time is a significant risk factor in the occurrences of dental trauma. Therefore, injury prevention programmes should be in place for both training and games. The program should also create awareness on the importance of treating traumatised teeth.

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R77

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Histone deacetylase inhibitors induced LPS-regulated differentiation of human dental pulp stem cells

Aim To investigate the effects of HDAC inhibitors (HDACis) on differentiation of human pulp stem cells (DPSCs) under infection and inflammation conditions and the potential signaling events in these processes.

Methodology Alizarin Red S staining was used to assess mineralized nodule formation in DPSCs in response to LPS and HDAC inhibitors (HDACis). The gene expression of osteogenic/odontogenic markers were investigated using quantitative real-time reverse-transcriptase polymerase chain (qRT-PCR). We also developed a biomimetic approach to prepare three-dimensional (3D) nanofibrous gelatin (NF-gelatin) hybrid scaffolds for the study *in vivo*. Haematoxylin and eosin (H&E) and von Kossa staining, were used to reveal ECM secretion and mineral deposition *in vivo*. Western blot analysis was used to measure the phosphorylation of proteins stimulated by LPS and HDACis. For the purpose of studying the role of HDAC2/5 in LPS-induced differentiation of hDPSCs, an siRNA for HDAC2/5 was prepared and transfected into hDPSCs. Moreover, chromatin precipitation (ChIP) analysis was performed using antibodies to HDAC2/5 and primers for DSPP promoters.

Results HDACis valproic acid (VPA) and Trichostatin A (TSA) promoted LPS-regulated differentiation of hDPSCs through increased mineralized nodule formation and mRNA expression of several osteogenic/odontogenic markers. The results were further confirmed *in vivo*. During this process, MAPK signalings were involved in the effect of VPA and TSA in hDPSCs. Moreover, LPS stimulation resulted in a remarkable change in the expression of HDAC2/5. In fact, HDAC2 silencing with si-RNA led to a decrease in the effect of LPS on the differentiation of hDPSCs. However, VPA/TSA could reverse the effect of HDAC2 siRNA. HDAC5 si-RNA performed the opposite effect. Moreover, chromatin precipitation assays were performed HDAC2/5 could bind at the Osteogenic/odontogenic marker DSPP gene.

Conclusions The effect of VPA/TSA on LPS in hDPSCs is mainly depended on HDAC2/5 binding with the target gene DSPP. These results may provide valuable targets for novel management strategies for the bone/dentine regeneration under infection and inflammation conditions.

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R78

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Stem cell responses to three root-end filling materials

Aim To investigate the responses of primary human bone marrow mesenchymal stem cells (hBMMSCs) to selected root-end filling materials.

Methodology Cultures of hBMMSCs were divided into 4 groups. Three were exposed to either white ProRoot MTA (wMTA), Ortho MTA (oMTA), or Intermediate Restorative Material (IRM). The last group served as the control. The effect of testing materials on the morphology (day3), proliferation ability (day 1, 3, 7 and 14) and osteogenic differentiation ability of hBMMSCs (day 7 and 14) were investigated via phase contrast microscopy ($n = 6$), MTT assay ($n = 6$), Real-time polymerase chain reaction (RT-PCR) ($n = 3$) and Alizarin Red S assay ($n = 3$) respectively. The data of cell proliferation was analyzed using a regression model and the data of osteogenic differentiation capability was analyzed by one-way ANOVA and Bonferroni procedure.

Results Exposure of wMTA or oMTA to the cell culture had no effect on cell morphology, while the IRM group revealed features of cytotoxicity, compared to the control. The MTT assays indicated that the oMTA group proliferated significantly faster than wMTA group on day1, 7 and 14 ($P < 0.05$). Osteogenic marker genes Runx2, ALP and BSP expression were similarly observed on wMTA and oMTA groups ($P > 0.05$). Both MTA groups were associated with a higher degree of calcification than the control group after 14 days or more ($P < 0.05$). IRM was cytotoxic to hBMMSCs with significantly lower figures or results in all tests, compared with the two MTA materials and the control groups.

Conclusions hBMMSCs survived and proliferated faster when cultured with oMTA. With wMTA, there was an initial retardation of proliferation at the first week. oMTA appeared better than wMTA in promoting osteogenic differentiation of hBMMSCs.

R79

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Application of calcium silicate-base material containing bioactive molecules in exposed pulps of minipig teeth

Aim To investigate the effects of calcium silicate-based material (CSM) enriched with Bone Morphogenetic Protein-7 (BMP-7) or Transforming Growth Factor (TGF)- β 1 on dental pulp cells, in the experimental model of mechanical pulp exposure in miniature swine teeth.

Methodology Deep cavities were prepared and pulpal exposures were performed in 28 permanent teeth of two miniature swines. After control of haemorrhage teeth were divided in 4 groups and treated with freshly mixed CSM (Biodentine, Septodont, France) or conical-shaped pieces of CSM after their setting for 24 h at room temperature. The materials were applied, as follows:

Freshly mixed CSM in the control group G1, set CSM soaked in PBS-albumin solution in the control group G2, and set CSM soaked in PBS-albumin solutions containing $50 \mu\text{g mL}^{-1}$ of recombinant human BMP-7 or TGF β 1 (Perprotech, USA) in the experimental groups G3 and G4, respectively. Reparative and

reactionary dentine responses (thickness and form of newly deposited matrix and morphology of the associated formative cells) were assessed at a post-operative period of 3 weeks by light microscopy. Data were statistically analysed by the Kruskal Wallis and the Mann-Whitney U-tests.

Results Severe inflammatory response or tissue necrosis was not found. Newly deposited mineralized matrix was seen at the implanted material-pulp interface and the surrounding pulp periphery in all groups. Rates and morphological characteristics of osteodentine, reparative dentine formation with the associated odontoblast-like cells differentiation and reactionary dentine formation were significantly different between G2 and all other groups ($P < 0.05$). No differences were found between G1 and G3 groups. Teeth of G4 group had a thin osteotypic structure surrounding the implanted CSM, followed by a well-organized zone of tubular matrix, which had been deposited in a predentine-like pattern in association with a layer of highly elongated polarized odontoblast-like cells.

Conclusions This study indicates that set CSM could be used as carrier for biologically active molecules and therapeutic drugs in the field of dentine-pulp complex tissue engineering.

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R80

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Effects of calcium hydroxide and other factors on attachment and differentiation of dental pulp stem cells: *in vitro* study

Aim Sodium hypochlorite (NaOCl) is an excellent bactericidal agent, but it is detrimental to stem cell survival, whereas intracanal medicaments such as calcium hydroxide (Ca(OH)₂) promote the survival and proliferation of stem cells. This study evaluated the effect of sequential NaOCl and Ca(OH)₂ application on the attachment and differentiation of dental pulp stem cells (DPSCs).

Methodology DPSCs were obtained from human third molars. All dentine specimens were treated with 5.25% NaOCl for 30 min. DPSCs were seeded on the dentine specimens and processed with additional 1 mg mL⁻¹ Ca(OH)₂, 17% ethylenediaminetetraacetic acid (EDTA) treatment, file instrumentation, or a combination of these methods. After 7 days of culture, DPSC morphology was examined using scanning electron microscopy and the cell survival rate was determined with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell adhesion gene expression levels after 4 days of culture and odontogenic differentiation gene expression levels after 4 weeks were measured using quantitative real-time polymerase chain reaction.

The Mann-Whitney U test was used to determine the statistical differences between the experimental groups with SPSS software version 21.0 (SPSS, Chicago, IL, USA). Adjusted P values less than 0.05 were considered statistically significant.

Results DPSCs did not attach to the dentine in the NaOCl-treated group. The gene expression levels of fibronectin-1 and secreted phosphoprotein-1 gene in both the Ca(OH)₂- and the EDTA-treated groups were significantly higher than those in the other groups ($P < 0.05$). All Ca(OH)₂-treated groups had higher expression levels of dentine matrix protein-1 than that of the control. ($P < 0.05$) The dentine sialophosphoprotein level was significantly higher in the groups treated with both Ca(OH)₂ and EDTA. ($P < 0.05$)

Conclusions The application of Ca(OH)₂ and additional treatment such as EDTA or instrumentation promoted the attachment and differentiation of DPSCs after NaOCl treatment.

R81

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Growth factor release from dentine after ultrasonic activation

Aim During tooth development, odontoblasts secrete growth factors which become entrapped in the dentine matrix and can be re-activated after demineralization. EDTA has been proven to be a suitable agent to release growth factors such as TGF- β 1 (transforming growth factor β 1) from human dentine. The objective of this study was to test whether ultrasonic activation of the EDTA solution can increase the release TGF- β 1 from root canal dentine.

Methodology In forty single-rooted human teeth, access cavities were prepared, and root canal preparation was performed using RECIPROC R50 files (VDW GmbH, Munich, Germany) to a working length of 16 mm under constant irrigation with 5.25% sodium hypochlorite. The standardized procedure allowed for equal geometry and volume (45 μ L) for each canal. Following canal preparation teeth were treated as follows: Group 1: Phosphate-buffered saline (PBS), 10 min, no activation; Group 2: EDTA, 10 min, no activation; Group 3: EDTA, 1 min, activation (VDW Ultra, Munich, Germany, IIRI S); Group 4: EDTA, 3 min, activation; Group 5: EDTA, 5 min, activation. Subsequently, test solutions were removed from the canals and amounts of TGF- β 1 were quantified using ELISA (R&D Systems, USA). The procedure was performed three times per root canal and group. TGF- β 1 was normalized to the computed root canal surface area and expressed in μ g mm⁻² per dentin.

Results The amount of TGF- β 1 was higher in EDTA compared with PBS, and significantly higher after ultrasonic activation. The highest amount was measured after 3 min of activation (median: 0.53 μ g mm⁻²). Similar amounts of TGF- β 1 were released even after repetitive treatment.

Conclusions Growth factors such as TGF- β 1 can be released from root canal dentine more efficiently by ultrasonic activation of EDTA solution, even after repetitive treatment, which might be advantageous for regenerative endodontic therapy.

R82

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The effects of antibiotic pastes used in endodontic regeneration on the microhardness of dentine

Aim The aim of this study was to evaluate the effect of various endodontic regeneration medicaments on the microhardness of human root dentine after contact for different time intervals.

Methodology Thirty-five extracted human maxillary incisors were selected. The canals were enlarged using a primary One Shape (MicroMega, Besancon, France) size 25, .06 taper file. The roots were fixed in acrylic resin blocks and cut transversally in 2-mm sections using a water-cooled diamond-coated band saw. Two sections were obtained from the middle-third of each root ($n = 70$). The root discs were divided randomly into three groups of 20 and a control group of 10. Baseline microhardness testing

was completed using a microhardness tester. The previously divided root discs were placed into Petri dishes, then assigned randomly to triple antibiotic paste (TAP), double antibiotic paste (DAP), calcium hydroxide paste (CH), and the control group. CH and both antibiotic pastes were placed in the Petri dishes, and discs were covered completely with the mixtures. Microhardness tests were repeated in the same manner after 7, 14, and 28 days. Data were analyzed statistically by Chi square or Fisher's exact test at 5% significance level and post hoc analysis was performed.

Results No significant change in dentine microhardness occurred in the control group ($P > 0.05$). Overall dentine microhardness values decreased after treatment with CH and antibiotic pastes over all time intervals. The DAP group had reduced KHN values compared with the TAP group at first week measurements ($P < 0.01$). Significant differences were found among the control, DAP, and TAP groups, but no difference was seen between the control and CH group at the first week examination. No significant difference was found between the DAP and TAP groups at the fourth week.

Conclusions The four-week application of DAP and TAP medicaments reduced the microhardness values of dentine discs significantly compared with baseline values.

R83

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In vitro evaluation of ProRoot MTA, Biodentine and MM-MTA on human alveolar bone marrow stem cells in terms of biocompatibility and osteogenic differentiation

Aim The aim of this study was to compare the effects of alternatives for ProRoot MTA, Biodentine and MM-MTA, on human alveolar bone marrow mesenchymal stem cells (BMMSCs) when used as root perforation repair materials *in vitro*. In this regard biocompatibility and osteogenic potential of the materials were evaluated.

Methodology The characterization of BMMSCs was performed using flow cytometry analysis. All the materials were prepared according to the manufacturer's instructions under sterile conditions. The materials were then placed in sterile Teflon discs of 5 mm diameter and 2 mm thickness. The effect of the materials on cell viability was assessed using MTS assay on the 1st, 3rd, 7th, and 14th days of the study. Differentiation was evaluated on day 14, by alkaline phosphatase (ALP) activity, alizarin red and Von Kossa staining, immunocytochemistry and quantitative real time polymerase chain reaction analysis.

Results ProRoot MTA, Biodentine and MM-MTA had no toxicity and similar results were obtained in terms of cell viability. ProRoot MTA and MM-MTA were two materials which enhanced ALP activity compared to the positive control where differentiation media contained no materials. All materials promoted mineralized nodule and extracellular matrix protein formation. All materials had increased Runx-2 gene levels, however osteonectin and collagen type 1A levels were similar to those of the positive control.

Conclusions ProRoot MTA, Biodentine and MM-MTA had similar biocompatibility and osteogenic differentiation potential when treated with human alveolar BMMSCs.

R84

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The effect of Biodentine on fracture strength of cracked-roots with re-attached fragments

Aim The aim of this study was to compare the effect of AH Plus and Biodentine on fracture strength of cracked roots with re-attached fragments.

Methodology Root canals of thirty-six extracted single-rooted human teeth were decoronated and instrumented with ProTaper files to F3. The roots were vertically cracked and the fragments re-attached using Super Bond C&B (Sun Medical, Japan) and randomly divided into three groups ($n = 12$). Group 1; cracked roots prepared, re-attached and served as control. Groups 2; roots prepared as in group 1 and filled with AH Plus sealer (Dentsply, UK) in combination with a single gutta-percha cone. Group 3; roots prepared and filled with Biodentine (Septodont, France). The samples were stored in an incubator for 72 h under 100% humid conditions, and vertically loaded until failure (1 mm min^{-1}). The data was recorded as Newton and analyzed statistically with one way ANOVA.

Results There was no significant difference among the groups. Fracture resistance of the test groups were as follows; Group 1; 304.462 ± 82.77 Group 2; 315.81 ± 119.00 and Group 3; 385.23 ± 180.30 ($P = 0.438$). Fractures occurred mainly at the initial crack line.

Conclusions Biodentine sealer use did not have a reinforcing effect on fracture strength of vertically cracked roots with re-attached fragments.

R85

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Traumatic dental injuries in an dental emergency service from 2010 to 2013

Aim The incidence of traumatic dental injuries (TDIs) is higher on evenings and weekend. They require an early and appropriate management to minimize complications and to save the affected tooth. Consequently many patients present in the dental emergency services which are operating outside regular consulting hours. The aim of this study was to analyze the TDI-cases in a dental emergency service unit of a University hospital over 4 years.

Methodology All patients with TDIs between 2010 and 2013 were determined from the hospital database and subjected to further analysis. Demographic data, classification of trauma (according to WHO), teeth involved and cause of trauma were evaluated.

Results Of 16 301 patients who received treatment in the dental emergency service over a period of 4 years 1305 (8.0%) came due to TDIs. Average age was 14.7 ± 15.7 years (range: 0.6–88.1). More than half of the trauma patients (54.2%) were under the age of ten, about two-third (74.5%) under twenty. Males experienced trauma more often than females (range 1.5 : 1). Nearly half of cases (48.2%) occurred on weekends. Most common diagnosis was subluxation (27.8%), followed by uncomplicated enamel-dentin -fracture (25.9%) and lateral luxation (21.7%). 355 patients (27.2%) had an additional soft tissue injury. In 48.6% of cases only one tooth was involved, in 33.5% two. The permanent dentition was injured in 56.6% of cases, the primary dentition in 41.1%. The maxillary central incisors were

affected in 79.2% of cases. The most common causes of TDIs were falls (54.6%) and sport-accidents (13.4%).

Conclusions The prevalence of TDIs in the dental emergency unit was high. Due to the complexity of management, possible complications and lifelong consequences the dentist on duty as well as the one providing further treatment have to be aware of the endodontic and surgical measures. A regular update of the dentists' knowledge about traumatology is required and more attention should be given to prevention of trauma.

R86

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Duration-dependent effectiveness of intracanal medicaments used for regenerative endodontic treatment on dislocation resistance of MTA

Aim The aim of the present study was to evaluate duration-dependent effectiveness of the intracanal medicaments used in regenerative endodontic treatment (RET) on the dislocation resistance of mineral trioxide aggregate (MTA).

Methodology One hundred ninety-two extracted human maxillary incisor teeth were sectioned apically 12 mm below and coronally 2 mm above the cemento-enamel junction. Root canals were enlarged to size 40 (ProTaper F4). Next, Peeso reamers from size 1 to 6, were used sequentially. Sodium hypochlorite (2.5%), EDTA (17%) and distilled water were used as the final irrigation.

The specimens were randomly divided into four groups ($n = 48$): Group 1, triple antibiotic paste (TAP) (ciprofloxacin+metronidazole+minocycline) prepared and was delivered into the canal using a lentulo spiral. Group 2, Double antibiotic paste (DAP) (ciprofloxacin+metronidazole), was placed into the canal Group 3, Calcium hydroxide (CH) paste was introduced into the roots Group 4 (control), no medication was applied to root canals.

Then samples were kept in saline solution for 2, 4 and 12 weeks. After, 2, 4 and 12 weeks, 16 roots were selected randomly from each group representing the samples of each time point. After removal of the medicaments, MTA was placed into the coronal third of root and incubated for 7 days. A push-out test was used to measure the dislocation resistance (DR) of MTA. The data were analyzed using two-way Anova followed by Tukey's pairwise comparisons ($P = .05$).

Results Time had a significant effect on DR of MTA ($P < 0.05$). All medicaments resulted in significantly smaller DR values after 12 weeks compared to 1 week ($P < 0.05$). A significant unfavorable effect of TAP and DAP was observed as early as four-week after the application, while two and four-weeks after the application of CH there was no effect on the DR of MTA. No significant differences were found between the time points of the control group ($P > 0.05$).

Conclusions The type and intracanal duration of medicaments used for regenerative endodontic treatment should be chosen carefully to provide maximum antimicrobial effect while creating a favorable environment both for stem cell attachment and MTA adhesion.

R87

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Histomorfometric analysis of the revascularization procedure – experimental study

Aim The aim of this work is to determine the regenerative potential of immature teeth. Additionally, a novel regenerative strategy was characterized based on inducing a vital supporting structure, composed of blood clots and chitosan scaffolds, within the root canal space.

Methodology Pulp necrosis and apical periodontitis were induced by infecting immature teeth in a total of 96 root canals in 4 Beagle dogs. Teeth were divided into four test groups according to the treatment protocol: 1- MTA apical plug; 2- revascularization protocol; 3- revascularization enhanced with a sodium hyaluronate:chitosan scaffold; and 4- revascularization with a pectin:chitosan scaffold. Following root canal disinfection with a triple antibiotic paste protocol, revascularization treatment was performed. Eleven weeks post-treatment, the animals were sacrificed and the jaw blocks harvested for processing and observation by light microscopy. Morphological and the histomorphometric analysis of mineralized tissue on the canal space was carried out. Statistical analysis (Kruskal-Wallis and Mann-Whitney U tests) were performed with a level of significance set at $P < 0.05$.

Results Group 1 revealed the lumen of the root canal completely filled with MTA and the creation of an apical barrier formed mainly from cellular cementum. Best results for mineralized tissue inside the canal were observed for group 2, with significant differences compared to group 3 ($P = 0.006$), and group 4 ($P = 0.025$). Groups 2, 3 and 4 showed the recovery of vital state in the pulp canal, allowing root growth apical to the restoration. Root growth was seen as a significant increase in thickness (85.6%), moderate increase in length (45.6%) of the root canal walls; together with a large number of apical closures (66.7% of cases). Regardless of the scaffold used, the canal space had large areas of highly vascularized soft tissue and mineralized hard tissues with different characteristics and locations. This intracanal cellular cement was often accompanied by a layer of a connective tissue similar to periodontal ligament, and contained some epithelial rests of Malassez.

Conclusions Revascularization of disinfected immature dog root canal systems is possible. The revascularization procedure allowed the continued development of roots in teeth with necrotic pulps.

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R88

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Impact of the cultivation period on gene expression patterns of human pulp derived stem cells

Aim To analyze gene expression of human pulp derived stem cells (hpdscs) in the course of cultivation with focus on the putative stemness of cells influenced by cell aging.

Methodology Cells were derived from pulp tissue of freshly extracted human third molars. Teeth were caries free with incomplete root development. Donor's age was 15–22 years. Pulp tissue was cultured after digestion with collagenase type I (Worthington, USA)/dispase solution (Invitrogen, Japan) based on the Gronthos protocol for obtaining hpdscs. After cells reached confluence they were trypsinized, counted with a cell counter (Casy, Germany) and transferred to the following T-Phase. Three groups of different stages were analyzed: group 1: T-phase 0–3 ($n = 16$), group 2: T-phase 4–7 ($n = 16$) and group 3: T-phase 8–11 ($n = 16$). RNA was isolated with RNeasy Mini Kit (Qiagen, Germany) as requested in the manual. An additional DNase digestion step was conducted using RNase-Free DNase Set (Qiagen, Germany). RNA purity and quantity was achieved by Nanodrop (Thermo Fisher scientific, Germany). For gene analysis customized arrays (SAB, Qiagen, Germany) with 43 genes known to be involved in the metabolism of hpdscs and additionally blanks, controls and Housekeeping genes included were used. For qRT PCR analysis an ICycler (BioRad, Germany) was used. Statistical analysis was achieved by one-way ANOVA followed by a post hoc analysis (Tukey's HSD Test). Level of significance was $\alpha = 0.05$.

Results Housekeeping genes were constantly expressed. Blanks and controls were inconspicuous. Genes significantly downregulated in the course of cultivation were: BGLAP, BMP2, 3, 7, 6, CXCL12, MMP8 + 13, FGFR2, SPP1, CD34 (group 2 versus 3). Genes significant upregulated were: CD 34 (group 1 versus 2) Col1A1, SPARC. Equally expressed were: BMP4, MMP2 + 9, Notch3, TGFb1 + 2, COL4A3. No measurable expression was detected for AMBN, DMP1, DSPP.

Conclusions Downregulation of genes indicating differentiation of human pulp derived cells (e.g. BMPs, SPP1, BGLAP) and upregulation of genes which indicate stemness of cells (e.g. CD34) are an indication of remaining, respectively developing stem cell increase in several transferred hpdscs populations. It has to be taken in consideration that hpdsc cultures contain a various population of differentiating cells (osteogenic direction) but contain also an increasing number of cells with stem cell properties.

R89

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Detection and characterization of mineralized formations observed after apexification and revascularization procedures in immature teeth

Aim The purpose of this study was to highlight the presence of large number of dental mineralized structures, partially or totally separated from the corresponding main root, observed after apexification and revascularization procedures in immature beagle dog's teeth with apical periodontitis.

Methodology Pulp necrosis and apical periodontitis were induced by infecting immature teeth in a total of 96 root canals of 4 Beagle dogs. Teeth were divided into four equal test groups, according to the treatment protocol: 1-MTA apical plug; 2-revascularization protocol; 3-revascularization enhanced with a scaffold of sodium hyaluronate:chitosan; and 4-revascularization with a pectin:chitosan scaffold. All root canals were disinfected with a triple antibiotic paste protocol prior to revascularization. After each protocol treatment, the healing of periapical lesions, the root thickness and closure of apical foramen were assessed and evaluated with the aid of follow-up periapical radiographs and histological analysis.

Results At the end of the experimental period, full radiographic evaluation of the periapical area commonly revealed the presence of mineralized formations, surrounded by a radiolucent band, located in close proximity and continuity with corresponding roots. Histological studies showed that these mineralized formations, which are usually separated from the corresponding main root, were essentially made of cellular cementum and dentine, surrounded by periodontal ligament, containing epithelial rests of Malassez, exhibiting a similar composition to the walls of any ordinary root apex, including the presence of foramina.

Conclusions These mineralized tissue areas, consistently observed after apexification and revascularization procedures, arise as a result of differentiation and maturation process of the apical papilla. The partially or totally separation of these formations from their corresponding root, may be due to the fact that apical papilla can be easily detached by mechanical trauma or iatrogenic factors, applied with the treatment protocol. Histologic observations showed that even when detached they were able to initiate the development of an independent root.

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SESSION 2: FRIDAY 18TH SEPTEMBER

APEX LOCATORS/WORKING LENGTH

R90

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In vitro comparison of working length determination by three electronic apex locators

Aim The aim of this study was to compare, *in vitro*, the accuracy of Apex ID (SybronEndo, USA), Apex Pointer+ (MicroMega, France), and Raypex5 (Dentsply Maillefer Switzerland) electronic apex locators (EALs).

Methodology Thirty five single-rooted extracted human lower premolar teeth with mature apices were used. After pulp extirpation the teeth were decoronated at the cemento enamel junction and actual canal lengths were determined by introducing a size 10 K-File into the canal until it emerged at the apical foramen. The teeth were embedded in floral foam soaked in 0.9% saline. All teeth were then measured with each EAL and the results compared with the corresponding actual canal lengths. The data were analyzed using the intraclass correlation coefficient (ICC) and the repeated measures analysis of variance (rANOVA) tests. The significance level was set at 0.05.

Results There was no significant difference between Apex ID measurements and actual length ($P = 1.00$) or between Raypex5 measurements and actual length ($P = 0.231$), but there were significant differences between Apex Pointer+ and other EALs measurements ($P < 0.001$) and actual length ($P < 0.05$). The ICC test confirmed that the most accurate working length determination was obtained by Apex ID (ICC=0.996), followed by Raypex5 (ICC=0.993); the Apex Pointer+ was the least accurate among the devices tested (ICC=0.986).

Conclusions Under the conditions of this study, Apex ID and Raypex5 had better accuracy of working length measurement than Apex Pointer+.

R91

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Evaluation of working length determination in teeth with large periapical lesions: CBCT versus apex locators

Aim The aim of this controlled clinical study was to evaluate the endodontic working length measurements in teeth with large periapical lesions using preexisting cone-beam computed tomography (CBCT) scans and to compare them with clinical root canal length determination using two electronic apex locators (EAL).

Methodology All patients had received a CBCT scan independent of the present study and needed root canal treatment of at least 1 tooth visible in the field of view. Forty teeth with single roots and canals were studied. Clinically, the root canal length was measured with two different EAL's (Raypex 6 [VDW, Germany] and Propex Pixi [Dentsply Maillefer]) by an endodontist. The measurements were repeated 3 times with digital caliper and the mean was recorded. This measurement was compared with the root canal length as measured on mesiodistal CBCT sections by an oral radiol-

ogist not involved in the endodontic treatment. The CBCT measurements were repeated once for analysis of intrarater reliability. The data was analyzed by repeated measurements ANOVA test.

Results There were no significant difference among the three measurements ($P > 0.05$).

Conclusions In teeth with large periapical lesions root canal length measurement using CBCT was as reliable as measurements derived from using apex locators. However, future studies should evaluate this issue under different circumstances.

R92

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Working length determination by Tri Auto ZX: an ex vivo investigation

Aim To compare the accuracy of two modes of Tri Auto ZX (the manual and auto-stop functions) when used to determine the working length of single-rooted teeth *ex vivo*.

Methodology Under 2.5X magnification, the actual length of 49 extracted single-rooted teeth was determined by inserting size 8 K-file with double rubber stoppers until its tip was seen at the most coronal border of the apical foramen. Stoppers were adjusted to stable coronal reference points then the file was withdrawn and measured using a caliper to an accuracy of 0.1 mm. This step was repeated thrice and averaged (RL). Working length (WL) was calculated by subtracting 0.5 mm from RL. Tri Auto ZX was first used in its manual mode combined with size 8 K-file that had double stoppers. Teeth were placed in freshly-mixed alginate model, canals were irrigated with 2 mL 5% sodium hypochlorite and the file was advanced in the canal until the APEX mark then withdrawn to the 0.5 mark. Stoppers were adjusted and length was measured as above. This step was repeated three times and averaged. Canals were then enlarged using SC1 rotary nickel-titanium file to approximately 2/3 of their WL. Tri Auto ZX was then used in the auto-stop function with SC2 rotary file and the length at which it stopped was measured. A paired t-test was used to compare the means of measurements with level of significance set at 0.05.

Results Mean lengths (\pm standard deviation) for WL, size 8 and SC2 were 13.53 ± 1.47 mm, 13.32 ± 1.59 mm and 13.33 ± 1.48 mm, respectively. Mean lengths for size 8 and SC2 were significantly shorter than WL ($P = 0.002$ and 0.021 , respectively). There was no significant difference between SC2 and size 8 mean lengths ($P = 0.79$).

Conclusions Although the working length determined with the manual and auto-stop modes of Tri Auto ZX were comparable, they were significantly shorter than desired. However, this statistical significance could be clinically insignificant because the differences between the electronic lengths and WL were less than 0.5 mm.

R93

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In vitro comparison of the electronic apex locators Apex ID and Root ZX

Aim The aim of this study was to compare *in vitro* the accuracy of two electronic devices for root canal length determination, Apex ID and Root ZX, using the marks 'APEX' and '0.5'.

Methodology Fifty extracted single-rooted mandibular premolars were selected. The crowns were removed and the teeth were cut in order to obtain roots with an average length of 15 mm. Cervical pre-flaring was performed with a rotary SX instrument. The actual root canal length (RCL) was obtained by placing the tip of a 15 K-file at the apical foramen under magnification (4x); the working length (WL) was established 0.5 mm short to the RCL. The roots were embedded in alginate and irrigated with 2.5% sodium hypochlorite. The electronic measurements of the two devices were performed using a 15 K-file attached to a digital caliper, with a precision of 0.01 mm. The electronic measurements were made in both 'APEX' (or '0.0') and '0.5' marks of the two devices, and compared respectively to the RCL and WL, to obtain the percentage of accurate measurements (within ± 0.5 mm range). Data were statistically analyzed (Wilcoxon and z test, significance set at 0.05).

Results The mean RCL was 15.3 mm, and mean WL was 14.8 mm. The electronic mean lengths obtained respectively at 'APEX' and '0.5' marks, were 15.43 mm and 14.55 mm for the Apex ID, and 15.31 mm and 14.58 mm for Root ZX. The means were not significantly different ($P > 0.05$). The percentage of accurate electronic measurements obtained at APEX mark, was 82% and 88% respectively for the Apex ID and Root ZX. The accuracy in the WL determination of Apex ID was 80%, and 82% for Root ZX. There was no statistically difference between the two devices ($P > 0.05$).

Conclusions The electronic devices, Apex ID and Root ZX were considered accurate both for the root canal length determination (using the 'APEX' mark), and to establish a working length, using the '0.5' mark.

R94

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In vitro evaluation of the accuracy of three electronic apex locators; RayPex 6, Propex PiXi, Ipex II by using different irrigation solutions

Aim The aim of this study was to evaluate the accuracy of three EALs in the presence of different irrigation solutions; 2.5% NaOCl, 5% EDTA, 17% EDTA.

Methodology Sixty human single rooted mandibular premolar teeth with mature apices were used. The actual root canal length was determined by inserting a size 10 K-file into each canal until its tip became visible through the major foramen under dental operating microscope at $\times 12$ magnification. The true working length (TWL) was determined by subtracting 0.5 mm from the actual root canal length. The electronic working length (EWL) was determined with the RayPex 6, Propex PiXi and IPex II EALs in the presence of three different irrigation solutions, 2.5% NaOCl, 5% EDTA and 17% EDTA. Group A ($n = 20$): the samples were irrigated with 2.5% NaOCl. Group B ($n = 20$): the samples were irrigated with 5% EDTA. Group C ($n = 20$): the samples were irri-

gated with 17% EDTA. The results were compared with the corresponding TWL, which was subtracted from the EWL. Data were analyzed using paired-samples t-test, Chi-square test and repeated measure analysis of variance evaluation at the 0.05 level of significance.

Results All EALs yielded accurate findings in all groups. There was no significant difference among EALs groups and irrigation solutions ($P > 0.05$).

Conclusions The use of 2.5% NaOCl and different concentrations of EDTA did not influence the accuracy of EALs used in this study. All devices had an acceptable determination of electronic working length between the ranges of ± 0.5 mm in the presence of 2.5% NaOCl, 5% and 17% EDTA solutions.

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LOCAL ANAESTHESIA

R95

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The limits of computerized intraosseous anaesthesia using the Quick Sleeper-S4 system as a primary technique in emergency treatment of irreversible pulpitis in adult patients

Aim To investigate the limits of primary computerized intraosseous anaesthesia (I.O.) using the Quick Sleeper-S4 system in emergency dental services to anaesthetize mandibular premolars and molars with irreversible pulpitis.

Methodology Twenty-four emergency patients, who were diagnosed with irreversible pulpitis of a mandibular posterior tooth and who were experiencing moderate to severe pain, were included. Patients received nearly 1.8 mL of 4% Articaine with 1 : 100 000 epinephrine by intraosseous (intra-septal) injection using the Quicksleeper-S4 system (DHT, Cholet, France) and underwent emergency treatment. Pulpal anaesthesia was tested via both cold and electric pulp testing at 1 and 2 min intervals after the injection. Pulpal anaesthesia was considered successful if there was a negative cold test and 80/80 on the electric pulp tester. A second clinical evaluation of anaesthesia was carried out while preparing the access to the pulp chamber. Anaesthesia was considered successful if the pulpotomy was performed without or with mild pain. The time needed to complete the treatment was registered and heart rate changes were observed. Patients were questioned on their satisfaction with the procedure.

Results Intraosseous injection using the Quick Sleeper-S4 system was applicable in 83.3% (20 out of 24) of patients and resulted in successful anaesthesia in 70% of patients. The technique was not used with 4 patients: three patients expressed anxiety and refused the I.O. injection and one patient exhibited thin septum (mesial and distal). Heart rates in all patients increased after injection: the mean value of this increase was 14.5 beats per minute (minimum: 4, maximum: 28). 70% of patients reported a perceived transient increase in heart rate. This technique allowed the management of emergency treatment in a relatively short time, 23 min being the mean time. 70% of patients found the intervention was pain-free due to the efficacy and comfort offered by this technique and would choose this anaesthesia for future interventions.

Conclusions Despite the nonapplicability of I.O. anaesthesia using the Quick Sleeper-S4 in a small number of patients, this method can be successfully used to manage irreversible pulpitis in the emergency dental services.

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PREPARATION: FRACTURE RESISTANCE

R96

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Fracture resistance of root filled teeth with two different access cavity designs

Aim To compare the fracture resistance of root filled teeth with traditional and conservative access cavity preparations.

Methodology Thirty maxillary and 30 mandibular first molars and 30 maxillary and 30 mandibular first premolars were selected based on similar dimensions. Average tooth size was calculated for each subgroup to minimize the influence of size and shape variations on the results. The specimens were subsequently assigned to 3 subgroups ($n = 10$) for each tooth type: Group A, acted as the negative control group and were left intact, without cavity preparation or root canal treatment; Group B, a traditional access cavity was prepared on the occlusal surface; Group C, a conservative access cavity was prepared on the occlusal surface. After cleaning, shaping and filling of the root canals, all the access cavities were restored with adhesive direct resin composite restorations (EsthetX; Dentsply-Italia, Italy). The specimens were held in *ad-hoc* grips and placed in a material static-testing machine and subjected to compressive force until fracture occurred. The fracture (breaking) load of the specimens was measured (N) and the data were analyzed by with one-way analysis of variance (ANOVA) and Bonferroni tests ($P < 0.05$).

Results No significant differences were found among groups ($P < 0.05$). Fracture resistance was similar among intact teeth, teeth with standard access cavity and conservative access cavity in all specimens analyzed: maxillary molars (Group A: $1172 \pm 598\text{N}$; Group B: $1143 \pm 506\text{N}$; Group C: $1170 \pm 432\text{N}$); mandibular molars (Group A: $1572 \pm 639\text{N}$; Group B: $1401 \pm 495\text{N}$; Group C: $1459 \pm 278\text{N}$); maxillary premolars (Group A: $913 \pm 188\text{N}$; Group B: $821 \pm 324\text{N}$; Group C: $784 \pm 204\text{N}$); mandibular premolars (Group A: $1006 \pm 310\text{N}$; Group B: $929 \pm 384\text{N}$; Group C: $945 \pm 267\text{N}$).

Conclusions An extreme conservative access cavity opening in intact teeth did not increase the fracture resistance of maxillary and mandibular molars and premolars compared to a traditional access cavity, when occlusal restoration was performed.

R97

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Effect of thermal treatment on K3, K3XF and HyFlex NiTi files after simulated clinical stress

Aim To investigate the effect of various thermal treatments on heat-treated files after simulated clinical stress.

Methodology Three NiTi rotary file systems were examined: K3 (SybronEndo, Orange County, CA, USA), NiTi traditional alloy; K3XF (SybronEndo), R-phase alloy; HyFlex (Coltene-Whaledent, Allstetten, Switzerland), controlled memory wire. Sizes 30, .06 taper instruments were selected and each file system was used at the setting recommended by the manufacturer (K3 and K3XF at 300 rpm, Hyflex at 500 rpm). To determine the fatigue and torsional resistance of the HyFlex file system, new files ($n = 10$) were rotated until fracture (previous data was used for K3 and K3XF file systems). To evaluate the effect of thermal treatment on cyclic fatigue and torsional resistance, the 3 file systems were preloaded until 50% of the number of cycles of failure (NCF) or ultimate torque load (N cm) before thermal treatment. In group I, a control group for each file type was not subjected to thermal treatment. The remaining 3 groups were thermally treated with autoclave sterilization, Nd-YAG laser treatment, and glass beads sterilization. After the thermal treatment, all groups were subjected to cyclic fatigue or torsional resistance test. The fractured instruments were examined under the scanning electron microscope (SEM) for topographic characteristics of the fractured surface. Data were analyzed statistically using one-way ANOVA test and post-hoc tukey test.

Results In the cyclic fatigue test, only K3 files were significantly different between the thermal treatment groups. In the K3 group, only laser treatment was associated with significantly reduced NCF compared with the positive control ($P < .05$). According to SEM images, the fractured surface of K3 files treated with laser had crater-like defects. In the torsional fracture test, Hyflex files had significantly extended resistance than the other groups after thermal treatment. There was a significant difference in the autoclave and laser groups ($P < .05$). However, there was no significant difference in the glass bead group ($P > 0.05$).

Conclusions Additional heat treatment on NiTi files did not change cyclic fatigue resistance and torsional resistance. However, thermal treatment increased the ultimate torque of the Hyflex file system.

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R98

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Effect of novel thermo-mechanical processes for rotary instrument manufacture on cyclic fatigue resistance

Aim The aim of this study was to isolate the effect of the alloy in cyclic fatigue (CF) by comparing times to failure of size 20, .08 taper rotary instruments (prototype TRUShape Orifice Modifiers, Dentsply Tulsa Dental Specialties) manufactured with the same design, cross-section and manufacturing process but using four different alloys (SE-Wire, M-Wire, Blue Wire and Gold).

Methodology A total of 80 equally designed rotary instruments (size 20 tip diameter, .08 continuous taper) were divided into four groups ($n = 20$) according to the type of alloy (SE-Wire, M-Wire, Blue and Gold groups). CF resistance was tested in curved canals (stainless steel, radius 60° , $r = 3$ mm). All files were tested 5 mm from their tips and rotated at 300 rpm until fracture. Time to failure was registered; mean half-life, beta and eta parameters were calculated for each group and compared with Weibull analysis.

Results Gold files lasted significantly longer than M-Wire, Blue and SE-Wire files with a probability of 93%, 94% and 97% respectively. There were no significant differences between the mean life of Blue and M-Wire files, but both were significantly more resistant to CF than SE-Wire (74% for Blue and 73% for M-Wire). SE-Wire instruments were significantly the least resistant to CF.

Conclusions Endodontic instruments manufactured with novel thermo-mechanical processes had higher CF resistance than the same instrument made of conventional NiTi. Use of the advanced metallurgy Gold alloy resulted in the most CF-resistant instruments.

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R99

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Influence of symmetry and variability of cross-sections along the blade of nickel-titanium rotary instruments in cyclic fatigue resistance

Aim The aim of this study was to compare the influence of uniform or variable (symmetrical and asymmetrical) cross-sections along the blade of nickel-titanium (NiTi) rotary instruments in cyclic fatigue (CF) resistance.

Methodology Two NiTi rotary systems with uniform and two with variable (one symmetrical and the other asymmetrical) cross-section along the blade were selected for the study due to the comparable cross-sections. 40 Mtwo (uniform S-shaped cross-section), 40 RevoS RC1 (uniform modified triangular section) 40 OneShape (OS) (variable symmetrical cross-section: modified triangular section at the tip that progressively changes to S-shaped section) and 40 OneShape New Generation (OSNG) (variable asymmetrical cross-section that decreases the mass of the file: modified triangular section at the tip that progressively changes to S-shaped section) instruments were tested. All files had the same size 25 tip diameter, .06 continuous taper. A total of 8 groups ($n = 20$ instruments each) were tested. CF resistance was tested in stainless steel curved canals (radius 60° , $r = 3$ mm) at 5 mm from the tip in groups MTWO-5, REVOS-5, OS-5 and OSNG-5 and at 10 mm in groups MTWO-10, REVOS-10, OS-10 and OSNG-10 at 10 mm. All files were rotated until breakage at 300 rpm, and times to fracture were registered. Mean half-life, beta and eta parameters were calculated for each group and compared with Weibull analysis.

Results MTWO-5 and OSNG-5 had a significantly higher mean life at 5 mm from the tip than OS-5 and REVOS-5, which showed the lowest. At 10 mm from the tip, OSNG-10 lasted significantly longer than OS-10, MTWO-10 and REVOS-10 with a probability of 88%, 97% and 100% respectively.

Conclusions S-shaped and new advancements such as asymmetrical cross-sections demonstrated an improvement in CF resistance of rotary NiTi instruments in simulated curved canals.

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R100

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Effects of ProTaper Next, Reciproc and self adjusting file systems on dentinal microcrack formation: a micro-computed tomographic study

Aim To evaluate the frequency of dentinal microcracks observed after root canal preparation with ProTaper Next (PTN) (Dentsply Tulsa Dental, Tulsa, OK, USA), Reciproc (VDW, Munich, Germany) and Self-Adjusting File (SAF) system (ReDent-Nova, Ra'anana, Israel) using micro-computed tomographic analysis.

Methodology Thirty mesial roots of mandibular molars with a type II Vertucci canal configuration were scanned at an isotropic resolution of 13.68 μ m. The samples were randomly assigned to 3 experimental groups ($n = 10$). The root canals were prepared using ProTaper Next in group 1, Reciproc in group 2 and the SAF system in group 3. After canal preparation root canals were scanned at an isotropic resolution of 13.68 μ m again. Then cross-section images of the roots were screened to identify the presence of dentinal microcracks. Statistical analysis was performed using SPSS version 22.0 software (SPSS Inc, Chicago, IL, USA). Propagation ratio of microfracture among the groups was analyzed using the Kruskal Wallis test ($P < 0.05$).

Results All of the instrumentation systems created microcracks in root dentine. In postoperative cross-section images, dentinal microcracks were observed in 7.3% of group 1 (PTN), 10.93% of group 2 (Reciproc) and 14.42% of group 3 (SAF). There were no significant differences among the groups ($P = 0.432$).

Conclusions Under the conditions of this study, PTN, Reciproc and SAF instrumentation systems created microcracks in root dentine. There is no difference among the instrumentation systems according to new microcrack formation.

Acknowledgements The authors deny any conflicts of interest related to this study.

R101

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HyFlex EDM: innovative electro discharge machined rotary instruments. Fatigue testing, superficial and microstructural characterization

Aim To test the fatigue resistance and to evaluate the surface and microstructural alterations of new and *in vitro* used HyFlex EDM NiTi rotary prototypes.

Methodology Thirty electro discharge machined HyFlex EDM (Coltène/Whaledent, Switzerland) prototypes, and 20 HyFlex CM (Coltène/Whaledent, Switzerland) files were subjected to cyclic fatigue test in a standardized 70° artificial metal canal. Results

were validated using Mann-Whitney tests (α -level 0.05). Fractographic analysis on broken samples was conducted using environmental scanning electron microscopy (ESEM) imaging. Fifteen new HyFlex EDM prototypes were used for *in vitro* instrumentation of severely curved root canals of extracted multi-rooted teeth. Surface and microstructural characteristics of files were analyzed by ESEM equipped with energy dispersive x-ray spectrophotometry (EDS) and optical metallographic imaging. The same analysis was conducted on new and used instruments to assess the usage-induced degradation.

Results The cyclic fatigue test revealed an increase of fatigue resistance up to 700% of EDM files compared to CM ($P = 0.0001$). Fractographic analysis of fatigued EDM instruments disclosed multiple crack origins in correspondence of the cutting edges, with a limited fatigue striation zone and a noteworthy dimpled area. Surface and microstructural characterization of new instruments revealed the typical features of a NiTi ED-Machined alloy with a 'crater-like' surface. No fractures were registered during instrumentation of curved canals. Surface and microstructural characterization of used files revealed no wear and no degradation of the size 25, .12 taper and size 25, .08 taper files. The tip segment was confirmed as the most mechanically stressed portion of size 10, .05 taper files. The metallographic inspection on the cross section of brand new HyFlex EDM files showed an homogeneous martensitic phase. The microstructure appeared uniform from the surface to the bulk, and no microcracks or defect were identified, even at high optical magnification (1000X).

Conclusions HyFlex EDM exhibited high values of cyclic fatigue resistance and a safe *in vitro* use in severely curved canals. Unaltered spark-machined surface and low microstructural degradation are the main features of HyFlex EDM.

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R102

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Torsional and cyclic fatigue resistance of the new Hyflex EDM instruments and their comparison with Reciproc and WaveOne

Aim To investigate the torsional strength, angular rotation and cyclic fatigue resistance of the new Hyflex EDM OneFile, manufactured by CM-wire and a process novel to nickel-titanium (NiTi) file production called electrical discharge machining (EDM), and compare these findings with Reciproc R25 and WaveOne Primary (both made with M-wire).

Methodology Thirty new Hyflex EDM OneFile, Reciproc R25 and WaveOne Primary files were tested for torsional and cyclic fatigue resistance. Cyclic fatigue testing was performed with the instruments ($n = 15$ each) operating until fracture inside an artificial canal with 60° angle of curvature and 5 mm radius of curvature in continuous rotation at 500 RPM for Hyflex EDM and in the reciprocating motion for the Reciproc R25 and WaveOne Primary files as recommended by each manufacturer. The number of cycles to fracture (NCF) was determined by measuring the time to fracture.

Instruments ($n = 15$ each) were tested for torsional strength and angular rotation to fracture following the ISO 3630-1 guide-

lines by using a torsionmeter. Kruskal-Wallis and Dunn's multiple comparison post-hoc tests at 0.05 were used to evaluate all data. The fracture surface of each fragment was examined with a scanning electron microscope (SEM).

Results Cyclic fatigue resistance of Hyflex EDM was significantly higher than that of Reciproc R25 ($P < .001$). Reciproc R25 cyclic fatigue resistance was higher than WaveOne ($P < .01$). Hyflex EDM OneFile had a significantly higher angular rotation to fracture and lower maximum torsional strength than Reciproc R25 and WaveOne Primary ($P < .05$ and $P < .001$ respectively). No significant difference was found in the torque and angular rotation to fracture of the reciprocating instruments tested.

Conclusions Hyflex EDM OneFile had higher cyclic fatigue and angular rotation, but lower maximum torque to fracture than Reciproc R25 and WaveOne Primary, due to their alloy and production processes.

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PREPARATION: CLEANING ABILITY

R103

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Efficacy of SAF, BtRace and ProTaper in eliminating bacteria from infected root canals *ex vivo*

Aim The aim of the study was to compare the efficacy of the Self-adjusting file, BtRace and ProTaper Next, in eliminating bacteria from oval single rooted teeth.

Methodology One hundred caries free, single rooted oval teeth (bucolingual to mesiodistal ratio $>2.5 : 1$, at 5 mm from the apex) were cleaned, sterilized and contaminated with *Enterococcus faecalis* (ATCC 29212). After random division into four groups ($n = 25$), the canals were shaped with four different techniques: Group1: manual preparation, Group2: SAF preparation, Group3: ProTaper Next preparation and Group4: BT-Race preparation. Irrigation was performed during shaping, for each tooth with 15 mL of NaOCl 5.25%, smear layer was removed with 5 mL EDTA 17%, followed by 5 mL of 5.25% NaOCl. Sodium hypochlorite was then inactivated by 5 mL of 10% Na2O3S2 and washed away by distilled water. For each tooth, two microbial samples were taken with sterile paper points: S1 after determination of the working length, and S2 at the end of the instrumentation. Bacterial counts were transformed in logarithmic scale and analyses of covariance (ANCOVA) were implemented. Post hoc pair wise comparisons using the Tukey method were used after the main analysis.

Results The reduction in bacterial counts in S2 for Group 1 was 80% for Group 2, 96% for Group 3 96% and 97% for Group 4. The canal preparation in Group 1 was less effective compared to the other methods, this difference was significant ($P < 0.001$). In a pairwise comparison between NiTi shaping methods (Groups 2, 3 and 4) no significant difference was found.

Conclusions Under the conditions of the present study, the 3 rotary methods were equally effective in reducing the bacterial load of infected oval root canals. Even though the SAF is considered the golden standard in shaping oval canals, the new designs in rotary files also provide good alternatives.

R104M. Biney^{1,*}, E. Guez², C. Ricci¹ & E. Medioni³¹Department of Postgraduate Endodontic, Pôle Odontologie, CHU NICE, ²Department of Restorative and Endodontics, Pôle Odontologie, CHU NICE & ³EA 7354 MICORALIS Laboratory, Faculté d'Odontologie, Université de Nice and Pôle Odontologie, CHU NICE, Nice, France**In vitro evaluation of apical debris extrusion during root canal shaping: single reciprocating Ni-Ti instrument versus rotary Ni-Ti sequence****Aim** The purpose of this *in vitro* study was to evaluate apical debris extrusion during root canal shaping when using WaveOne[®](WO) compared to the ProTaper Next[®](PN) system (DentsplyMaillefer, Ballaigues, Switzerland).**Methodology** Twenty single-rooted teeth were selected. The teeth were inserted in an Eppendorf tube in order to collect extruded apical debris during root canal shaping. Two groups of ten each were randomly created. The root canals were prepared with different instruments according to the manufacturer's recommendations and the gold standard irrigation technique was applied: 9 mL of irrigant solutions were used for each canal. The protocol of Tanalp *et al.* (2006) was slightly modified in order to measure the quantity of extruded debris. Eppendorf tubes were weighed empty and after evaporation of liquid; the teeth were also weighed before and after root canal shaping using an electronic microbalance (high precision 10–8 g). The data were analysed statistically using the Mann and Whitney test (non parametric) to compare the weights of extruded debris and the loss of weight of the teeth after preparation. The level of significance was set as $P < 0.05$. A ratio was calculated between loss of weight and weight of extruded debris.**Results** The two instrumentation techniques removed approximately the same quantity of dentine, $0.0108 \text{ g} \pm 0.0021$ for the WO, and $0.0113 \text{ g} \pm 0.0017$ for the PN; no significant difference ($P = 0.4963$). No significant difference was found for the weight of extruded debris ($P = 0.1988$); mean value for the WO group $0.0020 \text{ g} \pm 0.0010$ and for PN group $0.0005 \text{ g} \pm 0.0007$; but the amount of extruded debris was four times more for the WaveOne group than for the ProTaper Next group.When the ratio between the weight of extruded debris and the weight of dentine removed during shaping was compared there was a significant difference ($P = 0.0009$). For the WO group approximately 1/5 of the removed dentine during root canal shaping was extruded, whilst for the PN group it was significantly less with only 1/20 of removed dentine being extruded apically.**Conclusions** Under the conditions of this experiment, the use of a full sequence of rotary Ni-Ti instrumentation was associated with less apical extrusion of debris than the use of a single reciprocating instrument.**PREPARATION: SHAPING ABILITY****R105**D. Bakr^{1,*}, A. Sultan² & S. Saleem³¹Department of Conservative, Hawler Medical University, Erbil, ²Department of Conservative, Mosul University, Mosul & ³Department of Pedodontic, Orthodontic and Prevention, Hawler Medical University, Erbil, Iraq**A comparison between two types of endodontic single file technique (WaveOne and SAF) by means of centering ability and percentage of untouched canal surfaces****Aim** This study evaluated the canal centering ability and amount of remaining untouched root canal walls with WaveOne and the SAF.**Methodology** A micro computed tomography (Micro-CT) was used to select 60 mandibular first premolars out of a total of 160. The selective characteristics depended on the similarities in canal configuration and 20-degree apical curvature. The samples were divided into two groups. In group one, the canals were prepared with WaveOne files according to the manufacturers' recommendations. The root canal system was irrigated with 4 mL of 2.5% NaOCl; 2 mL of irrigation during the instrumentation intervals and 2 mL at the end of instrumentation as the final flush. Group 2 was prepared with Self Adjusting Files for 2 min with continuous irrigation with 4 mL of 2.5% NaOCl. A comparison between pre- and postoperative micro computed images was done by DICOM CS 3D imaging software. For centering ability, the pre- and postoperative images were transferred to DICOM CS 3D imaging software, to measure the change by instrumentation according to the mathematical formula: $\beta = (D1-D2/D) \times 100$, where parameter β represents centering propensity of the prepared canal. For determining the ratio of the touched-untouched surfaces, superimposed images of the surface area voxels of the canal before and after preparation were examined. The surface voxels remaining in the same place represented the untouched parts of the canal walls. The t-test was used for statistical analysis ($P < 0.05$).**Results** Self Adjusting Files had significantly ($P < 0.05$) better centering abilities than WaveOne files. In addition, the percentage of untouched areas of the canals prepared with WaveOne files were significantly ($P < 0.05$) higher than those prepared with SAF files.**Conclusions** SAF files had better results in terms of preserving canal configuration and centering ability. In addition, SAF files were better able to prepare larger surface areas of canal walls compared to WaveOne files.**R106**E.J. Silva^{1,*}, F.G. Belladonna², E.M. Souza³, M.A. Versiani⁴ & G. De-Deus¹¹Department of Endodontics, Grande Rio University, Rio de Janeiro, ²Department of Endodontics, Fluminense Federal University, Niterói, ³Department of Dentistry II, Maranhão Federal University, São Luiz & ⁴Department of Restorative Dentistry, University of São Paulo, Ribeirão Preto, Brazil**Micro-computed tomographic evaluation on the effect of ProTaper Next and Twisted File adaptive systems on dentinal cracks, hard tissue debris accumulation and non-instrumented canal areas****Aim** This study evaluated the frequency of dentinal micro-cracks, the amount of hard tissue debris accumulation and non-instru-

mented canal surface after mechanical preparation with ProTaper Next (PTN) and Twisted File Adaptive (TFA) systems through micro-computed tomographic analysis.

Methodology Twenty moderately curved mesial roots of mandibular molars with 2 independent canals were selected and scanned at an isotropic resolution of 14.25 μm . The sample was assigned to 2 experimental groups ($n = 10$) according to the tested system: PTN and TFA. Second scans were taken after the canals were prepared up to PTN X2 (25/0.06) and TFA SM2 (25/0.06). The matched images of the mesial canals, before and after preparation, were scrutinized from the furcation level to the apex to identify the presence of dentinal defects (micro-cracks). The amount of hard tissue debris (%) and non-instrumented area were also evaluated. Data were statistically compared using a general linear model for repeated-measures with a significance level set at 5%.

Results Dentinal micro-cracks were observed in 38.72% ($n = 5150$) and 30.27% ($n = 3790$) of the cross-section images in PTN and TFA groups, respectively. All dentinal defects identified in the postoperative cross-sections were also observed in the corresponding preoperative images. Instrumentations systems *per se* did not influence in the amount of hard tissue accumulation ($P > 0.05$) or in the percentage of untouched canal surface ($P > 0.05$).

Conclusions No causal relationship between dentinal micro-crack formation and canal preparation procedures with PTN and TFA was observed. The systems showed similar performance regarding the amount of hard tissue accumulation and in the percentage of untouched canal surface. None of the systems yielded root canals completely free from packed hard tissue debris or was able to prepare all the surface area of the mesial root canals of mandibular molars.

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R107

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The incidence of dentinal defects after root canal preparation of severely curved roots with the Reciproc single-file system with and without prior creation of a glide path

Aim To assess the incidence of dentinal defects after preparation of severely curved root canals using the Reciproc single-file system with and without prior creation of a glide path.

Methodology Mesial roots from extracted mandibular first molars were collected and scanned with CBCT to assess the morphology of the root canal systems. Three groups of 20 anatomically comparable specimens were generated. The control group was left unprepared, while the experimental groups were prepared with Reciproc R25 with and without a glide path (groups R and RG, respectively). A size 15 C-file was used to create a manual glide path to WL using a watch-winding motion. Roots were sectioned perpendicular to the long axis at 2, 4, 6, 8 and 10 mm from the apex and colored photographs of the sections at 40X were obtained. Two blinded examiners registered the presence of dentinal defects twice at 2-weeks interval. Data were statistically analyzed using the Fisher exact and Cochran's q tests.

Results The kappa values for inter-observer and intra-observer agreement were 0.93 and 0.91, respectively. No defects were observed in the control group. The overall incidence of dentinal

defects was 26% in group R and 24% in group RG, with no significant differences between these two groups ($P > 0.05$). Dentinal defects occurred significantly more often at the 6, 8 and 10 mm levels compared to the 2 and 4 mm levels ($P < .05$).

Conclusions Prior preparation of a glide path had no impact on the incidence of dentinal defects when using Reciproc files.

R108

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Shaping ability of the Reciproc and self-adjusting file systems in mandibular second molars with C-shaped root canals: a micro-CT study

Aim To compare using Micro-CT the shaping ability of the Reciproc and Self-adjusting file (SAF), on mandibular second molars with C-shaped merging type canals.

Methodology Twenty mandibular second molars with fused roots and C-shaped merging type canals were chosen. One group ($n = 10$) was prepared with a R25 (size 25, 08 taper) Reciproc instrument and 20 mL of 2.5% sodium hypochlorite (NaOCl) as an irrigant. The other group was instrumented with the SAF system for 4 min according to the manufacturers' instructions with continuous 2.5% NaOCl irrigation at 5 mL min⁻¹ with a VATEA peristaltic pump. Preoperative and post treatment scans were made using a micro-CT device. The reconstructed images were superimposed over each other and the volume increase (VI) and uninstrumented surface (US) of the root canal walls after instrumentation were analyzed using the Ctan software. Percentages of the VI and US were calculated in the total root canal length and also individually at the (1–3), (3–6) and (6–9) mm sections. Comparisons of the two groups were performed using the Mann Whitney test ($P < 0.05$).

Results Reciproc instrumentation resulted in a higher volume increase percentage 20.36 (3.88–44.20) when compared to the SAF group 7.79 (4.97–29.71) ($P = 0.037$). When analyzed by thirds, the volume increase with Reciproc was only higher in the 1–3 mm ($P = 0.008$) and 3–6 mm ($P = 0.014$) sections compared to the SAF. Furthermore, no significant differences between Reciproc and SAF were observed in the total US area of the canals ($P = 0.520$), and neither when the canal sections were analyzed separately. The median and range of the Reciproc uninstrumented surface in the root canal was 31.34 (10.02–63.18) while the SAF system left 33.87 (9.84–76.52) of uninstrumented surface.

Conclusions None of the instrumentation systems were able to shape completely the C-shaped merging type canals of mandibular second molars. Nevertheless, the SAF system had a more conservative enlargement of the root canal according to its volume increase.

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The authors deny any conflicts of interest on this study.

R109

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Ex vivo assessment of root canal deviation and instrumentation centering when shaping oval canals with new prototype instrumentation

Aim The purpose of this *ex vivo* pilot study was to assess the 3D root canal transportation and the centering of a new prototype Ni-Ti vibrating endodontic instrumentation (Micro-Méga, Besançon, France) compared to a Ni-Ti rotary instrumentation, the Revo'S system (Micro-Méga).

Methodology Twenty sound mandibular incisors with a single root canal were selected. Two groups of 10 teeth were randomly formed. After embedding the teeth in an epoxy resin model, Cone Beam examination (Planmeca- PROMAX 3D-1000) before and after shaping was achieved. Using the software Planmeca Romexis Viewer (3.0.1.R) Coronal and Axial sections at 3, 6 and 9 mm from the apex were examined to analyse the respect of the canal path and centering of the instrumentations. Measurements were registered in the Bucco-Lingual (BL) and Mesio-Distal (MD) directions at each level for root canal deviation and centering. Statistical analyses was done with using Kruskal-Wallis and Mann-Whitney tests at each level in BL and MD directions in each group (intra-group comparison) and between the groups at each level (inter-group).

Results In the group of prototype instrumentation, comparisons between each level did not reveal significant differences for transportation and centring ($P > 0.05$). The rotary Ni-Ti instrumentation transported significantly the root canal on the mesial side at 9 mm from the apex ($P = 0.0428$ and $P = 0.0156$) more than at 6 and 3 mm. Comparisons between the 2 groups at each level (inter-group) showed a significant difference at 9 mm: the prototype instrumentation was centred, whilst the rotary instrumentation transported the root canal toward the mesial side ($P = 0.0001$).

Conclusions The Ni-Ti vibratory prototype instrument was centered and respected the trajectory of oval root canals in mandibular incisors when compared to Ni-Ti rotary instrumentation.

R110

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Shaping ability of two nickel-titanium instrument systems in S-shaped mesial canals of mandibular molars assessed by micro-CT

Aim To evaluate the shaping ability of two different nickel-titanium instrument systems during the preparation of S-shaped mesial canals of mandibular molars.

Methodology Twenty extracted first and second mandibular molars with an S-shape in the mesial roots were scanned before and after instrumentation procedures using a micro-computed tomography system with a voxel size of 16.8 μm . The specimens with similar morphological dimensions were randomly divided into two groups ($n = 10$) after an anatomical study. ProTaper Next (NiTi MWire) and TF Adaptive (NiTi phase R) systems were used to shape the mesiobuccal and mesiolingual canals until size

0.25 mm instruments. The analysed parameters included the root canal volume, remaining dentine thickness at three different levels (1, 3 and 6 mm) and uninstrumented root canal surface (%). Preparation time and instrument failures were also recorded. Preoperative and postoperative data were compared using Kruskal-Wallis and Tukey's tests with the significance level set at 5%.

Results The volumetric analysis showed no significant difference ($P > 0.05$) among the systems. Both systems maintained the original shape of the root canals in the apical third. No significant difference was found between the mesial and distal dentine thickness for the analyzed parameters. No differences ($P > 0.05$) were found in the percentage of uninstrumented root canal surface among groups. The preparation times were similar ($P > 0.05$) and no instrument failure was found.

Conclusions ProTaper NEXT and TF Adaptive systems prepared canals rapidly and were able to maintain the canal curvature.

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R111

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Root canal preparation with ProTaper Next and one shape new generation in mandibular molars: a micro-computed tomography study

Aim To assess and compare root canal preparation with two rotary nickel titanium instruments in curved root canals using micro-computed tomography.

Methodology Thirty mandibular molars were selected for this study and submitted to standardized radiographs and micro-computed tomography (SkyScan 1172), before and after canal preparation. Based on radiographs taken prior instrumentation with the initial instrument inserted into the canal, the teeth were randomly divided into 2 homogeneous groups, with respect to the angle of canal curvature. The first group included teeth instrumented with ProTaper Next (Dentsply Maillefer) and the second group with One Shape New Generation (MicroMega). The teeth were three-dimensionally reconstructed (Nrecon v.1.6.3, Bruker-microCT) and evaluated for: changes in canal volume, percentage of shaped canal walls and canal transportation. The time taken for each method and working length for each tooth, before and after instrumentation was recorded. Statistical analysis was carried out with ANOVA (Bonferroni and Tamhane tests) with 0.05 level of significance.

Results There were no significant differences concerning root canal curvature and volume, preoperatively. Instrumentation with ProTaper Next and One Shape New Generation led to enlarged canal shapes with no evidence of preparation errors. Postoperatively, volume and surface area increased significantly in all root canals ($P < 0.05$). There was no significant difference between the two groups with respect to canal path (canal transportation). Prepared canals were rounder in cross-section and more tapered.

Conclusions *In vitro* preparation of root canals in mandibular molars with the two rotary nickel-titanium instruments was effective and safe, with little canal transportation.

R112

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Effects of ProTaper Universal and ProTaper NEXT instruments on dentinal crack formation in curved roots

Aim To evaluate dentinal crack formation with the ProTaper NEXT and ProTaper Universal systems in curved roots, and analyze the cracks generated at the point of maximum canal curvature.

Methodology Sixty human mandibular premolars extracted for periodontal reasons with curvatures between 30–49° and radii between 2–4 mm were randomly divided into two experimental groups and one control group ($n = 20$). The external root surface was inspected using a stereomicroscope (Leica MZ16F; Leica Microsystems Heidelberg GmbH, Mannheim, Germany) to exclude the possibility of any defects before the procedure. The root canals were instrumented using the ProTaper Universal® (Dentsply Maillefer) and ProTaper NEXT® (Dentsply Maillefer) systems, with the aid of the Proglider® (Dentsply Maillefer) system. The samples were sectioned transversely before subsequent analysis 2 mm and 8 mm from the apex and at the point of maximum canal curvature. The slices were examined through a stereomicroscope (Leica MZ16F). Defects were scored: 0 for no defects; and 1 for cracks. Pearson's chi-squared test was performed to compare the incidence of cracks between the experimental groups.

Results Root defects were not observed in the control group. The ProTaper NEXT system caused fewer defects (16.7% of root canals with micro-cracks) than the ProTaper Universal system (40%) ($P < 0.05$). With the ProTaper NEXT system, fewer dentinal cracks were formed in the apical third and at the point of maximum curvature in the coronal area ($P < 0.05$). The ProTaper Universal system caused significantly more cracks at the point of maximum canal curvature than the ProTaper NEXT system ($P < 0.05$).

Conclusions Rotary instrumentation systems often generate root defects, but the ProTaper NEXT system generated fewer dentinal defects than the ProTaper Universal system in teeth with curved roots. A higher prevalence of defects was found at the point of maximum curvature in the ProTaper Universal group.

R113

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In vitro comparative study of NiTi systems assessing shaping ability and preparation time in curved root canals

Aim The present study compared transportation, centering ability and preparation time of the ProTaper Universal system, the reciprocating WaveOne system and an association of these motions.

Methodology Sixty root canals of extracted human molar teeth, with curvatures ranging between 20° and 40°, were divided into 3 groups, according to the preparation system. Canals were irrigated with 5% sodium hypochlorite (NaOCl) and prepared until apical size 25 using the ProTaper Universal system (G1), WaveOne (G2) or a hybrid technique (G3) associating reciprocating preparation with rotary glide path and cervical pre-enlargement. Teeth were scanned pre and post-operatively using computed tomography. The images were transferred to Adobe Photoshop software (v. 8.0, Adobe Systems, San Jose, CA, USA)

and direction of transportation and centering ability of canals were measured using an adaptation of the method described by Gambill *et al.* (1996). The results were analyzed statistically using one-way ANOVA. The time for canal preparation was measured, including total active instrumentation, instrument changes within the sequence, cleaning of the flutes of the instruments and irrigation and was analyzed by ANOVA and the Tukey test.

Results No differences regarding transportation and centering ability were found among the tested protocols. Preparation with WaveOne (G2) was significantly faster ($P < 0.05$) in comparison to the other groups.

Conclusions The single-file reciprocating instrument was capable of providing more rapid root canal preparation with similar transporting and centering ability when compared with continuous and an association of motions in curved canals.

R114

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Lateral cutting efficiency of NiTi instruments used in reciprocation and continuous rotation

Aim The aim of the present study was to compare the lateral cutting efficiency of Twisted File Adaptive instruments used with two different motions: reciprocation versus continuous rotation.

Methodology Twenty new ML1 TF Adaptive (SybronEndo, Glendora, CA, USA) were randomly divided in two groups of ten each: Group 1 (TFA) were activated using the TFA reciprocating programme using the Elements motor (SybronEndo, Glendora, CA, USA), while group B (TF) used a continuous rotation motion programme (500 rpm). The device used for the cutting test consisted of a mainframe to which a mobile plastic support for the handpiece is connected and a stainless-steel block containing a Plexiglas block against which the cutting efficiency of the instruments was tested. The length of the block cut in 1 min was measured in a computerized program with a precision of 0.1 mm. Mean and standard deviations of each group were calculated and data were analyzed statistically using the T-student test and Bonferroni t test ($P < 0.05$).

Results TFA (reciprocating) motion cut the Plexiglas block to a mean depth of 8.7 mm (Standard Deviation – SD = 0.5 mm), while TF motion instruments cut the Plexiglas block to a mean depth of 10.5 mm (SD = 0.7 mm). TF motion displayed significantly greater maximum penetration depth than TFA motion ($P < 0.05$).

Conclusions Even if TFA (reciprocating) motion allows good lateral cutting efficiency, continuous rotation (TF) had better cutting efficiency, and could be the ideal clinical motion for brushing.

R115

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Prediction of contact points and pressures against canal walls of NiTi rotary files by virtual modeling and finite elements analysis

Aim The objective of this study was to predict contact points and pressure distribution against canal walls when using NiTi rotary instruments by FEM virtual simulation.

Methodology ProTaper Next (PTN) X1 and ProTaper Universal (PTU) S2 were compared. Canal configuration was based on an *in vitro* testing device. The *in vitro* canal and file geometries were replicated with computer aided design (CAD) software. CAD geometries (geometrical model) were imported and discretized (numerical model). The typical material model of dentine and M-Wire alloy was applied. The numerical models of the device and file geometries were used for finite element analysis (FEA). Experimental data from PTN X1 and PTU S2 were used to set-up the material parameter in the numerical model. Stress histories on the root canal contact points patterns due to the PTN X1 and PTU S2 were compared.

Results The pressure distribution against canal walls was mainly influenced by the geometry of the file. The material of the file did not affect significantly the results. The PTN X1 was associated with a pressure 80% lower than PTU S2.

Conclusions The pressure distribution against canal walls may be predicted by FEA. Virtual design, testing and analysis of file geometries could save considerable time and resources during instrument development.

R116

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Dentinal microcrack formation after root canal preparation using WaveOne, ProTaper Next and BT Race: a human cadaver study

Aim The aim of this study was to compare formation of dentinal microcracks after root canal preparation while using reciprocating Ni-Ti instruments (WaveOne) and rotary Ni-Ti instruments (ProTaper Next and BT Race) on human cadavers.

Methodology Fourteen central mandibular incisors, fourteen lateral mandibular incisors and eight buccal roots of eight maxillary first molars of seven human heads were selected. Six teeth were left unprepared and served as controls (Group 1) and the remaining thirty teeth were divided into three groups: Group 2 was instrumented by using WaveOne reciprocating files ($n = 10$), Group 3 using ProTaper Next rotary files ($n = 10$) and Group 4 using BT Race rotary files ($n = 10$). Roots were embedded in resin and sectioned perpendicular to the long axis 1, 3, 6, 9 mm from the apex. The sectioned surfaces were evaluated under a stereomicroscope at x15 and x40 magnification and checked for the presence of dentinal microcracks by three independent examiners. Data were analyzed with chi-square tests at $P < .05$.

Results No complete cracks were observed in the experimental or control groups. The difference between the control group and the experimental groups was significant for incomplete dentinal microcracks ($P < .05$). Group 4 had the lowest incidence of both the defects registered when compared with Group 2 and Group 3. The BT Race system (Group 4) produced significantly fewer incomplete cracks than the ProTaper Next and WaveOne systems in the 3 mm and 9 mm sections ($P < .05$).

Conclusions Within the limitations of this study, more data must be collected to find a correlation between the shaping techniques (WaveOne, ProTaper Next and BT Race) and incomplete microcracks formation.

R117

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Comparison of the cutting efficiency of instruments manufactured with conventional nickel-titanium and novel gold metallurgy in lateral action

Aim The purpose of the present study was to compare lateral cutting efficacy of two different rotary systems manufactured with either conventional nickel-titanium or novel gold alloy, ProTaper Universal (PTU) and ProTaper Gold (PTG) (Dentsply Tulsa Dental Specialties).

Methodology Seventy-eight PTU and PTG Shaping Files instruments (SX, S1 and S2), with the same exact geometries but manufactured with two different alloys were used. An Instron machine was used to test the bending behavior for each instrument in triplicate to determine the ideal displacement to generate a standardized force of 2N against the substrate. Ten instruments of each size were used in simulated lateral action for 60 s (s) against an acrylic substrate engaged in a computer-controlled testing platform at 300 rpm. The process was repeated four times producing 4 notches in each plastic block after 60s, 120s, 180s and 240s of action. Notch areas and depths were measured under a stereomicroscope, and Student T test was used to compare data between alloys. Repeated measures ANOVA was used to compare cutting efficiency for each instrument across the four different time points.

Results For all three Shaping Files (SX, S1 and S2) PTG instruments were significantly more cutting efficient ($P < 0.05$) at the four different times point, except for S1 that did not show significant differences at the 240s time point. No significant differences were found in cutting efficiency for any specific instrument at the four time points.

Conclusions ProTaper Gold had higher cutting efficiency in lateral action probably due to the advancements in the proprietary metallurgy after area and length analysis of notches produced in a plastic substrate. None of the instruments had a significant loss in cutting efficacy over the 4 mins of testing.

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FILLING: MTA

R118

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Bond strength of MTA to root dentine after different irrigation activation regimens for the removal of triple antibiotic paste

Aim The aim of this study was to evaluate the bond strength of mineral trioxide aggregate (MTA) to the root canal dentine after various irrigation procedures to remove triple antibiotic paste (TAP).

Methodology In total, 56 single-rooted human mandibular premolars were prepared using a rotary system to size 40. The speci-

mens were divided randomly into a control group (without intracanal dressing) and three experimental groups that had received an intracanal dressing with TAP. The intracanal dressing was then removed by rinsing with 10-mL 2.5% NaOCl with three irrigation systems (Vibringe sonic irrigation, CanalBrush irrigation and Syringe irrigation system). Two-thirds of the root canals were then filled with MTA. After storing for a week, all specimens were embedded in acrylic blocks and each of them were sectioned horizontally in 2 mm-thick slices at two levels (coronal and middle) using a low-speed saw with continuous water irrigation to prevent overheating. In each group, 28 samples were assessed using a universal testing machine with a push-out test to measure the bond strength between the root canal dentine and MTA. Comparisons between the four groups were applied using one-way ANOVA and Tukey test. Results were presented as mean \pm SD.

Results The mean values of push-out bond strength of MTA in Control, Vibringe, CanalBrush and the Syringe irrigation groups were 14.47, 13.96, 9.89 and 9.45, respectively. There was a non significant difference between push-out bond strength values of Vibringe sonic irrigation system and the control group ($P = 0.997$). The push-out bond strength value of MTA was significantly higher in the Vibringe sonic irrigation group compared with the CanalBrush and Syringe irrigation groups ($P < 0.001$).

Conclusions The results indicate that the push-out bond strength values were affected by the irrigation technique. Irrigation performed with the Vibringe sonic irrigation system to remove TAP from root canals increased the push-out bond strength of MTA compared to the CanalBrush and Syringe irrigation systems.

R119

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Cellular response to MTA alone or in combination with collagen gel

Aim To investigate the response of baby hamster kidney fibroblast (BHK) cell line to MTA (ProRoot tm MTA, Dentsply Tulsa, USA) mixed with either sterile water or collagen gel (Biocollagen gel, BCG-GEL1, Bioteck, Italy).

Methodology Extracts of the tested materials were prepared at three time points: 1, 3 and 7 days after mixing. Cells were seeded into 96-well plates at 2×10^4 cell/well incubated with 100 μL extracts from each group ($n = 9$). Cells cultured in culture medium only served as control. After 24 h incubation, cell viability was assessed using the MTT assay and cell numbers were assessed using the crystal violet proliferation assay. The effect of direct contact of specimens of materials on cells in culture was observed using inverted microscopy. Statistical analysis involved Student-*t* test and repeated-measures ANOVA to assess the effect of material and time on tested parameters.

Results One-day extracts of MTA with collagen gel resulted in significantly higher cell viability and cell number ratios than MTA ($P < 0.05$); no significant differences existed between the two materials for the 3- and 7-day extracts ($P > 0.05$). There was a significant increase in cell viability for MTA alone or combined with collagen over time ($P < 0.05$), while a significant rise in cell number over time occurred with MTA alone ($P < 0.05$). Three zones were identified around discs of each material after 1 day, namely: a cell-free zone, a zone of loosened round cells then a zone of normal cell growth; the cell-free zone narrowed and the intercellular spaces decreased after 3 days then, by the 7th day, the cell-free zone disappeared and the cells grew in multilayers.

Conclusions Mixing MTA with collagen gel may improve its early biocompatibility compared to mixing with sterile water; such addition might affect the outcome of MTA in its various clinical applications.

R120

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Dental pulp response to capping with MTA and β -tricalcium phosphate alone or incorporated in collagen

Aim To assess the inflammatory response and proliferative activity induced by MTA (ProRoot tm MTA, Dentsply Tulsa, USA) and β -tricalcium phosphate granules alone (β -TCP) [R.T.R[®] syringe, Septodont, France] or coated with collagen (β -TCP/C) [R.T.R[®] Cone, Septodont] in the dental pulp of dogs.

Methodology Sixty premolars in 12-to-18-month-old dogs were used. Class V cavities were prepared on the buccal surfaces of the teeth, the pulp was exposed and covered with one or other of the three materials ($n = 18$). Intact teeth were considered as controls ($n = 6$). Dogs were sacrificed after 1, 3 and 8 weeks ($n = 6$). Histopathological evaluation of inflammation was done in terms of inflammatory cellular infiltration, vasodilatation, tissue disorganization and hard-tissue formation. Area percentage of Ki-67 immunoreactivity was used to assess proliferative activity. Statistical analysis was performed using the Kruskal–Wallis test followed by Mann–Whitney test for pair-wise comparisons.

Results All the materials were associated with early mild-to-moderate inflammatory cell infiltration and vasodilatation, which significantly resolved over time ($P < 0.05$); no differences were observed among the materials at each time point. MTA had significant recession in tissue disorganization and more complete dentine bridge formation starting from 3 weeks onwards ($P < 0.05$); β -TCP materials induced only deposition of dentine calcifications. MTA had significantly higher proliferative cellular activity than the control and β -TCP materials at all time points ($P < 0.05$). β -TCP materials had significantly higher proliferative cellular activity than the control starting from 3 weeks ($P < 0.05$); β -TCP/C was associated with significantly higher proliferative cellular activity than β -TCP only at the 3-week time point ($P < 0.05$).

Conclusions MTA and β -TCP materials had similar biocompatibility to dental pulp, yet, MTA had a higher potential to completely seal pulpal exposures. Collagen addition to β -TCP did not seem to have affected its biological properties.

R121

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Fracture resistance of simulated immature teeth filled with bioactive materials: Tech Biosealer apex, Biodentine and MTA

Aim The aim of this *in vitro* study was to evaluate the effects of ProRoot MTA, Biodentine and TechBiosealer apex on fracture resistance of simulated immature teeth.

Methodology Forty-six maxillary anterior teeth were used. Access cavities were prepared and the apical parts of the roots were sectioned 9 mm below the cemento-enamel junction. All root canals were instrumented with rotary files. To simulate an immature tooth

a no. 6 peeso reamer was introduced until it passed 1 mm beyond the apex. Calcium hydroxide paste was introduced into the root canals. After 7 days, root canals were filled with TechBiosealer apex in group 1, with Biodentine in group 2 and with ProRoot MTA in group 3. A compressive load at a crosshead speed of 5.0 mm min⁻¹ was delivered at a point 3 mm above of lingual CEJ. The maximum fracture loads were recorded in Newton (N) and evaluated statistically using Kruskal–Wallis and Hollander–Wolfe tests ($P = 0.01$).

Results The positive control group had the lowest fracture resistance ($Z > \pm 2.58$). There was no significant difference between the experimental groups and the negative control group ($Z < \pm 2.58$). All the materials had reinforcing effect on simulated immature teeth.

Conclusions Under the conditions of this *in vitro* study, Biodentine and TechBiosealer apex had similar fracture resistance to ProRoot MTA.

R122

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Micro-CT volumetric solubility and chemical characteristics of root-end filling cements

Aim To evaluate the volumetric solubility and chemical characteristics of 4 root-end filling materials with alternative radiopacifiers and vehicle in comparison with MTA Angelus.

Methodology Three calcium silicate-based cements [white Portland cement (PC) with 30% zirconium oxide (ZO); PC with 30% calcium tungstate (CT); MTA (Vitalcem)] and an epoxy resin-based cement (Sealepox RP) were tested in comparison with MTA Angelus. Sixty acrylic teeth with root-end cavities filled with the materials ($n = 12$) were scanned twice by Micro-CT, before and after immersion in flasks containing 10 mL ultrapure water for 7 days. The digital data was reconstructed and the volume of the samples was obtained by using CTan software. The root-end fillings of the acrylic teeth were also analysed by scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDX) after setting and repeated 28 days after immersion in ultrapure water. Statistical analysis was performed using the Anova and Tukey's tests ($P < 0.05$).

Results MTA Vitalcem had the highest solubility values in comparison with all the materials ($P < 0.05$). Sealepox RP had the lowest values of solubility with significant differences in comparison with MTA Vitalcem and PC with 30% CT ($P < 0.05$). SEM–EDX analysis displayed morphological changes after 28 days immersion with dissociation of radiopacifier particles and other surface elements from the cements.

Conclusions All the materials had values complying with the minimum solubility requirements and presented similarity in comparison to MTA Angelus, with the exception of MTA Vitalcem. The chemical changes were observed in all materials after 28 days of immersion independently of their composition.

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R123

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Push-out bond strength of MTA canal filling with and without a single-cone

Aim To compare the bond strength of mineral trioxide aggregate (MTA) canal fillings with/without single cone.

Methodology Fifty one single-rooted human mandibular premolars were shaped with Ni-Ti rotary files to a size 50 and irrigated with NaOCl, EDTA and distilled water. Roots were randomly divided into 3 groups ($n = 17$). In Group 1, all canals were filled with MTA. In Group 2, canals were filled with a single cone technique (size 50, 0.02 taper) and MTA sealer. In Group 3, canals were filled with lateral condensation of cold gutta-percha using AHPlus sealer. After storing in an incubator for 1 week, each root was sectioned horizontally from apical, middle and coronal third of the root canal to obtain 3 slices of 1 mm \pm 0.1 mm. In all, 153 slices were prepared for push-out tests to measure the bond strength between dentine and filling material. Data were analyzed using Kruskal–Wallis and Mann–Whitney *U* tests ($P < 0.05$).

Results In Group 1, push-out bond strengths at the apical third were significantly lower than the coronal and middle thirds ($P < 0.05$). In Group 2, the bond strengths in the coronal third was significantly greater than the middle and apical thirds ($P < 0.017$). No significant difference was detected between coronal, middle and apical slices in Group 3 ($P > 0.05$).

Conclusions Push-out bond strength of MTA changed according to its location in the root canal. Gutta-percha cones did not affect the push-out bond strength of MTA in the coronal third. On the other hand, they decreased the push-out strength of MTA in the middle and apical thirds. The amount of MTA may be a decisive factor in the push-out bond strength of canal fillings.

R124

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Influence of blood and saline solution in solubility, pH and ionic component of white MTA Angelus in simulated root-end fillings

Aim To investigate the influence of contact of blood and saline solution with white MTA Angelus on the solubility, pH level and ionic component.

Methodology Thirty acrylic teeth with a simulated root-end cavity 3-mm in depth were filled with white MTA Angelus. The specimens were divided into three groups ($n = 10$), according to the liquid used to immerse them. Group 1: control group (CG), left without liquid exposure; Group 2: samples immersed in 1 mL saline solution (EG-S); Group 3: samples immersed in 1 mL of fresh human blood (EG-B). Samples were immersed at 37°C for 24 h. For solubility analysis, the samples were scanned twice using a micro-CT scanner (SkyScan). Scanning was performed after immersion in 10 mL ultrapure water for 24 h and 168 h. Initial and final volumes were used to determinate the solubility. Ultrapure water was used to evaluate the pH at 24 and 168 h immersion. At the end of the storage period, the specimens were removed from the ultrapure

water, sectioned at 2-mm and dehydrated in a vacuum dissector. The sectioned specimens were analysed under scanning electron microscope (Aspex Corporation). Scanning electron micrographs of the different material microstructural components in back-scatter electron mode were captured and energy dispersive spectroscopy (EDS) was carried out. Statistical analysis for solubility and pH tests was carried out using ANOVA/Tukey tests ($P < 0.05$).

Results The EG-B group had the least solubility, followed by EG-S. No significant differences were noted for solubility among groups ($P > 0.05$). For pH, all groups had an increase in pH level from 24 to 168 h assessment. Experimental groups had significantly high pH in comparison with the control group ($P < 0.05$). In elemental analysis, MTA Angelus was composed of calcium (Ca) and silicon (Si) ions, with traces of aluminium (Al). A high concentration of Ca was found in the control group (42.8%). For Si, EG-S presented the highest percentage (16.5%).

Conclusions Blood and saline solution in contact with white MTA Angelus did not interfere in the solubility, but increased pH levels and resulted in small variations in elemental composition.

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R125

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Colour stability of mineral repair materials: an *in vitro* study

Aim The aim of this study is to assess the colour change induced by four mineral repair materials in contact with dentine and immersed in three solutions.

Methodology Four mineral materials (MTA Caps, Pierre-Rolland; ProRoot MTA, Dentsply; EndoSequence Root Repair Material syringe and RRM-putty, Brasseler) were inserted in the canals of six slices from single-rooted teeth (2 mm thick) that had been prepared with a size 6 Gates Glidden drill and irrigated with 17% EDTA solution. All samples were cured in 100% humidity for 48 h and then immersed in three solutions [Sodium Hypochlorite 2.5%; Artificial Saliva (Artisial[®]) and Pure Water]. Three slices without filling were immersed as controls. Pictures were taken on the side of each sample at 1, 3, 5, 7 days and 3 months. Different levels of grey of the materials and dentine were measured using Image J software. Results were analyzed with non-parametric Wilcoxon test and a 5% alpha risk.

Results Slight changes in the grey-levels of dentine and materials were observed in the MTA-Caps and EndoSequence group in contact with all solutions (<5% in average). Dentine tended to whiten in sodium hypochlorite except in the ProRoot group where colour was almost constant. No changes in colour were generated by the dentine in EndoSequence and MTA-Caps groups unlike the ProRoot group where the grey-level increased ($P < 0.0001$).

Conclusions The new generation of materials did not result in colour change unlike ProRoot-MTA in agreement with the studies of Camilleri and co-workers. They did not interfere either with dentine or any solution type.

R126

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Tooth discoloration analysis induced by two calcium silicate-based materials: 1 year *in vitro* study

Aim To evaluate over time the chromatic alterations/discolouration in tooth crowns induced by two Calcium silicate based-materials (CSMs) – White mineral trioxide aggregate (WMTA- ProRoot[®] MTA) and Biodentine[™] – applied in tooth pulp chambers.

Methodology Twenty-eight premolar human teeth were sectioned 2 mm below cemento-enamel junction (CEJ). Pulp tissues were extirpated through the cervical cut, and occlusal standardized cavities were prepared. The specimens were split according to a stratified random sampling by value into four groups: Group 1 – Negative control (filled with dry sterile cotton pellets) ($n = 4$); Group 2 – Positive control (filled with blood moistened sterile cotton pellets) ($n = 4$); Group 3 – WMTA (ProRoot[®] MTA) ($n = 10$); Group 4 – Biodentine[™] ($n = 10$). Colour measurements were recorded with a Colorimeter (PR[®]-650 SpectraScan[®] Colorimeter, USA) at 4 time points: baseline (T0), immediately after material placement (T1), at 6 weeks (T2) and 1 year (T3). The measurements were taken under standardized conditions: dark room and a white cardboard box illuminated with a LED light of 5500 K and 6 watts. Data were transformed into values of the CIE L*a*b* colour system, and the corresponding colour differences (ΔE) values were calculated. Between the measurements the specimens were stored in tap water, in the dark, in a 100% humidity environment at 37°C, with normal atmospheric gas levels. The results were analysed using repeated ANOVA measurements, unilateral t-Student test considering a cut point of 2.3, one-way ANOVA with Tukey *post-hoc* tests. The significance level was set at $\alpha = 0.05$.

Results No significant differences were found in ΔE between the two CSMs (group 3-WMTA and 4-Biodentine[™]), for each time interval. Relative to L* parameter it was possible to observe significant differences between WMTA and Biodentine[™], being the greater L* variation being associated with WMTA.

Conclusions Concerning global colour variation (ΔE) over time, no significant colour variations were detected between the two CSMs (WMTA and Biodentine[™]). However WMTA was associated with greater tooth discolouration in terms of value (L* parameter) than Biodentine[™].

FILLING: LEAKAGE

R127

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A comparison of the quality of root fillings using lateral condensation and greater taper single cone gutta-percha techniques

Aim The aim of this study was to evaluate the quality of root fillings performed with cold lateral condensation and greater taper single cone gutta-percha techniques.

Methodology Forty-eight single round root canals of human extracted teeth were prepared with ProTaperUniversal[®] (Dentsply Maillefer[®]) rotary instruments to a final size F3. The roots were then randomly divided into two experimental groups ($n = 16$) and two control groups ($n = 8$). The root canals were filled with

the cold lateral condensation technique (group A) or with the greater taper single cone (ProTaper[®] gutta-percha cones) technique (group B). For the negative control group, eight root canals were filled as in group A. All roots of this group were completely coated with nail varnish. Another 8 roots were filled using lateral condensation, but without any sealer, as the positive control. AH Plus[®] (Dentsply Maillefer[®]) sealer was used in groups A, B and in negative controls. A glucose leakage model was used for quantitative evaluation of the coronal-to-apical microleakage at 24 h, and then at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks. The data was analyzed statistically with the Mann-Whitney *U*-test ($P < 0.05$). Then, roots were horizontally sectioned at 2.5 mm from the apical foramen. The samples were metallographically prepared and their photomicrographs were taken. Using a computer program, the surface areas of the gutta-percha, sealer, and voids were calculated. The results were subject to the statistical analysis using the Mann-Whitney *U*-test ($P < 0.05$).

Results For the two experimental groups (A and B), the amount of leakage increased gradually throughout the test period. No significant difference in the cumulative amount of leakage was found between groups A and B at all observation time points. In the apical third of the canal, the percentage of gutta-percha, sealer and void-filled canal area was comparable with the cold lateral condensation and the single cone ProTaper[®] technique.

Conclusions In round canals, the quality of root fillings was comparable after using the cold lateral condensation and single cone ProTaper[®] techniques.

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FILLING: OTHER

R128

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Effect of calcium hydroxide dressing on tubular penetration of two different endodontic sealers: a confocal laser scanning microscopic study

Aim The aim of this study was to evaluate the effect of the calcium hydroxide dressing (CH) on tubular penetration of two endodontic sealers, AH Plus and MTA Fillapex.

Methodology Seventy-two mandibular premolars with a single root canal were prepared with rotary Profile .04 taper instruments to a size 40 and divided into four groups ($n = 18$). In two groups a CH dressing was placed for 15 days. The sealers were mixed with rhodamine B dye. The root fillings were performed with lateral condensation of gutta-percha associated with one of the sealers. The roots were sectioned transversally at apical (3 mm) and middle (6 mm) levels. The percentage of sealer penetration in the root canal walls and the percentage of impregnated dentine area in transverse sections were obtained using a confocal laser scanning microscopy. Statistical analysis was performed using one-way ANOVA and Games-Howell tests.

Results The CH dressing reduced the mean of tubular penetration in the middle third of the teeth filled with AH Plus

($P < 0.01$), while no difference was observed at the apical sections for both sealers.

Conclusions The CH dressing did not interfere the apical penetration of both sealers, however it decreased tubular penetration in the middle third of AH Plus root fillings. Overall, MTA Fillapex was associated with greater tubular penetration than AH Plus.

R129

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Thermo-mechanical properties, sealing ability and void measurements of a new carrier-based root filling: an *in vitro* study

Aim The aims of this study were to determine the dynamical thermo-mechanical behaviour of an experimental hydroxyapatite-polyethylene (HA/PE) root filling system and compare its sealing ability and void formation to the Gutta-Core system.

Methodology The experimental HA/PE composite was specifically designed via a melt-extrusion process and characterised. The thermo-mechanical properties of HA/PE were determined using a differential scanning calorimeter with a dynamic mechanical analyser used to determine viscoelastic properties. Human single-rooted teeth and simulated root canals were instrumented and filled with the experimental Hydroxyapatite-reinforced Polyethylene (HA/PE) or Gutta-Core(GC) system. The sealing ability was evaluated using a fluid filtration system at different time intervals. Micro-CT was also used to quantify the voids within root canal space. The data were statistically analysed by one-way ANOVA and *post hoc* comparison tests ($\alpha = 0.05$).

Results The experimental HA/PE had a melting point of 111–115°C. The viscoelastic behaviour of the composite revealed both modulus and damping changes with an increase in temperature and HA filler content. The fluid filtration of the experimental system revealed no significant differences ($P > 0.05$) compared with the GC system. The void volume percentage in the GC system was lower than that of the experimental HA/PE system; however, the difference was non-significant ($P > 0.05$).

Conclusions The experimental hydroxyapatite polyethylene carrier-based filling system exhibited comparable sealing ability and voids to the commercial Gutta-Core, offering an adequate root filling
Acknowledgements I would like to express my sincere thanks to the staff in Biomaterial and Tissue Engineering Department at King's College London Dental Institute for their technical support. Also, special thanks go to the Ministry of Higher Education and Scientific Research.

R130C.N. Carvalho^{1,*}, T2. Du, Y. Shen², G. Gavini³ & M. Haapasalo⁴¹University Center of Maranhão-UniCeuma, School of Dentistry, São Luís, Brazil, ²Division of Endodontics, Oral Biological and Medical Sciences, UBC Faculty of Dentistry, Vancouver, Canada, ³Department of Restorative Dentistry, School of Dentistry, University of São Paulo, São Paulo, Brazil & ⁴Department of Oral Biological and Medical Sciences, UBC Faculty of Dentistry, Vancouver, Canada**Effect of bioactive root filling materials on *Enterococcus faecalis* disinfection in dentine using a confocal laser scanning microscopy****Aim** To evaluate the antimicrobial effects of different gutta-percha and bioceramic sealers on *Enterococcus faecalis* biofilms in dentinal tubules after exposure of 7 and 30 days by viability staining and confocal laser scanning microscopy (CLSM).**Methodology** Dentine canals in 80 semicylindrical specimens were infected with *Enterococcus faecalis*. An equal amount of the materials was placed on the root canal wall of the specimens for 7 and 30 days according the groups ($n = 5$): OBT (Obtura pellets, Shoreline); GNB (experimental gutta-percha associated with niobium phosphate glass); GBC (EndoSequence BC gutta-percha, Brasseler); BC (EndoSequence BC sealer, Brasseler); BC + OBT; BC + GBC; BC + GNB and PBS (phosphate buffered saline). The proportions of dead and live bacteria inside the dentinal tubules after exposure to materials were assessed by viability staining and CLSM. In addition, the materials ($n = 3$) were immersed in distilled water and the pH was monitored for 30 days with and without human dentine powder and analyzed with ICP-OES (Inductively Coupled Plasma - optical emission spectrometry) to determine elemental release. Data were analyzed statistically by 2-way ANOVA and Tukey test at 5% significance level.**Results** Significantly more bacteria were killed in all groups at 7 days (OBT 10%; GBC 24%; GNB 42%; BC 29%; OBT + BC 27%; GBC + BC 31%; GNB + BC 42%) than in the control group (PBS 5%) ($P < 0.05$). Thirty days of exposure to groups GNB (45%), BC (35%), OBT + BC (32%), GBC + BC (36%) resulted in significantly more dead bacteria in dentine than 7 days, whereas no significant increase of the proportion of dead bacteria was detected between 7 and 30 days in groups PBS (5%), OBT (10%), GBC (23%) and GNB+BC (41%) ($P > 0.05$). GNB and GNB + BC resulted in significantly more dead cells at both experimental times than the other groups ($P < 0.05$). Dentine lowered the pH of all groups combined with BC and also for GBC, except for the GNB material that had its pH raised. BC and GNB had a higher level of calcium release than the other groups. GNB had the highest release of zinc at all time intervals ($P < 0.05$).**Conclusions** The GNB had superior antibacterial effects compared with the other groups.**Acknowledgements** This study was supported by FAPESP 2012/18220-4 and FAPEMA 00625/14.**R131**M. Pawińska^{1,*}, A. Kierklo², E. Luczaj-Cepowicz³, G. Marczuk-Kolada³ & K. Leszczyńska⁴¹Department of Conservative Dentistry, ²Department of Dentistry Propaedeutics, ³Department of Paedodontics & ⁴Department of Microbiology, Medical University of Białystok, Białystok, Poland**Antibacterial activity of root canal sealers against bacterial strains associated with symptomatic apical periodontitis – an *ex vivo* study****Aim** To investigate the antibacterial effect of various sealers on standardized strains of anaerobic bacteria: *Fusobacterium nuclea-**tum* ATCC 25586, *Porphyromonas gingivalis* ATCC 33277, *Pep-
tostreptococcus anaerobius* ATCC 27337.**Methodology** The materials tested in this study included AH Plus Jet (AH), Apexit Plus (AP), Endomethasone N (EN), Epi-phany (EP), GuttaFlow (GF), Roeko Seal Automix (RSA), Sealapex (SP) and Tubliseal (TS). The antibacterial activity of the freshly mixed sealers against the above mentioned strains of bacteria was evaluated with the use of the agar diffusion method. After inoculation with the bacteria and applying the materials, the agar plates were incubated at 37°C for 1 week in an atmosphere of 5–10% CO₂. To control the growth of the standardized strains on agar used for the experiment, positive control plates were streaked with bacteria, but no sealer was used. The results were obtained by measuring the diameter of the growth inhibition zone after 48 h, 72 h, 1 week (*F. nucleatum*) or 72 h, 120 h and 1 week (*P. gingivalis*, *P. anaerobius*) and analyzed statistically using one-way analysis of variance (ANOVA) followed by Tukey's test. A level of significance was set at $P < 0.05$.**Results** The mean of bacterial growth inhibition zones was significantly influenced by the type of material tested ($P < 0.001$). AH, EN, EP, SP and TS significantly inhibited all the bacterial strains over the experimental period. AP had a slight suppressive effect on *P. gingivalis* only. GF and RSA displayed no growth-inhibiting effect on any of the species investigated.**Conclusions** The antimicrobial activity of the sealers varied considerably depending on the type of material and the bacterial species tested. Most of the sealers had an antibacterial effect on the bacteria associated with symptomatic apical periodontitis.**R132**H.M. Bayram¹, E. Bayram¹, O. Atakol² & M. Kanber^{1,*}¹Department of Endodontics, Gaziosmanpaşa University, Tokat &²Department of Chemistry, Ankara University, Ankara**Atomic absorption spectrometry evaluation of calcium ion release from different calcium silicate-based endodontic materials used with new irrigants****Aim** The aim of the present study was to measure, using atomic absorption spectrometry (AAS), the concentrations of calcium ions released from three calcium silicate-based materials, white MTA (WMTA), Bioaggregate (BA), and Biodentine, after their immersion in 0.2% chitosan, 10% propolis, 1% acetic acid, and 17% EDTA solutions and also in distilled water.**Methodology** One hundred and fifty silicon tubes (10 mm in length and 2 mm in internal diameter) were prepared. The tubes were randomly divided into three groups and filled as follows. Group I: MTA, Group II: BA Group III: Biodentine. After that, the set material was removed from the silicon tube. Each group was then randomly divided into five subgroups of 10 samples each ($n = 10$). For subgroup 1, a fresh 0.2% chitosan solution was prepared. For subgroup 2, 10% propolis solution was prepared. Subgroup 3 was treated with distilled water. For subgroup 4, 1% acetic acid was used. In subgroup 5, 17% EDTA was used. The samples were immersed in the test solutions for 10 min. AAS (GBC-GF; model Avanta PM) was used to determine Ca ion release. The data were analysed using the SPSS 20 software. The Kruskal–Wallis test was applied, and $P < 0.05$ was defined as statistically significant.**Results** The lowest Ca release values were observed in distilled water, while the highest values were found in the samples incubated in the EDTA solution; there are no significant differences between propolis, chitosan, and distilled water.**Conclusions** Chitosan and propolis natural irrigation solutions may be preferred to EDTA, when used with MTA, BA, and Biodentine.

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R133

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Ex vivo comparative evaluation of the sealing ability of three filling methods in severely curved root canals

Aim The aim of this study was to evaluate the quality of root fillings in curved canals, *ex vivo*.

Methodology Thirty mesial roots of mandibular molars (angle of curvature > 35°) and 36 independent root canals were used. All canals were instrumented with BioRaCe instruments to size 35, 0.04 taper., and were randomly divided into 3 groups: Group A: filled with lateral compaction, Group B: filled with System B/Obtura, and Group C: filled with Thermafil. The roots were embedded in acrylic resin and sectioned with a microtome at 0, 3 and 5 mm from working length. Sections were observed in a stereomicroscope (×6) and analyzed with Image J. The total area of voids in the root canal and the maximum penetration of filling materials in isthmuses were measured. For statistical analysis One-Way ANOVA, Kruskal–Wallis and Wilcoxon tests were used. The level of significance was set at $P < 0.05$.

Results Total area of voids (and the percentages) were $13\,636.399\,\mu\text{m}^2$ (3.45%), $13\,462.473\,\mu\text{m}^2$ (4.49%), $16\,572.794\,\mu\text{m}^2$ (7.64%) for group A, B and C, respectively. There was no significant difference among the groups. Cross-sections at 0 mm produced significantly greater total area of voids than cross-sections at 5 mm. Group A demonstrated the greatest heterogeneity. In group B, a homogenous mass of sealer and gutta-percha was noticed. In group C total or partial denudation of the carrier was observed. Penetration of filling materials into isthmuses was deeper in group B.

Conclusions Under the conditions of this study no difference in the quality of root fillings was found among the examined methods, however, group B demonstrated deeper penetration of filling materials into isthmuses.

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R134

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Canal filling in curved roots treated with simplified techniques and restored with a fibre post: a microtomography study

Aim To evaluate quantitatively and qualitatively root fillings in curved roots using a single point technique and restored with a quartz fibre post.

Methodology Sample size was determined according to published data ($\alpha = 0.05$; $\beta = 0.20$; $\delta = 3.0$; $\sigma = 3.0$). Thirty-four single-rooted teeth with curved roots were cross-sectioned at the cervical level with a cutting disc. Canal curvature was measured with Pruett's technique with the aid of standardized radiographs. Root canals were shaped with size 40, 0.06 taper Reciproc files and rinsed with 5.25% sodium hypochlorite. For canal filling,

specimens were randomly divided into a control group (continuous wave of condensation technique, $n = 17$) and an experimental group (single point, $n = 17$). After post space preparation with a dedicated bur up to a depth of 7 mm, a No. 2 DT Light Post Illusion X-RO was luted with RelyX Unicem self-adhesive cement. Microtomographic scanning of the samples was used to calculate volumetric data of the filling volume, internal, external and combined voids. Statistical comparison between groups was performed with a Student's *t*-test. For qualitative analysis, three-dimensional reconstructions were obtained by means of dedicated software.

Results The mean percentage of canal filling was $96.45 \pm 2.55\%$ in the control group and $97.11 \pm 1.47\%$ in the single point group. Combined voids were the most prevalent type of voids, whereas minimal amounts of internal and external voids were observed. Voids were mainly distributed in the middle third of the roots. The difference between the two groups was not significant in relation to any volumetric parameter.

Conclusions The two filling techniques combined with DT Light Post Illusion X-RO cementation were similarly effective in filling curved canals. None of the techniques could avoid the formation of voids, which was nonetheless limited.

R135

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Comparison of two push-out test methods in measuring bond strength of calcium-silicate-based endodontic sealers

Aim To evaluate the dislocation resistance (push-out bond strength) of MTA Fillapex (Angelus), EndoSequence BC Sealer (Brasseler) and AH Plus (Dentsply) using two test methods.

Methodology The 'dentine disc' method included 11 dentine discs obtained from the middle thirds of eleven extracted third maxillary molars with fused roots. Three 1.5 mm large holes were drilled in each dentine disc and filled with the three sealers. The 'root thirds' method included 150 dentine discs obtained from the coronal, middle and apical third of 30 extracted anterior teeth, randomly allocated to 3 groups of 10 teeth. The root canal of each specimen was enlarged with a carbide bur to 1.5 mm in diameter. Discs of the same tooth were filled with the same sealer. Following setting, the push-out test was performed using a universal testing machine at a cross-head speed of $1\,\text{mm}\,\text{min}^{-1}$. Bond strength values (MPa) were calculated as force (N) divided by area (mm^2). Data were compared using two-way and one-way ANOVA with Turkey's *post-hoc* test ($\alpha = 0.05$).

Results Push-out bond strength values obtained by the 'dentine disc' method were $0.34 \pm 0.25\,\text{MPa}$ (MTA Fillapex), $2.45 \pm 1.38\,\text{MPa}$ (EndoSequence BC Sealer) and $6.46 \pm 0.89\,\text{MPa}$ (AH Plus). The 'root thirds' method resulted in $0.45 \pm 0.60\,\text{MPa}$ (MTA Fillapex), $3.43 \pm 2.54\,\text{MPa}$ (EndoSequence BC Sealer) and $8.22 \pm 5.36\,\text{MPa}$ (AH Plus). There were no differences between the two test methods for each sealer ($P > 0.05$). Both test methods revealed significant differences between the sealers ($P < 0.05$). AH Plus had significantly higher values in the coronal ($11.02 \pm 3.48\,\text{MPa}$) than the apical third ($5.45 \pm 4.39\,\text{MPa}$) ($P < 0.05$). Predominantly mixed failures were observed for both test methods.

Conclusions Although both test methods gave comparable results for each sealer, the 'dentine disc' method resulted in less dispersive data. Two calcium-silicate-based sealers performed worse in terms of bond strength compared to the resin-based AH Plus.

R136

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Micro-CT assessment on the effect of three root filling techniques on dentinal cracks

Aim This study evaluated the frequency of dentinal micro-cracks observed after root canal filling with GuttaCore (GC), cold lateral compaction (CLC) and warm vertical compaction (WVC) techniques through micro-computed tomographic analysis.

Methodology Thirty mesial roots of human mandibular molars (type II Vertucci canal configuration) were instrumented with Reciproc R40 at the working length. The sample was randomly assigned to 3 experimental groups ($n = 10$) according to the canal filling technique. GC group was filled with a size 40 GC obturator, while CLC and WVC used conventional gutta-percha cones. AH Plus root canal sealer was used for all groups. The specimens were scanned, at an isotropic resolution of 14.25 μm , before and after root canal obturation. Then, all pre- and postoperative cross-section images of the roots ($n = 41\ 660$) were screened to identify the presence of dentinal defects.

Results Dentinal micro-cracks were observed in 37.35% ($n = 5020$), 31.98% ($n = 4778$) and 7.23% ($n = 3012$) of the cross-section images in GC, CLC and WVC groups, respectively. Therefore, 30.75% ($n = 12\ 810$) of the images displayed some dentinal micro-cracks. However, all micro-cracks identified in the postoperative scans were already present in the corresponding preoperative images.

Conclusions Root canal filling with GC, CLC and WVC techniques did not induce the formation of new dentinal micro-cracks.

R137

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An observer-independent microcomputed tomography study of the voids associated with the lateral compaction and single cone techniques

Aim (i) To develop an observer-independent and reproducible three-dimensional image-analysis protocol for the evaluation of the porosity of root fillings by means of microcomputed tomography (μCT). (ii) To evaluate and compare the porosity (void%) of canals filled with the lateral compaction and single cone techniques in single rooted teeth using this protocol.

Methodology Twenty human canines with single straight root canals were instrumented according to a standard protocol up to a size 40, 0.06 taper and scanned by μCT (Scanco Medical, μCT 40) at 10 μm voxel size. The specimens were then randomly allocated to 2 equal groups ($n = 10$) and filled with a calcium silicate sealer and either lateral compaction of gutta-percha or a single tapered gutta-percha cone. The specimens were re-scanned after complete setting of the sealer. Pre- and post-obturation scans were spatially co-aligned. Subsequently, dentine and filling materials were independently segmented. The void% in each filled canal was quantified by morphometric operations and the two

groups were compared by non-parametric statistical tests (Mann-Whitney U).

Results The protocol provided a reproducible volumetric estimation of the voids associated with both treatment techniques. Significantly less void% were associated with the single cone than with the lateral compaction technique [median: 0.445% (interquartile range: 0.168–0.825%) versus 3.095% (1.027–5.072%), $P = 0.001$, $r = 0.727$].

Conclusions An observer-independent, reproducible three-dimensional image analysis protocol for evaluating the porosity of root fillings in μCT scans was developed. The single cone was associated with less porosity than the lateral compaction technique and may thus represent a promising method that could benefit the long-term outcome of root canal treatment.

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R138

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Comparison of void presence and sealer penetration into dentinal tubules when using a bioceramic sealer or an epoxy resin sealer with two filling techniques: an *in vitro* study using confocal laser microscopy

Aim To compare the presence of voids and sealer penetration with two sealer, AH Plus with conventional gutta-percha (GP) and Endosequence bioceramic (BC) with BC GP, as well as their penetration into the dentinal tubules, when used with two filling techniques, single cone or continuous wave of condensation (CWC).

Methodology The root canals of eighty single-rooted teeth were instrumented to size 35, 0.06 taper using the Mtwo system and irrigation with NaOCl and EDTA activated with an ultrasonic tip. The specimens were randomly divided into 4 groups. G1: Filled by CWC using AH Plus as the sealer and GP, G2: a single GP cone was used with AH Plus, G3: CWC using BC sealer and BC GP, G4: Single cone with BC sealer and BC GP. All samples were analysed in the apical (3 mm), middle (5 mm) and coronal (8 mm) thirds using confocal laser microscopy. One-way ANOVA was used to test for differences among the experimental groups.

Results Sealer penetration analysis revealed significant differences between G3 and G1 as well as between G3 and G2 concerning the presence of voids at 3, 5 and 8 mm ($P < 0.001$), and penetration of the sealer at 3 ($P < 0.018$) ($P < 0.001$) and 5 mm ($P < 0.001$) ($p < 0.001$), respectively.

Conclusions BC sealer and BC GP using CWC were associated with smaller voids and greater sealer penetration into the dentinal tubules than either of the AH Plus groups.

R139

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In vitro bioactivity test of five calcium silicate-based endodontic materials immersed in a phosphate-containing solution

Aim To evaluate the apatite-forming ability of TotalFill Root Repair Material (RRM), Biodentine, RetroMTA, MTA Fillapex and ProRoot MTA after immersion in phosphate-buffered saline (PBS),

analysing: amount, morphology and elemental composition of the precipitates.

Methodology Fifty human dentine discs with standardized canals, were divided randomly into 5 groups ($n = 10$). The specimens were filled with TotalFill RRM Putty, Biodentine, RetroMTA, MTA Fillapex and ProRoot MTA and immersed individually in plastic vials containing Ca and Mg-free PBS for 1 week ($n = 5$ of each group) and for 1 month ($n = 5$ of each group). All specimens were incubated at 37°C and the PBS was replaced every 7 days. Morphology and elemental composition of precipitates on the surface of materials were analysed using scanning electron microscopy with energy dispersive X-ray (SEM-EDX). In addition, the amount of precipitate was weighed. For statistical analysis, the Kruskal–Wallis test were used to compare the ratio Ca/P between groups for each period of time. Two and two comparisons were done with Duncan's test. In all the case for a value $P < 0.05$.

Results The formation of precipitates following 1 week and 1 month of immersion in PBS, was observed on the surface of all materials, except MTA Fillapex. After 1 month, the amount of precipitate increased substantially (range 3.5x–4.5x), being Biodentine and TotalFill RRM Putty which produced the greatest amount of precipitate (4.1x and 4.5x respectively). SEM-EDX revealed different surface morphologies depending on the material and the soaking time, with predominance of acicular precipitates. All precipitates were associated with high peaks of O, Ca, and P. The precipitates of Biodentine and TotalFill RRM Putty showed higher Ca/P ratio at week, being Biodentine which was significant different compared to other materials ($P < 0.05$). No significant differences were found between materials at month.

Conclusions Precipitates of apatite on the surface of TotalFill, Biodentine, RetroMTA and ProRoot MTA after being immersed in PBS, demonstrate that these materials are bioactive. MTA Fillapex did not form precipitate, indicating a lack of bioactivity. The greater amount of precipitate of TotalFill and Biodentine suggest they are highly bioactive.

R140

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Effects of EDTA and Er:YAG laser treatment on bond strength of three root canal sealers

Aim The aim was to evaluate the effects of 18% EDTA solution and Er:YAG laser irradiation on the bond strength of three different materials for root filling: AH Plus sealer, Acroseal sealer and RealSeal SE system.

Methodology The root canals of 180 extracted single-rooted human mandibular premolars were prepared with BioRaCe rotary instruments. The teeth were divided into 3 groups according to the filling material: AH Plus with gutta-percha, Acroseal with gutta-percha and RealSeal SE system. This three groups were divided into two subgroups according to the technique of smear layer removal (18% EDTA or Er:YAG laser). The filled roots were cut perpendicular to their long access to create 1-mm thick slices from coronal thirds. The bond strength was measured with a Push-out testing machine. The values were analyzed by one-way analysis of variance (ANOVA).

Results In the AH Plus sealer/gutta-percha group, higher adhesion values occurred when EDTA was used (1.47 ± 0.13 MPa) compared to the Er:YAG laser method (0.95 ± 0.09 MPa) with a significant difference ($P < 0.05$). In the Acroseal sealer/gutta-percha group, the adhesion of material after smear layer removal with EDTA solution (0.75 ± 0.06 MPa) was also better in comparison to the laser method (0.52 ± 0.04 MPa). The comparison

of two methods for smear layer removal on the adhesion in RealSeal SE group, EDTA (1.54 ± 0.20 MPa) and Er:YAG laser (1.22 ± 0.13 MPa), revealed no significant difference.

Conclusions Application of EDTA solution gave significantly better results of adhesion in samples filled with AH Plus and Acroseal sealer with gutta-percha. In samples filled with the RealSeal SE system the smear layer removing technique did not affect the adhesion of material to dentine walls.

R141

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Push out bond strength of new root-end filling materials. An *ex vivo* study

Aim The purpose of the present *ex vivo* study was to evaluate the bond strength of two recently introduced root-end filling materials using the push out test. The materials examined were MTA Caps (Acteon, Pierre Rolland), Biodentine (Septodont, Saint Maur des Fosses, France) and a more classic root-end filling material, Intermediate Restorative Material (IRM, Dentsply De Trey).

Methodology Seventy-five recently extracted single-rooted human teeth were collected for the study. According to the guidelines of root-end resection, the apical 3 mm of each root were removed. Then each specimen was cut perpendicular to its long axis with a diamond-coated slow-speed band saw (Isomet, USA) in order to obtain a homogeneous dentine slice of 2 mm width. Peeso – Reamers No 1, 2, 3 were used to prepare a cavity of 1.3 mm in diameter in each slice. The samples were divided into three groups ($n = 25$) according to the filling material used (Group A: MTA Caps, Group B: Biodentine, Group C: IRM). After incubation of 7 days, the push out test was conducted using the Ultra-tester push out testing machine (Ultradent Products Inc). Finally, the specimens were examined under the stereomicroscope at 40× magnification to evaluate the bond failure modes. One way ANOVA model (IBM SPSS Statistics 20.0) and the *post hoc* Bonferroni test were used for the statistical analysis, with a significance set at $P < 0.5$.

Results MTA Caps had significantly lower push-out bond strength (mean 5.58 ± 3.93) than IRM (mean difference 5.42, 95%CI: 2.00–8.84, $P = 0.001$) and Biodentine (mean difference 3.74, 95%CI: 0.32–7.16, $P = 0.027$). In contrary, there was no significant difference between Biodentine (mean 9.32 ± 5.35) and IRM (mean 10.99 ± 5.39).

Conclusions MTA Caps had the lowest bond strength values as a root-end filling material while Biodentine had similar results to IRM.

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R142

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Influence of different agents for smear layer removal in the wettability of a zinc oxide-eugenol-based sealer

Aim To assess the influence of two different solutions used for smear layer removal on the wettability of a wide spread used zinc oxide-eugenol-based endodontic sealer, Kerr Pulp Canal Sealer.

Methodology Twenty flattened dentine slices, corresponding to the cervical third of the root, were prepared and used. They were

irrigated with 6% NaOCl simulating the irrigation used during chemomechanical preparation, and then washed with distilled water. After this procedure, the samples were divided into 2 groups according to the solution used for smear layer removal: group 1, 17% EDTA; group 2, 37% phosphoric acid. The solutions were applied for 3 min (1 mL per minute). After the treatments, the samples were dried in nitrogen gas and attached to a glass base. A goniometer was used to measure the contact angle between chemically treated surfaces and the Pulp Canal Sealer. The sealer dynamic wettability was accompanied and computed during 1 minute (sixty measurements with 1 s interval between each measurement). The formula used to evaluate the sealer dynamic wettability (%) was $(\text{initial angle} - \text{final angle}) \times 100/\text{initial angle}$. Data were analyzed statistically by the *t*-test ($P < 0.05$).

Results The dynamic wettability values (Mean \pm standard deviation) of dentine treated using phosphoric acid and EDTA were 21.76 ± 10.9 and 12.81 ± 9.4 respectively. Statistical analysis revealed a difference between phosphoric acid and EDTA.

Conclusions The experimental method revealed that different solutions used to remove the smear layer may exert an influence in the spread of sealers. 37% phosphoric acid improved the wettability of Pulp Canal Sealer when compared with 17% EDTA.

R143

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A comparison of bond strength to radicular dentine of Resilon/RealSeal SE and EndoSequence BC Sealer/BC Points, using a push-out test

Aim To evaluate and compare the push-out bond strength to radicular dentine walls, of two endodontic filling systems, which both claim to offer a true monoblock filling of the root canal space.

Methodology Twenty eight single-rooted human teeth with circular canals were selected. The root canals were prepared using a reciprocating system, with WaveOne Large File, and irrigated with NaOCl 5.25%, EDTA 17% and saline solution as the final rinse. The teeth were randomly divided into two groups, and root filled according to the manufacturer's instructions as follows: RealSeal SE and Resilon, using the vertical compaction technique (Group 1); EndoSequence BC Sealer and BC Points, using the single cone technique (Group 2). Teeth were stored for 1 week in a moist environment using SBF solution and then embedded in acrylic resin. For the push-out test, teeth were transversally sectioned with a microtome, into 1 mm serial slices from the apex to the coronal part of the tooth. Each slice was examined, with a stereomicroscope, in order to observe and exclude damaged slices, and those with oval canals. The push out load was applied using a universal mechanical testing machine, with cylinder pistons of 0.3–0.7 diameter, and a constant load of 0.5 mm min^{-1} , in an apical-coronal direction. Bond failure was manifested by the loss of adhesion, at the interface sealant/dentine or sealant/core material, and the extrusion of the root filling. Failure forces were recorded for each slice. The data were analysed using the Student *t*-test, with the significance level set at $P < 0.05$.

Results Push out strength was significantly influenced by the canal filling system. The EndoSequence BC Sealer/BC Points group had significantly higher bond strengths than the RealSeal SE/Resilon group ($P \leq 0.05$).

Conclusions Adhesion of root canal filling materials to root canal dentine is crucial for the long term prognosis of root filled

teeth. EndoSequence BC Sealer and BC Points had a better adhesion to dentine walls than RealSeal SE and Resilon.

EVALUATION OF A TECHNIQUE/ MATERIALS

R144

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Comparison of two contemporary rotary systems in a preclinical student course setting

Aim To avoid the limitations of the common one-operator shaping approach, the aim of this study was to assess two contemporary rotary instrumenting systems subjectively and objectively in a preclinical student course setting.

Methodology Undergraduate dental students ($n = 44$) prepared mesiolingual canals of 3D-printed mandibular molar replicas (RepliDens). The six-file systems HyFlex and BioRace, both previously unknown to the students, were used under concealed designation according to manufacturers guidelines. Half of the students used either system first. A first-generation rotary system (ProFile .04) that the students knew from their previous education was then used to instrument the mesiolingual canal in a third 3D-printed molar. Questionnaires were issued to note subjective experiences immediately after instrumentation. Time to instrument to size 40, 0.04 taper and shaping outcomes were analysed. Categorical data was compared using Chi-squared and Fisher's exact tests, numerical data according to goodness of fit to the normal distribution, $P < 0.05$.

Results Subjectively, the students liked the file size and sequence designation in the BioRace system significantly ($P < 0.05$) better than in the HyFlex counterpart, whilst they found better controllability with the HyFlex ($P < 0.05$). Objectively, canal transportation was significantly less with the Hyflex (and the ProFile) systems compared to BioRace ($P < 0.05$), whilst both systems were similar in terms of procedural mishaps, length control, and instrumentation time.

Conclusions The systems under investigation both had their advantages and disadvantages, which may not be revealed by a simple instrumentation study.

R145

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Five year analysis of undergraduate education using tapered hand and rotary endodontic instrumentation techniques

Aim To evaluate the effectiveness of an undergraduate endodontic teaching module using NiTi tapered hand and rotary instrumentation over 5 years.

Methodology Fourth year BDS students (2009–2013) who had learned a crown-down step-back hand preparation technique in third year completed a preclinical module using ProTaper Universal tapered hand (PTH) and rotary instruments (Maillefer Dentsply). Evaluation of effectiveness included self, peer and teacher formative assessment, summative assessment and an anonymous

questionnaire about perceptions, clinical experience and understanding. Quantitative analysis used Chi square and Fisher's exact statistics.

Results The module was completed by 390 students and over 5 years. The questionnaire response rate was 86% with the module viewed as reinforcing endodontic understanding and updating practice. Live demonstrations of the tapered hand and rotary techniques were useful. Instrumentation of acrylic blocks and natural teeth with both techniques were described as 'easy' and 'efficient'. Some initial challenges were identified learning balanced-force techniques (PTH) and maintaining length control with rotary files. Students reported improved clinical confidence (70%) and an enhanced interest in endodontics (89%). There were significant relationships between perceived knowledge prior to the module and interest ($P = 0.001$) as well as clinical confidence ($P < 0.001$). Premolars were the commonest teeth treated. Over the 5 years treatment of premolars and molars increased (6% and 17% respectively). Students were reflective in critically evaluating their work. Summative grades increased by 5% for clinical practice and 12% for theoretical understanding.

Conclusions The module was effective in educating the use of tapered hand and rotary instrumentation and reinforced endodontic understanding. Uptake of the techniques by recent graduates involved in the module requires investigation.

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R146

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Cytotoxicity and genotoxicity of materials used in vital pulp therapy on Chinese hamster ovary (CHO) cells

Aim The purpose of this study is to evaluate the cytotoxicity and genotoxicity of materials used in vital pulp therapy on Chinese hamster ovary (CHO) cells using the MTT assay and micronucleus assay.

Methodology Cultured Chinese hamster ovary cells (CHO-K1) were treated with 100 μL of 10 $\mu\text{g mL}^{-1}$ tested materials, Dental Formocresol (Murakami, Japan), Calcipect II (Nishika, Shimonoeki City, Japan), ProRoot MTA (Dentsply, Tulsa, OK, USA) and ENDOCEM MTA (Maruchi, Wonju, Korea) for 24 h. Then, cell viability was determined by MTT assay ($n = 9$). Control groups in both assays were treated with the medium only and the positive control group in the micronucleus assay was treated with 1 $\mu\text{g mL}^{-1}$ methyl methanesulphonate (MMS). For the micronucleus assay ($n = 5$), the cultured cells were exposed to 10 $\mu\text{g mL}^{-1}$ of tested materials and 4 $\mu\text{g mL}^{-1}$ of cytochalasin B and incubated for 24 h. Then, the cells were harvested, fixed and stained for counting the micronuclei formed. Cell viability observed in MTT assay and nuclear division index (NDI), micronucleus frequency (MNF) calculated from the data acquired in micronucleus assay were analyzed by one-way ANOVA and Tukey HSD test at 0.05 significance level.

Results Cell viability rates observed in MTT assay were the highest in Calcipect II, ENDOCEM MTA, followed by ProRoot MTA and the control group and the lowest in Dental Formocresol ($P < 0.001$). MNF determined in the control group recorded the lowest value followed by ProRoot MTA and ENDOCEM MTA, then Calcipect II, MMS group, respectively. Dental Formocresol revealed the highest MNF value ($P < 0.001$). ProRoot MTA and the control group appeared to have similar NDIs ($P > 0.05$) and recorded the highest values. ENDOCEM MTA had similar NDI values to ProRoot MTA ($P > 0.05$) followed by the Calcipect II group. NDI

values observed in Dental Formocresol and MMS groups were the lowest and closest to 1, which means cells were more in cytostasis ($P < 0.001$).

Conclusions Dental Formocresol was most cytotoxic and genotoxic among the materials tested. Generally, ProRoot MTA and ENDOCEM MTA had similar results in the MTT and micronucleus assays and appeared to be less cytotoxic and genotoxic than the other materials used for vital pulp therapy.

R147

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Cytotoxicity of two bioactive root canal sealers

Aim The aim of this study was to investigate the cytotoxicity of two different bioactive root canal sealers: one based on mineral trioxide aggregate, MTA Fillapex (Angelus, Solucoes Odontologicas, Londrina, PR, Brazil), and the other based on bioceramics, Endosequence BC Sealer (Brasseler, Savannah, Georgia, USA), in culture of mouse L929 fibroblasts.

Methodology Mouse (L929) fibroblasts, obtained from subcutaneous connective tissue of mouse line C3Hf, were cultivated in plastic culture flasks in an incubator at temperature of 37°C, with 5% CO₂ and 90% of humidity. Freshly mixed materials (0.1 g), Endosequence BC Sealer and MTA Fillapex, were placed on sterile teflon discs, 6 mm in diameter. Teflon discs with the materials as well as empty discs, serving as a control group, were placed in wells of 12-well plate. After incubation time of 1, 6, 20 and 24 h, the teflon discs were removed from the wells and the number of viable cells was determined using trypan blue in Neubauer chamber. Mann-Whitney *U* test was used for statistical analysis and the level of significance was set at 5%.

Results There was a significant difference in the number of viable cells between the Endosequence BC Sealer and MTA Fillapex ($P \leq 0.05$). In comparison to the control group, MTA Fillapex had significantly less viable cells for all incubation periods ($P \leq 0.05$), while Endosequence BC sealer had significantly less viable cells from after 6, 20 and 24 hours of incubation ($P \leq 0.05$). MTA Fillapex exhibited significantly less viable cells in comparison to Endosequence BC sealer after the first hour and after 20-h hours of incubation ($P \leq 0.05$), while for the other incubation periods there was no significant difference ($P \geq 0.05$).

Conclusions MTA Fillapex and Endosequence BC sealer were both cytotoxic in cultures of mouse L929 fibroblasts.

R148

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Influence of keratin preparations on cementoblast OCCM-30 and fibroblast L929 cells

Aim Regeneration of the periradicular tissues is a goal in root canal treatment and the development of regenerative type materials is critical in achieving this. The aim of the study was to assess the effect of novel keratin preparations on important cellular

functions for tissue regeneration, namely cell viability and proliferation of cells from the periradicular region.

Methodology Cementoblast OCCM-30 cells were grown in Dulbecco's modified Eagle's medium and fibroblast L929 cells in minimum essential media with 10% FBS and 1% antibiotic-antimycotic. Cells were assessed for viability and proliferation in growth media supplemented with 10, 1 or 0.1 mg mL⁻¹ of keratin preparation using a LIVE/DEAD assay™ Kit and alamarBlue® proliferation assay. Experiments were replicated three times. For mineralisation the SensoLyte® pNPP alkaline phosphatase (ALP) assay was performed for cementoblasts grown with 1 or 0.1 mg mL⁻¹ keratin at 3, 6, and 10 days and Alizarin red assay was performed at 14 days to evaluate calcium-rich deposits in the media. Experiments were replicated twice. Growth characteristics in media with no added keratin acted as controls. ANOVA and Tukey's multiple comparison test were used for statistical analysis.

Results Fibroblast L929 cells remained viable and underwent proliferation at keratin concentrations below 1 mg mL⁻¹, at and above this level proved toxic to cells. Cementoblast OCCM-30 cells had similar viability and proliferation to the control group for keratin concentrations 1 mg mL⁻¹ and below ($P > 0.05$), above this proved toxic to cells. Mineralisation assays revealed that cells grown with keratin at 0.1 mg mL⁻¹ had similar ALP and alizarin red levels as controls ($P > 0.05$). In contrast, 1 mg mL⁻¹ of keratin had significantly lower ($P < 0.0001$) markers for mineralisation.

Conclusions Keratin preparations at low concentrations allowed normal cell proliferation of fibroblast L929 cells and cementoblast OCCM-30 cells and mineralisation functions of cementoblast OCCM-30 cells.

R149

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Quality of *in vitro* root fillings completed by undergraduate dental students using four different file systems

Aim To evaluate the treatment quality of *in vitro* root canal treatments done by four undergraduate students using four different file systems.

Methodology Four operators instrumented and root filled 5 single-rooted teeth with each of four different file systems (K-Flex handfiles, ProTaper Universal, ProTaper Next, and WaveOne) ($N = 80$). Time spent on access, canal preparation, and root filling was registered separately. Digital radiographic images of all teeth were obtained in different 2 directions: bucco-lingual and mesio-distal. Root filling quality was evaluated according to length (long, adequate, short) and density of the root filling on a four-level scale (1–4). All images were evaluated twice by six individual observers. The 24 evaluations of each tooth were combined to a proportion of adequate evaluations. Analysis of variance methodology was used to analyze the proportions of adequate evaluations and the time spend on access, preparation and root filling.

Results The proportion of root fillings with adequate density varied among the operators ($P = 0.004$) and file systems ($P = 0.03$). Root fillings performed after hand instrumentation had significantly more voids than after motor driven instrumentation ($P = 0.02$), but the motor driven systems were not significantly different ($P = 0.20$). The proportion of adequate length root fillings varied among the operators ($P = 0.02$), but not among file

systems ($P = 0.87$). Regardless of operator and file system there was a significant improvement of root filling quality (length: $P = 0.03$, density: $P = 0.02$) and a decrease in the duration of operation time with increasing number of teeth treated by the operator. The decrease in operation time was mainly caused by a decrease in time spent on canal preparation and root filling. These improvements indicate a learning curve for the procedures, peaking around tooth number 10, regardless of operator and file system used.

Conclusions Root fillings performed after hand instrumentation resulted in lower quality root fillings compared to root fillings performed after motor driven instrumentation. Root filling quality gradually increased and all operators improved their skills and working speed from first to last tooth.

R150

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Radiographic evaluation of root filling quality: conventional lateral compaction versus GuttaCore®

Aim The aim of this study was to investigate the radiographic quality of root fillings performed by students in preclinical laboratories, comparing conventional lateral compaction (LC) and GuttaCore® (GC).

Methodology Digital radiographs of extracted teeth that had been root filled were obtained. Root fillings were performed using two techniques: LC and GC. Teeth with procedural errors such as perforation, ledge and irretrievably broken instruments were excluded. Each canal was graded by three calibrated endodontic residents based on: (i) Length, (ii) Homogeneity, and (iii) Adaptation and taper. Generalized linear mixed model (GLMM) was used for statistical modelling.

Results Seven hundred and thirty seven root fillings from 473 teeth were reviewed and graded. Two hundred canals were filled using GC and 537 canals using LC. Root fillings with satisfactory length were found in 31% of the GC group and 42.5% of the LC group. 50.5% of GC and 45.9% of LC root fillings were dense and homogenous throughout the entire length of canal. 44.0% and 39.1% of root fillings, performed using GC and LC respectively were found to have satisfactory taper and adaptation to the canal wall. The order in which techniques were learnt and the choice of technique did not affect the result significantly. In terms of homogeneity and taper, no significant difference ($P = 0.157$ and $P = 0.202$) was found between GC and LC. However, there was a significant difference ($P = 0.007$) in terms of length control.

Conclusions The technical quality of GC and LC root fillings as assessed by radiograph was not significantly different in terms of homogeneity, adaptation and taper. However, LC performed better than GC in terms of length control.

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Influence of bleaching interval on the luminosity of long-term discoloured enamel-dentine-blocks: a laboratory study

Aim To investigate the influence of changing the sodium perborate tetrahydrate (PBS-4) at a 4-day-interval versus no change after 16 days of internal bleaching. The tested hypothesis was that there is no difference between change versus no change of the PBS-4.

Methodology 210 bovine enamel-dentine-blocks were discoloured for 3.5 years with 14 different endodontic materials. All groups with a discoloring index of $\Delta E(\text{mean}) \geq 5.5$ were included in the present investigation: ApexCal (APCA), MTA white + blood (WMTA + BL), Portland Cement + blood (PC + BL), blood (BL), MTA gray (GMTA), MTA gray + blood (GMTA + BL), Ledermix (LED), triple antibiotic paste containing minocycline (3Mix). 14 specimens of each group were randomly assigned into 2 subgroups (i) no change of the PBS-4 ($n = 7$); (ii) change of the PBS-4 every 4 days ($n = 7$). Colour measurements were taken at 10 different time intervals and the $L^*a^*b^*$ values were recorded with a spectrophotometer (VITA Easyshade[®] compact; VITA Zahnfabrik, Bad Säckingen, Germany).

A Welch two-sample t-test was used to assess significant differences between the treatment procedures change and no change of the PBS-4 after 16 days (ΔL^*t4) of internal bleaching ($\alpha < 0.05$).

Results In the group 3Mix significantly better results were achieved by changing the bleaching agent every 4 days ($P = 0.0049$), while the group WMTA+BL had better results by no change of the bleaching agent ($P = 0.0222$). All remaining groups had no statistical difference between the two treatment procedures, however a tendency of higher ΔL^*t4 values was observed in the subgroup no change with exception of PC + BL.

Conclusions Changing the sodium perborate tetrahydrate every 4 days was beneficial for severe discoloured enamel-dentine-blocks only. Moderate discolorations were treated successfully without changing the bleaching agent in this laboratory investigation.

R152F.S. Grecca^{1,*}, I.B. Carlotto¹, M.B.C. Ferreira² & F. Montagner¹¹Department of Conservative Dentistry, School of Dentistry &²Department of Pharmacology, Basic Science Health Institute, Federal University of Rio Grande do Sul, Porto Alegre, Brazil**Comparison of the surface tension and the soft-tissue dissolution capacity of calcium and sodium hypochlorite solutions**

Aim To compare, *in vitro*, the tissue-dissolution capacity and the surface tension values of 0.5%, 1%, 2.5% and 5.25% calcium hypochlorite (CaOCl_2) and sodium hypochlorite (NaOCl) solutions.

Methodology Bovine pulp specimens were prepared (weight range 0.03–0.04 g). Each concentration of CaOCl_2 or NaOCl represented a test group ($n = 20$ per group). In each group, fragments were placed into cell culture wells ($n = 10$) or were attached to dentine blocks ($n = 10$) for 10 min. The pulp specimens were blotted dry and weighed again. The percentage of weight loss was calculated and analyzed statistically using Kruskal–Wallis and Dunn *post hoc* and Mann–Whitney tests ($\alpha = 5\%$). The surface tension of each solution was measured

using a Du Nouy tensiometer. Statistical analysis was carried out using ANOVA and Tukey Tests ($\alpha = 5\%$).

Results Suspended pulp fragments were more susceptible to dissolution than the fragments in dentine blocks, except for the 0.5% CaOCl_2 solution ($P \leq 0.05$). The 5.25% and 2.5% calcium and sodium hypochlorite solutions were more effective in dissolving pulp tissue than the two lower concentrations of NaOCl (0.5% and 1%), the lowest concentration of CaOCl_2 (0.5%) and saline ($P \leq 0.05$). The 1% CaOCl_2 group had similar results to all the other test groups and better results than saline ($P \leq 0.05$). All the NaOCl solutions had higher values of surface tension than the CaOCl_2 ($P \leq 0.05$). All calcium hypochlorite solutions had higher values of surface tension than distilled water ($P \leq 0.05$), except 0.5% CaOCl_2 .

Conclusions Calcium hypochlorite solutions had a pulp dissolution capacity, which was reduced when the pulp fragments were attached to dentine blocks. However, surface tension values tended to be lower than the sodium hypochlorite values. Calcium hypochlorite has potential to be used as irrigation solution in the root canal system.

R153O. Abduljabbar^{1,*}, D. Bakr² & S. Saleem¹¹Department of Pedodontic, Orthodontic and Prevention & ²Department of Conservative Dentistry, Hawler Medical University, Erbil, Iraq**Temperature rise in the pulp chamber during orthodontic bonding with different light curing sources: an *in vitro* study**

Aim The aim of this laboratory study was to evaluate intra-pulpal temperature rise during bracket bonding using three different light sources.

Methodology Sixty intact-extracted mandibular premolars were used. The teeth were divided into three groups of twenty teeth. Orthodontic brackets (Roth 0.22) were bonded to the buccal surface of all the teeth with Transbond XT adhesive applying a constant force with the help of a surveyor. The first group were light cured with a halogen light for 40 s, the second group using a light emitting diode used for 20 s, and the third group using a plasma arc light for 5 s. The distance between the tip of light source and tooth surface was 2 mm. The measurements were taken with a J-type thermocouple wire, placed in the centre of the pulp chamber and connected to a data logger. The analysis of variance and Turkey HSD test were used to analyze the results.

Results The pulp chamber temperature changes were influenced by type of light source. The intra-pulpal temperature changes for halogen was $4.11 (\pm 1.24)$, for light emitting diode $2.30 (\pm 0.92)$ and for plasma arc $0.90 (\pm 0.61)$. All the groups revealed significant difference between each other ($P < 0.001$).

Conclusions Orthodontic bonding when using different light sources did not exceed the critical 5.5°C temperature rise that produces pulpal damage. Halogen produced significantly higher intra-pulpal temperature changes than light emitting diode and plasma arc.

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Sealing ability of chemical-cure versus dual-cure dentine bonding systems in resin cores

Aim The purpose of this study was to evaluate the sealing ability of a newly introduced chemical-cure bonding system, compared with a dual-cure system.

Methodology Seventy two human extracted single-rooted teeth were used. The crowns of the teeth were removed at the CEJ used with a low-speed diamond saw and pulpal tissue removed using endodontic files. A post space was prepared to a depth of 6 mm and the specimens were randomly divided into six groups. The dentine surfaces were treated with a chemical-cure (Estecore, CC) or a dual-cure (Estelite-Core Quick Bond, DC) type of single-step bonding system. The post spaces were filled with a dual-cure resin core material and irradiated with a light activation unit for 10 s. The dye penetration test was performed by immersing the specimens into 0.2% basic fuchsin solution: (i) immediately immersed after the light irradiation, (ii) after being stored in distilled water for 6 days or (iii) after 2000 thermal cycles at 5 and 60°C, for 30 s respectively. Each specimen was then cut parallel to the long axis and under a stereo-microscope, the sealing ability was evaluated based on the degree of dye penetration, scored into 4 grades from 0 to 3 by dividing the length between the top and the apex of the root into quarters. The data was analyzed statistically by Mann–Whitney *U* test (12 specimens in each group). The sectioned surfaces were also observed with scanning electron microscope.

Results In all groups tested, the scores of dye penetration were less than grade two. There were no significant differences among all groups ($P = 0.87$). No gaps between dentine and the bonding layer were revealed by SEM observation.

Conclusions The sealing ability of the chemical-cure system, Estecore was equal to the dual-cure system, Estelite-Core Quick Bond.

R155

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Dental microcrack incidences after instrumentation with five different NiTi systems

Aim The purpose of this study was to evaluate the incidence of dental microcracks after root canal preparation with TwistedFile Adaptive (adaptive motion technology), WaveOne (reciprocating motion), Reciproc (reciprocating motion), Typhoon CM (rotary motion) and ProTaper Universal (rotary motion) instrumentation systems.

Methodology Sixty mandibular incisors with single canals were selected: 10 teeth were left unprepared and served as control, and the remaining 50 teeth were divided into five experimental groups ($n = 10$). The apical preparations were completed with size 25, 0.08 taper for TwistedFile Adaptive, WaveOne, and Reciproc systems. In Typhoon CM and ProTaper Universal systems; apical preparations were completed with size 25, 0.06 taper, and F2 (size 25 multitapered design) respectively. After canal preparations, roots were sectioned 3, 6, and 9 mm from the apex, and the cut surface photomicrographs of these sections were taken under a Scanning Electron Microscope and inspected for the existence of dental microcracks. The chi-square test was performed to compare the appearance of microcracks between the experimental groups ($P < 0.05$).

Results No cracks were found in the control teeth. All instrumentation groups were associated with microcrack formation significantly more than the control group ($P < 0.05$). TwistedFile Adaptive, WaveOne and Reciproc groups produced significantly fewer dental microcracks than Typhoon and ProTaper Universal groups ($P < 0.001$). No significant difference was observed between TwistedFile Adaptive, WaveOne and Reciproc groups ($P > 0.05$).

Conclusions Under the conditions of this study, root canal preparations with both rotary and reciprocating instruments resulted in dental microcracks. Full-sequence rotary systems

were associated with significantly more dental microcracks than reciprocating files.

R156

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Torsional resistance and metallurgical characteristics of conventional NiTi wire and CM-wire based rotary NiTi files

Aim To compare the torsional resistance and metallurgical characteristics of conventional and CM-wire based NiTi files.

Methodology Conventional NiTi wire based V taper2 and CM wire based V taper2H (size 25, SS White, USA) were used. (i) Torsional resistance: the tip of each file was fixed in jig in torque tester. A uniform clockwise rotation (2 rpm) was applied to the file until fracture. The maximum distortion angle at fracture was measured. Paired T-test was used to compare the torsional fracture resistance between V Taper2 and V Taper2H. A *P* value < 0.05 was considered statistically significant. (ii) Differential scanning calorimetry (DSC): small segments of the files were placed in DSC. The thermodynamic energy flow was recorded from -100°C to 100°C . The *Af* (austenite transformation finishing point) were determined. (iii) Transmission electron microscopy (TEM) with selected area diffraction analysis (SADA): the DB-FIB system was used for sectioning and imaging of sample. The microstructure analysis was carried out by using a 200 kV TECNAI F20 G2 SuperTwin TEM.

Results (i) Torsional resistance: V2H had significantly higher maximum distortion angles at fracture than V2. (ii) Differential Scanning Calorimeter analysis: V2 had a small endothermic or exothermic peak implying that V2 is almost completely composed of Austenite at room temperature. V2H revealed remarkable endothermic and exothermic peaks implying that V2H could be composed of mixed phases of austenite and intermediate R-phase at room temperature. (iii) Transmission electron microscopy examination and selected area diffraction pattern analysis: on the surface of V2, 2 layers were identified (TiO₂, NiTi layer). The grain size was about 100 nm. The internal structure was mainly composed of NiTi BCC phase (austenite). On the surface of V2H, 3 layers were identified (TiO₂, NiTi, Ni₃Ti layer). The grain size increased from surface (100 nm) to inside (200 nm). The internal structure is mainly composed of NiTi B2 phase (austenite).

Conclusions V2H had higher maximum distortion angle at fracture than V2. DSC results revealed that these superior properties were based on the enhanced thermal characteristics of V2H.

R157

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Tooth staining potential of current endodontic materials: a laboratory study

Aim To investigate the discoloration potential of 21 endodontic materials using a bovine tooth model introduced by Lenherr *et al.* (2012).

Methodology A total of 345 bovine incisors were extracted, standardized cuboid enamel-dentine blocks were prepared and randomly assigned into 23 groups ($n = 15$). The standardized cavities were filled with the materials including calcium silicate based cements (groups C-H, L), endodontic sealer (groups I-K), antibiotic pastes (groups M,N) and endodontic disinfectants (groups O-W). Positive and negative controls were filled with blood (B) or left empty (A) respectively. All specimens were sealed with a self-adhesive resin and stored in physiologic solution. Colour measurements were performed with a digital spectrophotometer at 7 different time intervals over 12 months. A two-way analysis of variance was used to assess significant differences between the endodontic materials. The mean values of all groups were compared with the *post-hoc* Tukey's test ($\alpha = 0.05$).

Results The most intense discolorations were detected in the groups B (blood = positive control; ΔE 32.45), H (MedPc BiOx; ΔE 22.18), and in the antibiotic pastes N (BiMix; ΔE 14.90) and M (TreVitaMix; ΔE 14.88). All remaining groups were not significantly different compared to the negative control group A (empty; ΔE 5.03). Compared to group A, higher ΔE values were recorded in groups K (Gutta Flow; ΔE 8.59), V (Odontopaste; ΔE 7.57), C (Ledermix MTA; ΔE 6.67), O (EDTA SC; ΔE 6.17), S (Octenidine; ΔE 5.94), G (Med PC ZrO; ΔE 5.90), I (Seal Apex; ΔE 5.89), D (MM MTA; ΔE 5.59), U (Prontosan; ΔE 5.28) and Q (CHX 2%; ΔE 5.17), while lower ΔE values were recorded in the groups R (Betadine; ΔE 4.94), P (NaOCl 3%; ΔE 4.86), T (Octenisept; ΔE 4.86), W (Pulpdent; ΔE 4.73), L (Odontocem; ΔE 4.64), J (Real Seal; ΔE 4.18), E (Biodentine; ΔE 4.00) and F (Med PC; ΔE 3.33).

Conclusions Even antibiotic pastes without minocycline were associated with severe discoloration. Portland cement based materials were associated with different levels of discoloration dependent on their chemical composition. Within the limitations of the current experiment the endodontic sealers and disinfectants used were colour-stable.

R158

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Energy consumption of the Twisted File instrument used with rotary or reciprocating adaptive motion

Aim The purpose of the study is to compare the energy consumption of Twisted File endodontic instrument used with either rotary or reciprocating adaptive motion.

Methodology Forty simulated S-shaped Endo Training Blocks (Dentsply Maillefer) with double curvatures 30° coronally and 20° apically were divided into 2 groups. Twenty blocks were prepared with Twisted File size 25, 0.08 taper (TF; SybronEndo, USA) using rotary motion (RM group), and twenty were prepared using reciprocating adaptive motion (AM group) according to manufacturers' instructions. All preparation procedures were performed with the Elements Motor (SybronEndo) connected to a digital wattmeter. The required torque for root canal instrumentation was analyzed by evaluating the electrical power consumption (mW h^{-1}), elapsed time (seconds) and number of pecking motions required to reach the full working length were calculated. Differences between groups were analyzed statistically with Wilcoxon and Mann-Whitney *U* tests ($P < 0.05$).

Results There were no significant differences in the elapsed time and pecking motion between two groups ($P > 0.05$). According to electrical power consumption there was a significant difference between groups ($P < 0.05$). Rotary motion group significantly decreased electric power consumption compared with reciprocating adaptive motion group ($P < 0.05$).

Conclusions This study confirmed that endodontic motors used more electricity in adaptive motion. Torsional stress developed during mechanical preparation could not be directly related to electric consumption.

R159

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Comparison of two techniques for measuring apical irrigant extrusion: a methodological laboratory study

Aim The aim was to compare an established method (Myers & Montgomery) with an alternative method for measuring the volume of irrigating solution extruding from the apex, and to check whether results from these two methods were parallel.

Methodology Endodontic access cavities were prepared in 10 human maxillary incisor teeth extracted for periodontal reasons (tooth length: 22–24 mm, first binding file: size 10–15). The working length was established 1 mm short of the apical foramen. The canals were instrumented using ProTaper Universal rotary nickel-titanium files to F2, F3 and F4. A size 15 K-file was extruded 1 mm past the apical foramen to achieve apical patency. At each file size, the teeth were tested for irrigant extrusion successively using two methods. First, according to the Myers & Montgomery method, each tooth was mounted on a glass vial, the root canal was irrigated with 5 mL of distilled water, and the extruded irrigant was collected into a preweighed Eppendorf tube. Second, the same teeth were inserted into preweighed flower arrangement foam cubes, and irrigation was carried out identically. The tubes and the foam cubes were weighed on a precision micro-balance. The data were subjected to statistical analysis; a *P* value of 0.05 was considered statistically significant.

Results Extrusion was significantly greater with the conventional method at all preparation sizes ($P < 0.05$, paired samples *t*-test). In each method, no significant difference was found among the F2, F3 and F4 sizes for the volume of the extruded irrigant ($P > 0.05$, repeated measures ANOVA). A significant positive correlation was found between the yields of the two methods at each preparation size (F2: $r = 0.770$, $P = 0.009$, F3: $r = 0.721$, $P = 0.019$, F4: $r = 0.794$, $P = 0.006$; $n = 10$; Spearman's correlation) and also in total ($r = 0.740$, $P = 0.000$; $n = 30$).

Conclusions The alternative method, although yielded less volume of irrigant extrusion, revealed substantial correlation with the conventional method. Foam cubes may be considered for use in irrigant extrusion studies.

R160

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Influence of patients' considerations in the difficulty level of an endodontic case assessed through the AAE questionnaire

Aim To analyze the influence of factors related to 'patients's considerations' in the evaluation of the difficulty of a clinical endodontic case, following the criteria from the American Association of Endodontists (AAE).

Methodology A sample ($n = 642$) of questionnaires (AAE model), carried out between 2008–2014 with patients from the Master in Endodontics (ME) programme at the University of Valencia, Spain (UV), were used to analyze the difficulty of endodontic treatment. The influence of several factors were studied in the chapter

'patients' considerations': medical history (ASA), anaesthesia, patient disposition, ability to open mouth, gag reflex and emergency condition. Data were coded in three levels: minimal difficulty = 1, moderate difficulty = 2, high difficulty = 5. Adding the points in each item the final level of difficulty of the case (minimal difficulty <20 points, 20–40 moderate and >40 high) was scored. Relationship among difficulty and each one of the studied variables were compared through the ANOVA test. A linear regression was used to detect variables related with the explanation of the difficulty. In every case, a significant level of $P < 0.05$ was considered.

Results 27 cases (4.2%) had a low, 371 (57.8%) moderate and 244 (38%) high degree of difficulty. Average difficulty values revealed significant differences with all the studied variables at the three levels of difficulty ($P < 0.05$). The model's square vR was 0.408. Every variable was included in the model. Case difficulty can be explained in a 40% as the following formula: $15.78 + (1.147 \times \text{ASA}) + (0.838 \times \text{anaesthesia}) + (0.965 \times \text{patient disposition}) + (1.903 \times \text{ability to open mouth}) + (1.082 \times \text{gag reflex}) + (1.199 \times \text{emergency condition})$.

Conclusions The majority of endodontic cases treated at the ME of the UV in the study period had a medium or a high level of difficulty. Factors related to the 'patients' considerations' items of the AAE questionnaire influenced significantly the degree of difficulty.

R161

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Comparison of crown discolouration with triple antibiotic paste and minocycline paste using spectrophotometric analysis

Aim To evaluate the degree of crown discolouration induced by triple antibiotic or minocycline pastes.

Methodology The root canals of ninety extracted human incisors were prepared to size 30 using ProTaper rotary instruments. After pulp tissue removal, shaping and irrigation procedures, the specimens were randomly divided into two groups ($n = 45$), which received either triple antibiotic paste (TAP) or minocycline paste (MP) as the intracanal medicament. Following placement of the pastes, the teeth were sealed coronally with cotton pellets and a temporary filling. Spectrophotometric readings were obtained on the buccal surfaces of the crowns on day 0 to week 4 after dressing, and the differences in colour were recorded. Data were analysed with the Mann–Whitney U and Wilcoxon sign tests ($P < 0.05$).

Results Both groups had significant coronal discolouration from day 0 to weeks 1, 2, 3 and 4 ($P < 0.01$), but their final shades did not significantly differ between the groups ($P > 0.05$).

Conclusions Both antibiotic pastes induced crown discolouration, and neither was superior.

Acknowledgements The authors deny any conflicts of interest related to this study.

R162

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Spectrophotometric analysis of coronal tooth discolouration induced by Biodentine and BioAggregate

Aim To evaluate coronal tooth discolouration induced by Biodentine and BioAggregate when used in molar and incisor teeth.

Methodology A total of 42 extracted human maxillary incisors and 42 third molar teeth were selected. All teeth were randomly assigned to one of three groups: two experimental ($n = 14 \times 2$) and one negative control ($n = 14$). Molar teeth were sectioned 1 mm below the cemento-enamel junction. Pulp chambers were extirpated with an excavator, and pulp chambers were debrided with NaOCl. Materials were prepared according to the manufacturers' instructions and placed into the pulp chambers with an MTA carrier through the access and compacted with slight vertical pressure. Forty two incisor teeth were root filled with AHPlus and gutta-percha. Extruded cones were cut 3 mm below the orifice. In the experimental groups, 3 mm of Biodentine or BioAggregate plugs were placed in the root canal below the orifice. Following setting of the materials, teeth were restored with composite resin. Colour measurements were recorded with a spectrophotometer under constant laboratory light prior to material placement, 1 week after placement, and 1 month after placement. The Commission Internationale de l'éclairage (CIE) system was utilized to calculate differences in colour. Data were analyzed by two-way repeated-measures ANOVA with Bonferroni's method.

Results Both molar and incisor teeth in the experimental groups exhibited perceptible discolouration after 1 week. The control group had no perceptible discoloration and was significantly different than the experimental groups ($P < 0.05$). There was no significant difference between Biodentine and Bioaggregate at 1 week (for incisor teeth $P = 0.602$ and molar teeth $P = 0.326$) or 1 month (for incisor teeth $P = 0.982$ and molar teeth $P = 0.259$).

Conclusions Biodentine and Bioaggregate induced perceptible discolouration of teeth.

R163

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Ten year follow-up study on the adoption of endodontic technology amongst Danish general dental practitioners

Aim To re-assess the adoption rate of endodontic technology in a subpopulation of Danish general dental practitioners (GDPs) after 10 years.

Methodology In 2003 GDPs from Copenhagen Dental Association (CDA) ($n = 692$) responded to a questionnaire concerning use of endodontic technology. The questionnaire was repeated in 2013 where 25% ($n = 278$) of the members of CDA were approached randomly. Three options were available; often, occasionally and never. Responses were anonymous. In addition, the CDA 2013 group was compared with the response profile from GDPs voluntarily attending a national one day Endodontic Symposium (ES) arranged by the Danish Dental Association. The statistical analyses were performed using Chi-squared test.

Results The response rate of the CDA group was 39% ($n = 109$). The frequencies of 'often' rubber dam and apexlocator users significantly increased from 4% to 29%, and from 15% to 45%, respectively ($P < 0.0001$). A significant increase was detected for the adoption of rotary NiTi instruments from 10% to 61% ($P < 0.0001$). Although significantly decreased more than half of the responders used stainless steel instruments. Furthermore, there was a significant decrease in the number of treatment visits for molar instrumentation. In 2013 10% used more than 2 appointments for instrumentation ($P = 0.018$), and more GDPs filled a non-vital case within the first visit ($P = 0.0007$). No differences in response profile were noted between the randomly selected CDA group and the ES attenders.

Conclusions Over a 10 year period, there was a marked increase in the adoption rate of endodontic technologies amongst GDPs. The majority of the GDPs complete molar root canal treatment within 2 visits. However, progress towards high quality root canal treatment may still be obstructed as the majority still avoid consistent use of rubber dam.

Acknowledgements The Copenhagen Dental Association are gratefully acknowledged for the contribution to the study.

R164

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Comparative study of root canal shaping using ProTaper Universal and ProTaper Next rotary files in preclinical dental education in Turkey

Aim To evaluate and compare the ability of third year dental students to preserve original canal curvature and length, avoid ledging, instrument deformation and fracture, and to compare working times during the shaping of simulated root canals with ProTaper Universal (PU) and ProTaper Next (PN) rotary instruments.

Methodology A total of 395 simulated canals in resin blocks with 40° curvature (Dentsply Maillefer) were prepared by 79 dental students with PU and PN according to the manufacturer's instructions. Each student instrumented 5 blocks with 1 set of instruments and an electric motor (X-Smart, Dentsply Maillefer). The final apical preparation was F2 for PU and X2 for PN. Fractured instruments were replaced with new instruments. Canal straightening and the incidence of ledges was assessed on digital images with AutoCad 2007. Change of working length and incidence of fracture and deformation were also noted. Data were analysed by student-*t* test ($P < 0.05$)

Results The mean curvatures of canals after instrumentation with PU and PN were 24° and 26°, respectively. The incidence of deformation and fracture was 9 and 33 for PU and 2 and 18 for PN. Ledge formation and change of working length occurred in 19% and 59% of canals instrumented with PU and 4% and 71% instrumented with PN. Significant differences in canal straightening, incidence of ledging, change of working length, incidence of fracture or deformation, and working time were found between the groups ($P < 0.05$).

Conclusions Within the limits of this study, PN performed better than PU. However after doing more exercises with rotary instruments satisfactory results will be achieved.

R165

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Crown discoloration induced by endodontic sealers: an *in vitro* spectrophotometric analysis

Aim The aim of this study was to evaluate tooth crown discoloration induced by commonly used and new-generation endodontic sealers placed in pulp chambers.

Methodology The crowns of 60 extracted mandibular incisor teeth were removed 2 mm below the cemento-enamel junction. Pulp chambers were chemomechanically debrided through the apical access. The specimens were randomly divided into 4 groups ($n = 15$). The following materials were placed into the pulp

chambers: Group I: Pulpispad (Dentsply International, France), Group II: AH26 (DeTrey Dentsply, Germany), Group III: MTA Filapex (Angelus, Brazil), Group IV: EndoREZ (Ultradent Products Inc., USA). The apical access was sealed with glass-ionomer cement. Crowns were stored individually marked vials in standard conditions (% 100 humidity, 37°C). The colour of the crowns was measured using spectrophotometer (VITA Easyshade compact; VITA Zahnfabrik, Germany). The CIE L*a*b* parameters were obtained before sealer placement (baseline), after one month, six months, 1 year and 2 years respectively and data were transformed into ΔE values. Statistical analysis was performed using Multiple Comparison test and Kruskal-Wallis test ($\alpha = 0.05$).

Results All sealers caused varying chromatic alterations. Significant differences were detected amongst the groups after different time periods ($P < 0.05$). Pulpispad had the greatest and MTA Filapex had the least discoloration after 2 years ($P < 0.05$).

Conclusions After root filling, the clinician should be aware of the presence of remaining materials. Thorough debridement of the pulp chamber is essential for the prevention of sealer-induced discoloration.

OTHER

R166

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Evaluating hydroxyl radical diffusion, and thiourea as a scavenger during intracoronal bleaching of rootfilled teeth

Aim (1) To quantify hydroxyl radical diffusion to external root surfaces following intracoronal bleaching with various bleaching materials, and (2) to evaluate the effect of adding thiourea (a radical scavenger and reductive bleach) in reducing radical diffusion.

Methodology One hundred and twenty-two intact human premolars were prepared, stained with red blood cells, root filled, and subjected to various intracoronal bleaching materials as follows:

Group 1: 30% hydrogen peroxide, Group 2: acidified thiourea and 30% hydrogen peroxide, Group 3: neutral thiourea and hydrogen peroxide, Group 4: sodium perborate and hydrogen peroxide, Group 5: sodium perborate and water Group 6: water (negative control)

After placement of intracoronal bleaching agents, teeth were bathed in a terephthalic acid solution at 37°C for 48 h. Hydroxyl radicals that diffuse to the external root surfaces will react with terephthalic acid to generate hydroxyterephthalate, a fluorescent molecule. This was quantified using a Fluorescence Microplate Reader and high performance liquid chromatography with fluorescence detection for product confirmation.

Results Combination of sodium perborate and hydrogen peroxide had the greatest diffusion of hydroxyl radicals and was significantly greater than sodium perborate and water ($P = 0.0006$) and hydrogen peroxide alone ($P = 0.003$). Addition of acidified or neutral thiourea to hydrogen peroxide showed no statistically significant difference compared with use of hydrogen peroxide alone. Sodium perborate and water had the least diffusion and was significantly lower than when acidified thiourea was used with hydrogen peroxide ($P = 0.0011$). The pH of thiourea solution influenced radical diffusion.

Conclusions The sodium perborate and water mixture resulted in low radical diffusion and appears to be a safe bleaching material. Hydrogen peroxide and sodium perborate resulted in significantly greater radical diffusion and should be used with caution. The addition of acidified thiourea to hydrogen peroxide did not

reduce radical diffusion but may enable a reductive and oxidative bleaching process to occur simultaneously. This combination had lower radical diffusion than hydrogen peroxide and sodium perborate and may be a safer alternative bleaching protocol for an improved bleaching effect.

Acknowledgements Australian Society of Endodontology

R167

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Colorimetric evaluation of bacterial contamination on teeth stained with blood *ex vivo*: efficacy of two different bleaching regimes

Aim To assess the colorimetric effect of bacterial contamination on blood stained teeth; to compare efficacy of two bleaching regimes: sodium perborate and water (S) and acidified thiourea-hydrogen peroxide (T).

Methodology A total of 99 human premolars had their apices resected and pulp extirpated, leaving ~3 mm of root below the CEJ. Canals were injected with blood ($n = 49$), or blood/bacteria containing *Porphyromonas gingivalis* and *Prevotella intermedia* ($n = 50$). Teeth were sealed and stored anaerobically at 37°C. On day 35, teeth were accessed occlusally and divide into six groups: Group 1–3; blood stained and bleached with water ($n = 10$); S ($n = 16$) or T ($n = 16$) respectively. Group 4–6 teeth were stained with blood/bacteria and bleached as group 1–3. Teeth were re-bleached 7 days later. Colorimetric evaluation of tooth staining was assessed using digital imaging and CasMatch standardization at Pre-op, day 35 of staining, day 7 and 14 after bleaching. Data were transformed using the CIE L*a*b colour system and the clinical perceptible colour difference (ΔE) calculated. A linear mixed model with fixed effects of time, group and bleach was used to examine colour difference between groups and bleaching over time. Planned comparisons examining group and bleach differences over time were obtained.

Results Blood stained teeth were associated with a significant increase in redness ($P = 0.0006$) and were darker ($P < 0.0235$) on day 35 compared to blood/bacteria stained teeth. ΔE values were 20.94 and 17.20 respectively. At day 7 and 14 after bleaching, blood/bacteria stained teeth were significantly less red using either S [(day 7, $P = 0.0259$); (day 14, $P = 0.0169$)] or T [(day 7 $P = 0.0278$); (day 14, $P < 0.0001$)] compared to blood/bacteria stained teeth. At day 14 after bleaching, blood stained teeth became significantly whiter using either S ($P = 0.0005$) or T ($P < 0.0001$) compared to blood stained teeth. At day 14 after bleaching, T produced a significantly whiter and less red shade in blood stained [(L*, $p < 0.0001$); (a*, $p < 0.0001$)] and blood/bacteria stained teeth [(L*, $p = 0.0021$); (a*, $p < 0.0001$)] compared to S.

Conclusions Blood stained teeth were significantly darker and red compared to blood/bacteria stained teeth. They were also more resistant to bleaching using either S or T bleaching regime. T bleaching regime was more effective than S.

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R168

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Effect of aging on surface free energy and mineral composition on root canal dentine

Aim The purpose of this study was to evaluate the aging effects on surface free energy and mineral composition (calcium, phosphorus, magnesium and Ca/P ratio) of root canal dentine.

Methodology Ten sound human premolar teeth extracted from 13- to 27-year-old (average 16.2) patients scheduled for orthodontic treatment and 10 periodontally involved teeth extracted from 61- to 70-year-old (average 66.1) patients were used. Teeth were decoronized and the apical third of each root was removed. The remaining root was cut longitudinally into 2 equal segments. The sectioned surfaces were fixed in acrylic resin blocks, with the root canal dentine surface facing up. The surfaces of 40 root slices were polished under running water by using 600-, 800-, 1000-, 1200- and 2000-grit SiC polishing papers under running water to produce relatively smooth surfaces and washed in an ultrasonic water bath. The surface free energy was calculated by measuring the contact angles of the treated surfaces with water, diiodomethane, ethylene glycol and formamide according acid-base theory. The mineral compositions of root dentine surfaces were analysed by X-ray photoelectron spectroscopy over a wide scanning range. Data were analysed by *t*-test for statistical comparison.

Results Surface free energy of dentine was significantly higher in young samples ($61.84 \pm 5.44 \text{ mN m}^{-1}$) than aged ones ($48.89 \pm 5.30 \text{ mN m}^{-1}$) ($P < 0.001$). While calcium and phosphorus composition increased with age, the Ca/P ratio was not significantly higher in young samples (3.12 ± 1.31) than aged donor samples (2.8 ± 0.78).

Conclusions Surface free energy and mineral composition of dentine may alter depending on age. Further studies are needed to evaluate the effects of aging on dentine bonding of root canal sealers and dentine wettability of root canal irrigants.

R169

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Influence of root canal treatment procedures on the elasticity of teeth

Aim To determine the differences in compression and decompression loads within the elastic limit of teeth after different endodontic treatment procedures using hysteresis analysis.

Methodology Intact freshly extracted premolars were microCT-scanned to determine the dimensions and anatomy of the tooth structures. Finally, eight dimensionally similar premolars were selected. Individual stubs were fabricated for each tooth to allow exact replacement in the testing machine after different endodontic procedures. The elasticity of teeth was assessed in a universal testing machine before and after predefined six endodontic steps: access-cavity preparation, canal-orifice widening, canal shaping, irrigation with 5% sodium hypochlorite, calcium-hydroxide medication and canal filling including placement of an adhesive coronal restoration. After each procedure, teeth were subjected to four cycles of compression and decompression loads of 2–300 N. Loads

of each 0.5 micrometer tooth displacement were recorded. Analysis of variance was performed on load distributions between treatment steps. The change in elasticity after each endodontic procedure in the decompression cycle was compared using Wilcoxon/Kruskal–Wallis test.

Results Each endodontic procedure changed the hysteresis behaviour of the whole tooth. The largest differences in displacement loads were observed after access-cavity preparation and widening of the canal orifice ($P = 0.001$ and $P < 0.0001$ respectively). Displacement loads after canal filling and coronal restorations were significantly different from that of the intact tooth ($P < 0.0001$).

Conclusions Access cavity preparation and canal orifice widening had the greatest effect on tooth elasticity. Canal filling and coronal adhesive restorations did not restore the rigidity of teeth after root canal treatment.

R170

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Student confidence on multiple-choice questions in endodontics: are there more misconceptions in action questions or knowledge questions?

Aim One of the most widely used instruments in health profession education are multiple-choice questions (MCQs) that offer an efficient way to assess a large number of students over wide subject domains with reliability. Despite this, MCQs might foster superficial learning and students may focus on recognizing isolated facts rather than relationships or higher levels of learning. As the student performance on MCQs is based only on the correct answer, this type of test provides little feedback to the instructor about what students do not know or the confidence students have in their responses. Therefore, misconceptions by students who are confident in their incorrect answers are often not identified by conventional MCQs. The aim of this study was to identify students' misconceptions by compare confidence to correctness on two different types of questions: action and knowledge.

Methodology This cross-sectional study was conducted using a convenience sample of 105 third-year University of California, San Francisco (UCSF) dental students from the graduating class of 2011. Students completed an endodontic assessment that included 20 MCQs (10 action questions, focused clinical decision making and 10 knowledge questions). After they had chosen a correct answer among 4 alternatives, they indicated their confidence for each question (sure or unsure). Misconception was distinguished with the following combination: incorrect response and confident. Findings were analyzed descriptively (frequency and percentages).

Results For the action questions, the average confidence level was 88.5 on a 100 point scale, with an average percent correct of 87.4 and an average of 9.7 misconceptions on each question from the 105 students while for knowledge questions had values of 94%, 93.1% and 5.4%, respectively.

Conclusions Action questions had a higher number of misconceptions compared to the knowledge questions. Even adjusting for question difficulty, students were more miscalibrated on action questions than knowledge questions.

R171

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How popular is Endodontology among dental students in Slovenia?

Aim To investigate the popularity of Endodontology among dental students in Slovenia.

Methodology A questionnaire was designed and distributed among 180 fifth, sixth and final year dental students in the Section of Dental Medicine, Faculty of Medicine, University of Ljubljana, Slovenia. Students were asked how many root canal treatments (RCT) they had done, how much they enjoyed doing RCT, how they feel while doing RCT, how important they think Endodontology is now we have implants, and on a scale from 1 to 10 how confident and how competent they think they are in various RCT procedures and how important are precision, consistency, persistency, and aseptic work in RCT. Data were analysed using descriptive statistics, χ^2 test and ANOVA.

Results The response rate was 70%, 22.7% of students were males. 35.9% were fifth, 29.7% were sixth and 34.4% were final year students, with 3.17 ± 1.45 RCT, 9.61 ± 2.38 RCT and 10.48 ± 1.21 RCT completed, respectively. Among all students, 55.5% liked and 41.4% enjoyed doing RCT. While doing RCT, 14.1% of students became bored, tired or irritated, 15.6% became relaxed, 78.9% felt challenged with RCT and 22.7% were terrified of RCT. 95.3% of students found Endodontology important and 98.4% would choose RCT over an implant. Students were most confident in pain management, apical size and working length determination, but afraid of root perforations, missed canals and instrument fractures. They felt least competent in locating canals, achieving working length and access cavity preparation and most competent in pain management, rubber dam placement, apical size determination and canal filling. Fifth-year students had significantly more problems with pain management ($P = 0.025$) and canal filling ($P = 0.011$), while sixth year students were more concerned of root perforations ($P = 0.013$). All students believed precision, consistency, persistency and aseptic work were highly important in RCT.

Conclusions Endodontology is quite popular among dental students in Slovenia.

SESSION 3: SATURDAY 19TH SEPTEMBER

BASIC SCIENCE: PULP

R172

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Effects of short-period three-dimensional (3D) spheroid culture for odonto-/osteoblastic differentiation of dental pulp cells

Aim We have reported that 14 days of three-dimensional (3D) spheroid culture promoted odonto-/osteoblastic differentiation of dental pulp cells. The aim of this study was to evaluate the effects of short-period 3D spheroid culture of dental pulp cells on their odonto-/osteoblastic differentiation.

Methodology For 3D spheroid culture, mouse dental papilla cells (MDPs) that have the ability to differentiate into odontoblasts, were cultured for 6, 12, and 24 h in 96-well low-attachment culture plates (PrimeSurface; Sumitomo Bakelite, Tokyo, Japan). Flat-bottomed plates were used for two-dimensional (2D) monolayer culture as a control. Following RNA extraction and complementary DNA synthesis from samples, real-time PCR was performed to evaluate the expression of dentine sialophosphoprotein (Dspp), alkaline phosphatase (Alp), and bone morphogenetic protein2 (BMP2) using specific primers.

Results Accumulation of MDPs cultured in the 96-well low-attachment plate was observed in the bottom of the wells at 6 h. The cellular mass was relatively translucent at 6 h, and appeared translucent white or milky white following the culturing period of 12 or 24 h. Up-regulation of Alp and Bmp2 expression was observed at 12 h, and that of Dspp expression was observed at 24 h in the 3D spheroid-cultured MDPs. On the contrary, the expression of these genes was low in the 2D monolayer-cultured MDPs.

Conclusions 3D-spheroid culture for 12 h induced the odonto-/osteoblastic differentiation of MDPs.

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R173

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Inability of human dental pulp cells to differentiate into odontoclasts

Aim The aetiology and cell of origins responsible for internal root resorption are partially understood. Human dental pulp tissue is composed of various cell types capable of differentiation into odontoclasts, i.e. monocytes, dendritic cells, human dental pulp stem cells (DPSCs). The purpose of this study was to evaluate the ability of human dental pulp cells (hDPCs) to differentiate into odontoclasts.

Methodology In tissue culture plates, hDPCs were cultured in culture media supplemented with RANKL and M-CSF. Clast-like cells differentiated from the induced hDPCs were count after

7 days. On dentine discs, resorptive pits were observed to confirm the activity of the differentiated cells after 14 days. Peripheral blood mononuclear cells (PBMCs) were used as controls. Descriptive statistics was used.

Results After 7 days, no clast-like cell was found in the hDPCs group, but 78 odontoclast-like cells were observed in eight of nine wells, ranging from zero to 38 cells per well from the PBMCs group. After 14 days, no resorption pits were found in the hDPCs group, but were observed in the PBMCs group.

Conclusions Within the limitations of this study, it may be concluded that hDPCs do not differentiate into odontoclasts. Internal root resorption is likely to occur from mononuclear cells from the blood stream.

R174

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Inflammatory bone destruction in experimentally induced periapical lesions in ST2 deficient mice

Aim ST2 is a member of the interleukin (IL)-1 receptor family, and IL-33 is its natural ligand. ST2 signaling promotes Th2 immune response in allergy, autoimmunity, and chronic inflammatory disorders, but its role in the pathogenesis of periapical lesions is unknown. The purpose of this study was to investigate whether ST2 gene deletion affects the development of experimentally induced periapical lesions in mice.

Methodology Pulp of 15 mandibular molars from wild-type (WT) and 15 ST2 knockout (ST2^{-/-}) BALB/c mice were exposed and left open to the oral environment. After death, hemi-mandibles were isolated and prepared for histologic, immunohistochemical, and flow cytometric analysis. Student *t* test was used for statistical evaluation.

Results The expression of IL-33 and its receptor ST2 was higher in periapical lesions in WT mice compared with normal root apices (both *P* < 0.05). The increased periapical bone loss observed in ST2^{-/-} mice was associated with enhanced influx of neutrophils, CD3+ CXCR3+ Th1 cells, and CD3+ CCR6+ Th17 cells and increased number of tartrate-resistant acid phosphatase + osteoclasts (all *P* < 0.05). Furthermore, periapical lesions in ST2^{-/-} mice contained increased percentages of T cells expressing interferon- γ , IL-17, tumor necrosis factor- α , and IL-6 (all *P* < 0.05). In comparison with WT mice, CD3+ receptor activator of nuclear factor kappa B ligand+ T cells were increased, whereas CD3+ osteoprotegerin+ T cells were decreased in the lesions of ST2^{-/-} mice (both *P* < 0.05).

Conclusions ST2 deletion increased inflammatory bone loss in experimental periapical lesions in mice, which is associated with enhanced Th1/Th17 cell mediated periapical immune responses and increased osteoclastogenesis.

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Assessment and quantification of non collagenic matrix protein release from human dentine powder incorporated into a Si-HPMC biomedical hydrogel

Aim Non-collagenous matrix proteins are sequestered into dentine during dental formation. They can be released under pathological conditions but also by controlled demineralisation with bioactive materials. Dentine is now considered as a reservoir of growth factors and it may be interesting to use or design new biomaterials to extract them from the mineralized matrix. The purpose of this study was to investigate the ability of a biomedical hydrogel to extract and release these growth factors from crushed dentine.

Methodology Dentine was crushed with two different grinders (Zirconia Grinder RM100 RETSCH and Smart Dentine Grinder KometaBio). The particle size was measured by SEM. Different kinds of mixtures and different quantities of powder were incorporated into Si-HPMC hydrogel (100, 125 and 150 mg) and then immersed into PBS. Dentine powder immersed in PBS only served as control. The supernatant was collected and total released proteins was quantified by gel shift migration and Coomassie staining. The presence of TGF β 1 was investigated by Western Blot.

Results The mixture powder/hydrogel released total proteins (from 27.2 $\mu\text{g mL}^{-1}$ to 200.56 $\mu\text{g mL}^{-1}$ according to the mixtures) from the first day until stabilization at 14 days. The quantity of released proteins (max 200.56 $\mu\text{g mL}^{-1}$ was directly related to the size of the particles of the crushed dentine ($<50 \mu\text{m}$) and the amount of powder incorporated into the hydrogel. The negative control had no DMPs release. Gel shift with direct revelation by UV and Western Blot, targeting the latent form and the active form of the TGF β 1, had bands whose molecular weights correspond to the TGF β 1.

Conclusions Si-HPMC hydrogel was able to release DMPs from crushed dentine powder. The next step will be to investigate its biological effect on pulp cells. If biological effects of this mixture on cultured cells is confirmed, it might be considered as a new way of treatment for dentine pulp complex regeneration, and especially pulp capping.

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The effect of ozone on VEGF and nNOS levels in human dental pulp

Aim To assess the influence of gaseous ozone application in deep cavities on the level of vascular endothelial factor (VEGF) and neuronal nitric oxide synthase (nNOS) in healthy human dental pulps.

Methodology Thirty-eight intact and caries-free human teeth scheduled to be extracted for orthodontic reason were selected from healthy patients aged 18–40 (mean age \pm SD; 25.9 ± 6.1). Standardized Class I cavities were prepared on the occlusal sur-

faces of all teeth. After randomization procedures, 19 teeth were exposed to ozone gas (OzonyTron X-Bioozonix, Munich, Germany) for 40 s; 19 other served as controls. Pulp were removed 20 min after cavity preparation and ozone application in control and ozone group, respectively. The levels of VEGF and nNOS were determined in the pulp tissue lysates with enzyme-linked immunosorbent assay. Data were analysed statistically by independent-t test using the level of significance of 5%.

Results VEGF and nNOS concentrations in the pulp of healthy teeth exposed to 40 seconds of ozone were $1023.4 \pm 176.6 \text{ pg mL}^{-1}$ and $0.28 \pm 0.18 \text{ ng mL}^{-1}$ of tissue, respectively, and were significantly higher in comparison to controls ($776.2 \pm 207.1 \text{ pg mL}^{-1}$ and $0.12 \pm 0.12 \text{ ng mL}^{-1}$ of tissue, respectively).

Conclusions A single application of gaseous ozone in deep cavities affected the human pulp levels of VEGF and nNOS and may provide relevant data for ozone biocompatibility.

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Osr2 (odd-skipped related 2) induced up-regulation of odonto-/osteoblastic gene expression in mouse dental papilla cells

Aim The odd-skipped related (Osr) gene is a zinc-finger transcription factor that is involved in embryonic development and bone formation. Recently, Osr2 has been reported to be expressed in the mesenchyme of tooth germs. The aim of this study was to evaluate the role for Osr2 in odonto/osteoblastic differentiation of mouse dental papilla cells (MDPs).

Methodology MDPs were cultured in α -modified minimum essential medium containing 10% foetal bovine serum and antibiotics. The open reading frame of the mouse Osr2 cDNA was subcloned into the eukaryotic expression vector pEF-Dest51 (Invitrogen, USA), and the Osr2 expression vector was transfected into MDPs with Lipofectamine 2000 (Invitrogen). Stable cell lines were selected in a growth medium containing 4 $\mu\text{g mL}^{-1}$ blastidicin (Invitrogen). Expression of odonto/osteoblastic genes was measured by real-time PCR (VFX-96, Bio-Rad, USA) using specific primers. Nodule formation was induced by a mineralizing medium containing ascorbic acid (50 $\mu\text{g mL}^{-1}$; Wako, Japan) and beta-glycerophosphate (5 mM; Sigma, USA), and nodules were visualized by alizarin red S staining. Data was analysed with student's t-test and statistical significance was set at $P < 0.05$.

Results The Osr2 stable cell lines showed significantly higher expression of odonto/osteoblastic genes, alkaline phosphatase and dentine matrix protein 1, compared to mock gene transfected MDPs ($P < 0.05$). Alizarin red S staining revealed that the Osr2 stable cell lines formed more mineralized nodules than the mock gene transfected MDPs.

Conclusions Enhanced expression of Osr2 induced augmented odonto/osteoblastic differentiation of MDPs.

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R178

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The effect of calcium silicate-based cements on viability of stem cells within the apical papilla

Aim Endodontic treatment of immature permanent teeth results in an increased risk for tooth fracture. The use of stem cells in pulp, periodontal and hard tissues regeneration may promote normal root development, preserving tooth viability. Studies have suggested that adult stem cells submitted to an inductive medium can be differentiated in osteo/odontoblasts that may then be used to repair tooth defects. This study aimed to investigate the effects of three different calcium silicate-based materials (ProRoot[®] MTA, Biodentine[™] and MM-MTA[™]) on dental apical papilla stem cell viability.

Methodology Apical papilla were harvested from human immature third molars with incomplete rhizogenesis. Stem cells from the apical papilla (SCAP) were obtained after enzymatic digestion with dispase and type I collagenase and cultured in KnockOut-DMEM/FBS for 2 weeks. SCAPs were concentrated/purified by magnetic activated cell sorting using STRO-1. SCAP or fibroblast cultures were exposed separately to indirect contact with one of the three materials. Materials were placed inside Millicell[®] culture inserts and positioned in wells containing cultured cells for 36 h. In control conditions, no material was present in the insert. Toxicity was analysed using the Alamar Blue cell viability assay. The effect on cellular morphology was determined by microscope observation.

Results None of the three materials affected fibroblast cell viability (MTA: $81.4 \pm 10.40\%$; MM-MTA: $84.2 \pm 14.57\%$; Biodentine: $77.3 \pm 9.31\%$). Purified SCAPs in the presence of MTA and MM-MTA displayed cellular viability in the range of 75% (MTA: $76.8 \pm 3.38\%$; MM-MTA $75.8 \pm 8.21\%$). In the presence of Biodentine, SCAP survival was 46% (Biodentine: $45.9 \pm 0.483\%$). All values are presented as percent of control (no material) \pm SEM, $n = 2$. Microscope observation did not reveal overt changes in cellular morphology in any of the culture condition.

Conclusions SCAP cultures were associated with reduced cellular viability when incubated in the presence of Biodentine[™], compared to both ProRoot[®] MTA and MM-MTA[™] cements. Further studies are needed to assess dose-response effects and if there are potential molecular changes even in sub-toxic conditions.

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R179

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Hyaluronan induces mineralization of dental pulp cells

Aim The CD44 is a cell-surface glycoprotein involved in a variety of cellular functions. Recent studies have observed that the CD44 is expressed strongly in odontoblasts at the appositional stage of tooth development. Whether CD44 and its principle ligand, hyaluronan (HA), regulate the mineralization process of dental pulp cell (DPC) was investigated.

Methodology Five different doses of high molecular weight HA, 0.1, 0.5, 1, 1.5 and 2 mg mL⁻¹, were used to treat DPC to study the dose response effect of HA on alkaline phosphatase (APL) activity. 2 mg mL⁻¹ treatment showed the strongest effect and was selected for subsequent experiments. The time course effect of HA on mineralization of dental pulp cell was monitored by measuring APL activity at 3, 7 and 14 days after treatment and Alizarin red staining at 21 days after treatment. The Human Osteogenesis PCR array was used to identify the genes involved in HA induced mineralization and the candidate genes were further confirmed by shRNA knockdown experiments and western blot. The control medium, osteogenic medium, and gingival fibroblast were used as control and all experiments were repeated at least 3 times.

Results High molecular weight HA induced ALP activity in a dose dependent manner, in which 2 mg mL⁻¹ induced 2.1-fold increase in ALP activity ($P < 0.01$) and the activity was peak at 3 days after treatment. Strong mineral deposition could be observed at 21 days after treatment. Osteogenesis PCR array identified MMP13, COL15A1, IBSP, BMP7 and ALPL were upregulated by HA treatment. Knockdown HA receptor, CD44, by shRNA significantly reduced COL15A1, BMP7 and ALPL expression in both mRNA and protein level. These results confirmed the three genes were involved in HA induced DPC mineralization.

Conclusions High molecular-weight HA was a good inducer to stimulate DPC mineralization. HA induced DPC mineralization through upregulating COL15A1, BMP7 and ALPL expression.

R180

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Calcitonin gene-related peptide release when neuropeptide Y1 receptor is activated in acute pulp inflammation

Aim To evaluate Calcitonin gene-related peptide release when the Neuropeptide Y1 receptor is activated in acute pulp inflammation.

Methodology A total of 18 Wistar rats weighing 250–260 g and aged between 6 to 8 weeks were used. Acute pulp inflammation was induced by preparing cavities in the maxillary and mandibular incisors. Pulp exposures were confirmed with bleeding upon probing. NPY1 agonist (Leu31, Pro 34) was placed in the pulp exposures. The animals were sacrificed at periods of 1, 3 and 7 days, the teeth were then sectioned using a cylindrical diamond bur in a high-speed handpiece irrigated with saline solution, and the pulp tissue was obtained using a sterile excavator. RT-PCR (Real Time-polymerase Chain Reaction) analysis and ELISA were carried out to detect and quantify the expression of CGRP (Potent peptide vasodilator). The Kruskal–Wallis and Mann–Whitney's test was performed to establish significance differences in values obtained from the groups ($P < 0.05$).

Results Calcitonine gene-related peptide receptor expression was found in all pulp tissue samples. The Kruskal–Wallis test revealed significant differences between the different periods of time ($P < 0.001$), with or without the application of the agonist. The difference in the group of 1 day with NPY1 agonist placement, was also significant ($P < 0.001$).

Conclusions The application of agonist NPY1 in acute pulp inflammation had an inhibitory effect on the release of CGRP in the early stages of inflammation.

R181

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Vascular network formation by DPSCs after angiogenic differentiation

Aim To determine the angiogenic potential of dental pulp stem cells (DPSCs) and their vascular network formation capacity

Methodology Isolation of DPSCs was achieved using freshly extracted human and swine teeth. Human (h) DPSCs or swine (s) DPSCs at passage 3 reaching 70%–90% confluency were cultured in endothelial growth medium (EGM) for 7 days. The expression of endothelial marker Von Willebrand Factor (vWF) was determined by immunostaining. To analyze the ability of induced DPSCs to form vascular networks, induced and non-induced cells at different cell numbers (10 000, 15 000, 20 000, 30 000 cells per well of 96-well) were cultivated in Matrigel. The formation of vascular networks was analyzed after 4, 8, 12, and 18 h. Quantitative analysis of vascular network formation was performed by acquiring the vascular networks images at different time points and analyzed using ImageJ. The vascular networks on the images were segmented, skeletonized and followed by detection of nodes, segments, meshes, and total length of tubes of the cellular meshed network organization. Data were expressed as mean \pm SD relative to human microvascular endothelial cells (HMEC) as controls using one-way ANOVA.

Results vWF was detected by immunofluorescence in non-induced DPSCs and its expression was increased in induced DPSCs (both human and swine). Observation under the light microscopy over a period of 24 h revealed that the vascular network was formed by induced DPSCs, but not non-induced DPSCs. After 4 h, induced DPSCs revealed vascular network formation while non-induced DPSCs started to aggregate and form clusters. Induced hDPSCs displayed a similar capacity to form vascular networks in Matrigel as HMECs based on quantitative analysis ($P > 0.05$). Induced sDPSC formed a better network of vascular-like structures including more segments, nodes and mesh compared to HMECs ($P < 0.05$).

Conclusions Both hDPSCs and sDPSCs have the capacity to form vascular networks *in vitro* after angiogenic induction.

R182

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Catalase activity in healthy and inflamed pulp tissues of permanent teeth in young people

Aim This study evaluated the defensive ability of human dental pulp against H_2O_2 in healthy and inflamed dental pulp tissues through determination of catalase activity by spectrophotometric methods.

Methodology Twenty patients between 15–25 years of age that had been diagnosed as healthy, were source of the pulp tissue. Pulp status was assessed using thermal and electrical tests, radiographic evaluation, and clinical assessment. Patients were divided two groups from which healthy and inflamed pulp tissues were obtained; each participant provided one pulp tissue specimen. Ten normal healthy pulps were extracted from mandibular wisdom teeth; these teeth were fully erupted, had clinically healthy crowns without restorations and were surrounded by a clinically healthy periodontium. Ten specimens were retrieved from teeth with inflamed pulp tissues; these

patient had spontaneous pain, and prolonged episodes of pain were produced by sudden temperature changes. The specimens were collected during endodontic treatment or by longitudinally grooving and splitting the teeth (if extracted). When pulp specimens were removed from the experimental teeth, they were immediately placed in plastic vials and washed in ice-cold, heparinized, sterile saline to remove blood. Samples were then stored at -80°C until analysed. Prior to biochemical analysis, the specimens were weighed and homogenized in 1 mL of 50 mM potassium phosphate buffer (pH 7.0). This homogenate was centrifuged at 10 000 *g* for 15 min at 4°C , and the supernatant was recovered, and used for the enzymatic activity determinations. Catalase activity was determined through spectrophotometric methods and an independent sample *t* test assessed the significance of differences between the groups.

Results There was a significant difference between healthy pulp tissue and inflamed pulp tissue ($P < 0.05$, independent sample *t* test). The catalase activity of the healthy group was significantly lower than the inflamed pulp group.

Conclusions Significant increases in catalase activity was found in inflamed dental pulps in comparison to healthy pulps. An inherent biological defense system against H_2O_2 is present in human dental pulp tissue.

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Interleukin-1 beta in asymptomatic periapical lesions: correlations between radiographic findings and the stage of root canal treatment

Aim To determine the levels of an immunological mediator (IL- β 1) in the periapical exudate of teeth with asymptomatic periapical lesions in relation to radiographic findings and the stage of root canal treatment.

Methodology The study was performed on a group of 22 patients (age 22–54 years). Periapical lesions were diagnosed from panoramic radiographs using the periapical index (PAI) with PAI 1 and 2 excluded. Periapical radiographic examination using the bisecting-angle technique was performed for the selected 30 teeth. The teeth were divided in three periapical lesion categories: incipient (PAI 3), medium (PAI 4), severe (PAI 5). The root canal system were accessed under isolation with rubber dam. The sampling of periapical exudate was performed with sterilized paper points, one size 40 paper point was subsequently inserted into the root canal close to the established working length and held for 2 min. The volume of the fluid was calculated from a standard curve. Each paper point was immersed into sterile Ependorf vials containing 50 μL phosphate buffered saline (PBS), vortexed for 1 min and stored at -70°C until time of assay. A further stage was represented by the root filling with $\text{Ca}(\text{OH})_2$ paste, maintained for 2 weeks. After this interval the paste was removed with NaOCl 3% and EDTA 17% and collection of periapical exudate was performed using the same protocol. The assessment of IL- β 1 levels in periapical exudate was carried out using the ELISA test (Interleukin- β 1, IBL International GMBH, Germany). Measuring of IL- β 1 levels was performed at baseline, after 14 days and after 30.

Results The levels of IL- β 1 were significantly higher for severe periapical lesions (mean value 623.3 ng mL^{-1}) compared with incipient periapical lesions (mean value 120 ng mL^{-1}). Related to the baseline levels, the levels of IL- β 1 decreased to 24.20% after 14 days and 5.22% after 30 days of root canal treatment with $\text{Ca}(\text{OH})_2$ paste. Significant differences were found between IL- β 1 levels at baseline and after 30 days of treatment.

Conclusions IL-1 beta levels released in asymptomatic periapical lesions correlated both with radiographic findings and stage of root canal treatment.

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In vitro evaluation of cytotoxic effects of materials used in vital pulp therapies

Aim This study aimed to compare the cytotoxic effects of materials used in vital pulp therapy, including MTA (Angelus MTA), calcium hydroxide (Dycal), light-cured calcium hydroxide containing resin (Calciol LC), light-cured MTA containing resin (TheraCal LC) on 3T3 fibroblast cells in culture.

Methodology Angelus MTA (G1) and Dycal (G2) were prepared according to manufacturer's instructions, and samples of TheraCal LC (G3) and Calciol LC (G4) were polymerized by a halogen light curing unit. All materials were standardized and etched into 1 mm width and 6 mm diameter teflon blocks. Prepared specimens were placed into 24-well culture plates making direct contact with the cells. Cells cultured only with culture medium served as negative controls (C). After the procedures, test materials were evaluated at 24 and 72 h. The trial was performed in triplicate for each material and each time interval. The Trypan blue exclusion assay was used for cell viability. The proliferation of cells was evaluated by BrdU assay. Apoptotic effects of materials was determined with AnnexinV-PI and cell morphology was also evaluated via Scanning Electron Microscopy. Statistical analysis was performed using independent the Student's *t*-test ($n = 6$).

Results After 24 h, total numbers and proliferation rates of cells were ranked as highest in $C = G4 > G1 = G3 > G2$ and the rates of apoptosis were ranked as highest in $G2 > G3 > G1 > G4 > C$. After 72 h, viability and measures of cell kinetics were ranked as highest in $C = G1 > G4 > G2 > G3$ and the rates of apoptosis were ranked as highest in $G3 > G2 > G4 > G1 > C$.

Conclusions The cytotoxicity of TheraCal LC was highest. Calciol LC had low cytotoxicity and was similar to Angelus MTA. However, Angelus MTA was the most biocompatible one among these materials. The reason why Calciol LC was less cytotoxic than TheraCal LC may be explained by the difference between the monomer contents of Calciol LC and TheraCal LC and the interactions between the contents of each material. Further *in vivo* studies and long-term clinical trials are needed to confirm the biocompatibility of these materials.

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Effects of composite films of silk fibroin and graphene on the proliferation and osteogenic differentiation of dental stem cells

Aim To investigate the effects of different composite films of graphene, silk fibroin and graphene combined with fibroin in the mesenchymal proliferation rate and osteogenic differentiation of periodontal ligament stem cells (PDLSCs).

Methodology PDLSCs were cultured on graphene, silk fibroin or a combination of graphene and silk fibroin films up to 10 days. Then, the proliferation rate of the PDLSCs was assessed using the MTT colorimetric assay. Also, gene expression for some osteogenic and cementogenic markers reported in the literature were assayed by qPCR: BMP2, RUNX2, SOX9, OSX, ALP, BGALP, DSPP, CEMP-1, CAP/PTPLA and GAPDH (control housekeeping gene). Statistical analysis was carried out using SPSS statistical software version 19. Differences between multiple groups were analyzed by ANOVA with a Tukey post-test. For all studies, a $P < 0.05$ was deemed significant.

Results At day 10 of culture, a significant increase in PDLSCs proliferation rate on graphene films compared to plastic ($P < 0.05$) was observed, as well as on graphene plus fibroin compared to fibroin alone ($P < 0.001$). Concomitant up-regulation of RUNX2, SOX9 and OSX also suggested an intermediate chondrogenic differentiation similar to that reported during endochondral ossification. Noteworthy, the best results were obtained for graphene-coated plates, which resulted in the highest up-regulation of SP7/Osx and ALP. The lack of expression found for BGLAP (osteocalcin) and DSPP (dentine sialophosphoprotein) indicated that complete differentiation-related gene expression may not be completed after 10 days. Moreover, the use of fibroin as substrate was of particular interest as it was associated with an over-expression of BMP2 and SP/Osx. Importantly, lone-reduced-graphene and the sandwich preparation including reduced graphene/fibroin, strongly promoted CEP-1 over-expression.

Conclusions The findings support the potential of fibroin or graphene constructs as osteoinducers and vehicles to deliver PDLSCs or other dental stem cells.

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HISTOPATHOLOGY: PERIAPICAL LESIONS

R186

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Histological investigation of root filled teeth with apical periodontitis

Aim To study the extent of inflammation in biopsies of periradicular tissue from root filled teeth and to correlate the inflammation to symptoms and time elapsed since root filling.

Methodology The study sample consisted of 199 biopsies from 180 patients. Consecutive biopsies, consisting of periradicular tissue from the root tip of previously root filled teeth were sent to the Department of Oral Pathology, Malmö University, Sweden for histological diagnosis. One oral surgeon undertook all biopsies. The specimens were routinely processed for histopathological diagnostics and examined with a light microscope. The histological variables registered were the extent of lymphocytes and polymorphic nuclear cells (PMNs) as well as the proportion of inflammatory cells in the biopsy. Based on the information given in the referral, the clinical variables registered were symptoms and time elapsed from the root canal treatment to the time of the surgical removal of the periradicular tissue.

Results Approximately half of the sample had a considerable accumulation of lymphocytes, a quarter of the sample a considerable amount of PMNs and in another quarter of the sample there were only slight signs indicative of an inflammatory process. The area in which the different inflammatory cells were found varied among the biopsies. Preliminary results show 105 of the patients to have symptoms. For 85 of the patients the root filling was performed 6–50 years prior to apical surgery.

Conclusions The amount of lymphocytes and PMNs differed considerably in a sample of periradicular tissue from previously root filled teeth committed to apical surgery. The correlation of the extent of inflammation in the biopsies to symptoms and time elapsed since root filling is yet to be analysed.

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R187

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Influence of osteoporosis on experimental periradicular lesions in rats

Aim The aim of this study was to evaluate the effects of osteoporosis on the development of periradicular lesions in Wistar rats ($n = 24$).

Methodology Twelve animals were ovariectomized (OVX group) and the other 12 were sham operated (control group). One-hundred-and-twenty days after castration, the pulps of the left mandibular first molars were exposed in order to induce the development of periradicular lesions. Body mass was verified on a weekly basis. Following 21 and 40 days of lesion induction, the animals were sacrificed. Blood was collected for biochemical anal-

ysis (alkaline phosphatase, calcium and estradiol), and the mandibles were removed for radiographic analysis. Comparative analysis was performed using nonparametric tests (Kruskal–Wallis test, and Dunn multiple comparison test). Statistical significance was set at $P < .05$.

Results The periradicular lesions evaluated radiographically were larger in the 40-days OVX group when compared with both 40-days ($P < 0.05$) and 21-days control ($P < 0.001$) groups. Serum oestrogen levels were lower in the OVX group ($P < 0.01$), confirming the efficacy of castration. Oestrogen deficiency resulted in greater body mass gain ($P < 0.01$) in the 40-day OVX group when compared with 40-day control group. Serum concentrations of calcium and phosphorous were similar between the groups ($P > 0.05$). Alkaline phosphatase levels, although higher in the OVX groups (21 and 40 days), were not significantly different.

Conclusions Ovariectomized rats had significantly larger periradicular lesions after 40-days of pulp exposure when compared with controls. These findings suggest that osteoporosis can act as a disease modifier, influencing the progression of apical periodontitis.

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In situ detection of Epstein-Barr virus-encoded small RNA in periapical granulomas

Aim Epstein-Barr virus (EBV) is an oncogenic herpes virus that infects a significant percentage of the population worldwide and is the causative agent of infectious mononucleosis. On the other hand, an association between EBV and inflammatory reactions inducing cytokine expression by inflammatory cells has been demonstrated. A correlation between EBV infection and rheumatoid arthritis and Sjogren syndrome has also been shown. Thus, EBV infection could be related to tissue injury and cell damage in periapical granulomas. The aim of this study was to examine whether EBV infects inflammatory cells in periapical granulomas.

Methodology This study was approved by the ethics committee in the university, and written consent was obtained from patients prior to the collection of the lesions. Surgically removed periapical lesions ($n = 40$) were analysed by haematoxylin-eosin stains using the paraffin section. Healthy gingival tissues ($n = 5$) were obtained as a healthy control. EBV DNA was detected using real-time polymerase chain reaction (PCR). In situ hybridization (ISH) using Epstein-Barr virus-encoded small RNA (EBER)-specific probes was employed, and periapical granulomas ($n = 9$), showing more than 10 000 copies of EBV by real-time PCR were examined, because of the detection limit of ISH.

Results Of 40 specimens, 32 were histologically determined as periapical granulomas. EBV DNA was detected in 25 out of 32 periapical granulomas, whereas none of the healthy gingival tissues showed EBV. The median number of EBV DNA copies was 8688.01/microgram total DNA. EBER *in situ* hybridization of EBV-positive periapical granulomas demonstrated that six of nine periapical granulomas had positive expression of EBER; whereas, none of the healthy gingival tissues ($n = 5$) expressed EBER. EBER-positive cells were B lymphocytes and plasma cells, as determined morphologically.

Conclusions The data suggested the possibility that B lymphocytes and plasma cells are infected by EBV, and EBV could be associated with the pathogenesis of periapical inflammation.

R189

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The effect of melatonin on experimentally induced periapical lesions: radiological evaluation

Aim Melatonin has been investigated in relation to periodontal disease, oral cancer, osteointegration of dental implants, and inflammatory lesions of the oral cavity. Recent studies have shown that melatonin promotes osteoblast maturation and enhances bone formation. Because of the antioxidant and anti-inflammatory effects of melatonin, an increase in melatonin levels may improve an organism's defensive response to the inflammatory process and bone destruction. The aim of this preliminary study was to assess radiographically the effects of melatonin on periapical bone destruction in rats.

Methodology Thirty adult Sprague-Dawley rats were divided equally into negative, positive control and treatment groups, and the pulp chambers of mandibular first molars of positive control and treatment rats were exposed to the oral environment to induce periapical lesions. The treatment group received daily intraperitoneal injections of melatonin at a dose of 10 mg kg⁻¹, whereas the control groups received only the 10% ethanol vehicle. After 21 days following pulp exposure, the rats were sacrificed, and the mandibles were separated into hemi-mandible parts and radiographically evaluated for periapical bone loss area. Periapical lesion dimension and pixel values of the region of interest at the periapical region of each tooth were evaluated with Image J (version 1.48v; National Institute of Health, USA). Data were analyzed using Repeated Measurement Analysis ANOVA and Tukey's tests.

Results There was a significant difference between the positive control and treatment groups regarding the dimension of periapical lesion ($P < 0.01$). The periapical bone loss area was significantly smaller in rats that were given daily intraperitoneal injections of melatonin ($P < 0.01$). Positive control and treatment groups did not differ in terms of pixel value ($P > 0.05$), however the negative control group had a greater value of pixel ($P < 0.01$).

Conclusions Administration of melatonin to rats inhibited alveolar bone resorption associated with periapical disease. However, this must be supported with extended histological and immunological studies.

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R190

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The influence of antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) on the level of proangiogenic vascular endothelial growth factor (VEGF) in human periapical granulomas

Aim VEGF-induced angiogenesis can contribute to inflammatory and bone resorption processes with the following formation of granulomas in periapical tissues. Antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs) indicated in the treatment of chronic exacerbated periodontitis inhibit processes involved in inflammatory and pain signalling pathways. The purpose of this study was to investigate the influence of these medicaments on the levels of proangiogenic VEGF in periradicular lesions.

Methodology Granulation tissue biopsies were obtained from 56 patients with chronic periapical periodontitis at the time of tooth extraction. VEGF concentration was quantified by RayBio Human VEGF ELISA Kit (RayBiotech, Inc.) in 4 groups of patients: without any drug intake (control group, $n = 25$), with NSAIDs ($n = 7$), with antibiotics ($n = 5$) and with both, NSAID and antibiotic implementation ($n = 5$). Due to severe intake of a wide range of medications, biopsies of 14 patients were excluded from the study. The specimens were collected after at least 2 days of NSAIDs and 5 days of antibiotic therapy, were frozen at -70°C and immersed in liquid nitrogen after storage. Results were calculated to 1 g of granulation tissue and analyzed with the ANOVA test ($P < 0.05$). In all 56 samples the reverse transcriptase (RT) reaction was performed using the Enhanced Avian HS RT - PCR Kit (Sigma Aldrich, Germany). Presence of VEGF-A and VEGF-B genes expression was checked using the qualitative polymerase chain reaction method (PCR).

Results Enzyme-linked immunosorbent assay analysis revealed that VEGF levels in granulation tissue were significantly lower in patients classified into NSAIDs ($6096.7 \text{ pg mL}^{-1}$, $P = 0.008$) and antibiotics ($5660.9 \text{ pg mL}^{-1}$, $P = 0.01$) groups in comparison with the control patients without pharmacotherapy ($10\,432.4 \text{ pg mL}^{-1}$). Values for NSAIDs with antibiotics group ($7141.9 \text{ pg mL}^{-1}$, $P = 0.07$) were not significant. For determination of VEGF-A and VEGF-B gene expression in all studied specimens PCR product for reference gene – ACTB was shown. In the biopsies PCR product for VEGF-A gene was not demonstrated, however, it was present for VEGF-B gene in 37 samples.

Conclusions The use of NSAIDs and antibiotics in patients with chronic exacerbated apical periodontitis decreased VEGF levels in human periapical granulomas. These findings suggest that evaluated adjuvant pharmacotherapy may alleviate VEGF-connected consequences of apical periodontitis progression.

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R191

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Expression levels of matrix metalloproteinase-2 and gram-negative bacteria in symptomatic and asymptomatic periapical lesions

Aim To determine whether the expression of matrix metalloproteinase (MMP-2) is significantly elevated in patients with symptomatic apical periodontitis and to correlate this with the detected amount of gram-negative bacteria.

Methodology Twenty patients with periapical lesions were divided into 2 groups: ten symptomatic (SYM) group included 10 patients with painful periapical lesions, and the asymptomatic (ASYM) group included 10 patients with no pain. All teeth had been root canal retreated at least 2 year before the periapical surgery. The periapical lesions were collected and the periapical lesions were serially cut into 4 µm sections. At least 2 sections were processed for histological examination using haematoxylin-eosin stain. Other sections were processed for immunohistochemical examination. For MMP-2, the area fraction of the positive cells was measured, and the percentage of the MMP-2 immunopositive area to the total area of the microscopic field was calculated. For gram-negative stain cells, the number of bacterial cells showing the pink-red color was counted per microscopic field. The Student's test was used to compare SYM and ASYM groups. The Pearson correlation coefficient was used to determine a significant correlation between the number of bacterial cells and MMP-2 levels. The significance was set at $P < 0.05$.

Results For MMP-2 area percent the ASYM group had a significantly higher mean than the SYM group ($P = 0.027$). The SYM group had a significantly higher mean number of gram-negative cells ($P = 0.003$). There was no significant correlation between the number of gram-negative cells and the MMP-2 area percent ($P = 0.187$).

Conclusions Gram-negative bacteria may play an important role in symptomatic lesions. On the other hand, the expression of MMP-2 may have a significant influence in asymptomatic lesions.

R192

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Immunohistochemical detection of calcitonin-gene related peptide (CGRP), S-100 and PGP9.5 proteins in periapical cysts and periapical granulomas

Aim To evaluate the immunohistochemical expression of neuropeptide CGRP and neuronal markers S-100 and PGP9.5 in periapical cysts and periapical granulomas.

Methodology Positive staining and distribution of CGRP, S-100 and PGP9.5 were examined in 30 well-defined periapical cysts (PC), 5 periapical granulomas (PG) and 1 odontogenic keratocyst (OKC) using archived formalin-fixed, paraffin-embedded tissues. Monoclonal antibodies against human CGRP, S-100 and PGP9.5 were used to evaluate their expression.

Results CGRP was detected in perivascular neurons of 6 periapical cysts and in ciliated-epithelium remnants of a periapical cyst from the maxilla. S-100 protein was found to be expressed by Langerhans cells in suprabasal epithelial layers in 83.3% (25/30)

of PCs and by antigen-presenting cells of the surrounding connective tissue of all (100%) PCs and PGs, particularly in areas with intense inflammatory infiltration. S-100 was expressed by neurons or nerve fibres in the connective tissue of all specimens and was used as an internal positive control. Immunoreactivity for PGP9.5 was restricted to neurons in the connective tissue of all PCs and PGs, whereas in 33.3% (10/30) PCs PGP9.5 was expressed by subepithelial fibroblast-like attractoid cells. In the OKC, S-100 was detected in nerve fibres and antigen-presenting cells of the connective tissue and in basal melanocytes, whereas PGP9.5 was detected in neurons and nerve fibres.

Conclusions Taking into account the limitations of the experimental approach used in the present study, the presence of these proteins in PCs and PGs may participate in periapical inflammation and indicate their role in the development of the above mentioned lesions.

R193

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Cytokine expression in patients with acute apical abscess

Aim To compare serum levels of cytokines Th1, Th2, Th17 and Treg in patients with acute apical abscess (AAA) from endodontic origin, in onset and post antibiotic treatment.

Methodology Thirteen patients with diagnosis of AAA and trismus were included. Serum samples were obtained at baseline and 7 days after antibiotic management with Amoxicillin and clavulanic acid. Thirteen healthy subjects (HS) matched on age and gender, were included as controls. Quantification of cytokines (IL-1β, IL-2, IL-12p70, IFN-γ, TNF-α, IL-4, IL-13, IL-6, IL-17, IL-10) was performed using the Bioplex-MAGPIX[®] system (Bio-Rad Life Science, USA). Mann-Whitney U test and Wilcoxon test were used for nonparametric distribution data. Correlations were examined by Spearman's rank correlation test. Statistical significance was considered at $P < 0.05$.

Results The levels of Th2 cytokines IL-6 and IL-10 were higher at baseline in patients with AAA compared to HS ($P < 0.05$). No differences between patients with AAA and HS for the cytokines IFN-γ, IL-4 and IL-17 were observed. Cytokines IL-1β and IL-13 were undetected. While levels of IL-2, IL-12 and TNF-α were lower in patients with AAA respect to the HS ($P < 0.05$). On the other hand, AAA patients had higher levels of Th2 (IL-6, IL-10) and Th1 (TNF-α) cytokines at baseline compared to after antibiotic treatment. IL-6 had the highest difference levels. In addition, correlation between IL-6 and IL-10 was found ($r = 0.77$, $P < 0.001$) in AAA patients.

Conclusions IL-6 is an important pro-inflammatory cytokine, which participates in bone resorption by activation and differentiation of osteoclasts. IL-6 was associated with severity of the inflammatory response in AAA, decreasing when the aetiological agent was controlled. The results suggest that IL-6 could play an important role in the pathogenesis of AAA, and their homeostasis with IL-10 is necessary for resolution of inflammatory response.

R194

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Influence of cemental canal instrumentation on the repair of periapical lesions

Aim To evaluate the influence of cemental canal instrumentation on the repair of induced periapical lesions in a rat model.

Methodology Periapical lesions were induced in first mandibular molars of 22 rats. After 3 weeks, teeth were divided into two experimental groups: G1 (12 canals) – chemomechanical preparation was performed with hand instruments and 2.5% sodium hypochlorite irrigation, up to the apical foramen, including the cemental canal, which was established by an electronic apex locator; G2 (10 canals) – same procedures without instrumentation of the apical segment (1 mm short of the apex). Root canals in both experimental groups were filled with gutta-percha and AH Plus. The negative control group (22 canals) consisted of the same teeth on the opposite side, which were left open to the oral cavity and untreated. The animals were sacrificed on day 21, their jaws were dissected, and radiographs were taken to measure the area of periapical lesions. Histological analysis evaluated the following parameters: intensity of the inflammatory infiltrate, apical cementum resorption and formation of new cementum (biological sealing). Radiographic data were compared by Student t-test and histological results were analysed by Fisher exact test ($\alpha < 0.05$).

Results Instrumentation of the cemental canal was significantly effective in promoting the formation of new cementum/biological sealing ($P = 0.040$), and allowed decreased intensity of the inflammatory infiltrate when compared to the negative control group ($P = 0.038$).

Conclusions Instrumentation of the cemental canal can be an effective alternative to create more favourable conditions for apical tissue repair.

CLINICAL TRIALS

R195

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Infection control in retreatment cases: evaluation of the antibacterial effects of two different systems

Aim The aim of this *in vivo* study was to compare the antibacterial effects of two endodontic systems, the Self-Adjusting File (ReDent-Nova, Israel) and Twisted File Adaptive (SybronEndo, USA), used during the chemomechanical re-preparation of root canals in teeth with post-treatment apical periodontitis.

Methodology Forty-two single-rooted single-canal teeth with post-treatment apical periodontitis were selected according to stringent inclusion/exclusion criteria. For retreatment, teeth were randomly divided into two groups according to the canal instrumentation technique (the Self-Adjusting File and Twisted File Adaptive). In all groups, 2.5% NaOCl was the irrigant. Bacteriological samples were taken before (S1) and after chemomechanical

preparation (S2). DNA was extracted from the clinical samples and subjected to quantitative real-time polymerase chain reaction (qPCR) to evaluate the levels of total bacteria and streptococci. Statistical analysis from qPCR data were performed within groups using the Wilcoxon matched pairs test and between groups using the Mann–Whitney U-test and the Fisher's exact test, with significance level set at $P < 0.05$.

Results All S1 samples were PCR positive for bacteria. Both instrumentation protocols were associated with a highly significant intracanal bacterial reduction ($P < 0.001$). Intergroup quantitative comparisons disclosed no significant differences between the groups ($P > 0.05$).

Conclusions Both instrumentation techniques/treatment protocols were highly effective in significantly reducing the total bacterial counts. No significant difference occurred between the two systems in disinfecting canals of teeth with post-treatment apical periodontitis.

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R196

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Effect of different canal preparation techniques on postoperative pain in teeth with non vital pulps and periapical lesions

Aim The aim of the study was to compare the incidence and intensity of postoperative pain related to several root canal instrumentation techniques during single visit root canal treatment of teeth with non vital pulps and periapical lesions.

Methodology Ninety patients requiring root canal treatment in single root/canal with non vital pulps were included. The patients were assigned into three equal groups ($n = 30$). Different instrumentation techniques were used for each group during mechanical preparation of the root canals. In group 1, the canals were instrumented with a modified crown-down technique using hand instruments (HF); in group 2, the canals were instrumented with WaveOne (WO); and in group 3, the canals were instrumented with ProTaper Next (PTN) files. All canals were shaped, cleaned, and filled in a single-visit. The assessment of post-operative pain was carried out 48 h after the initial appointment by one independent clinician blinded to the groups. The presence or absence of pain, or the appropriate degree of pain was recorded by using a 4-point pain intensity scale. Statistical analyses were performed with SPSS 19.0 software and differences among the groups were analyzed by the Kruskal–Wallis test. The Dunn's test was used for *post-hoc* test after Kruskal–Wallis test.

Results Severe pain was only recorded at the 12 h period. The incidence and intensity of postoperative pain in all groups gradually reduced over the study period. Postoperative pain was not significantly different at the 12 and 24 h periods. The difference in 48 h period was significant among the groups ($P = 0.04$). The HF group had significantly lower postoperative pain than PTN ($P = 0.018$) and WO ($P = 0.020$) groups. However, no difference was found between WO and PTN groups ($P > 0.05$).

Conclusions The instrumentation technique did not affect the intensity of postoperative pain at 24 h. Hand file instrumentation was associated with lower postoperative pain than the other two instrumentation techniques at 48 h.

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R197

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Clinical and microbiological evaluation of 0.5% versus 3% sodium hypochlorite in root canal treatment. A randomised controlled trial

Aim To compare the antimicrobial and adverse effects of irrigation with 0.5% NaOCl versus 3% NaOCl during root canal treatment.

Methodology Consecutive patients referred to the Specialist Clinic of Endodontics, Public Dental Health, Gothenburg, Sweden were randomly assigned to have the root canal treatment performed with irrigation either with a buffered 0.5% NaOCl (Dakin's) or 3% NaOCl solution. Teeth with vital or necrotic pulps and retreatment cases diagnosis were included. Root canal treatment procedures followed the routines of the clinic. Immediately before the root filling a microbial sample was taken from the operative field and the root canal. The bacterial samples were processed at the laboratory as outlined by Möller (1966). After each visit the patient was instructed to fill in a questionnaire and assess his or her post-operative pain on a visual analogue scale (VAS), with endpoints 0 = 'no pain' and 10 = 'worst imaginable pain', for seven consecutive days. The Fischer's exact test was used for statistical analyses of the root canal samples and t-test for postoperative pain VAS levels.

Results So far 175 patients (98 women and 77 men) have been recruited. Mean age is 52 years (Range 18 years–89 years). 84 patients have been allocated to the 3%- group and 47 patients to the 0.5%- group. Results from 139 cultures and 120 questionnaires were available for analyses. The number of positive cultures in the 3%-group is 12 (25%) and in the 0.5%-group 12 (13%) ($P = 1.00$). No significant differences in postoperative pain were found between the groups.

Conclusions No significant differences between the two concentrations of NaOCl were detected either in terms of cultivable microorganisms or postoperative pain.

R198

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Prevalence of apical periodontitis in patients affected by inflammatory bowel diseases and under immunomodulators

Aim To evaluate the status of oral health, and the prevalence of apical periodontitis (AP), in patients with inflammatory bowel diseases (IBD) and treated either with Biologic medications (BM) or with corticosteroids (CS).

Methodology One hundred patients (45 males and 55 females, age 44 ± 14.1) from the Gastroenterology Unit of the University Hospital, with inflammatory bowel diseases (IBD) or with both IBD and rheumatoid arthritis (RA), treated with BM or with CS were included in the study. One hundred patients who registered for a dental check-up at the Dental Clinic, and matched for age, sex, and physical characteristics with the study group, without systemic diseases and not under medications were the control. Patients underwent a complete oral, dental and radiographic examination. DMFT and PAI indexes were recorded. The Chi-square test was used to determine the differences between the groups ($P < 0.05$).

Results DMFT was 9 in the control and 8.5 in the IBD patients. The prevalence of AP was 64% in IBD patients and 57% in the control and was significantly different. The the number of teeth with AP was significantly higher in women than in men with IBD. The prevalence of AP in patients treated with BM and with CS was 63.9% and 64.1% respectively. The mean PAI was 1.5 in the control and 2.8 in IBD (2.8 BM; 2.8 CS) group. In both groups the percentage frequency of endodontically treated teeth with AP was higher than in untreated teeth.

Conclusions Patients with IBD, and under immune modulators had a higher prevalence of AP than healthy subjects which in turn was significantly higher in women than in men with IBD. These data may emphasize the importance of the status of the immune system in the onset and response to treatment of AP. Further studies are needed to confirm these results.

R199

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Clinical effectiveness of two integrated systems for the treatment of maxillary central incisors with periapical lesions: 6-month results

Aim To assess the early success rate of root canal treatment of maxillary central incisors with periapical lesions making use of two integrated shaping and filling techniques.

Methodology The present study enrolled 43 patients (45.3 ± 18.5 years old) with an untreated maxillary incisor with chronic periapical pathosis having radiographic diameter smaller than 5 mm. By computerized randomization procedures, the patients were allocated to two treatment groups with integrated shaping and filling system and received single-session root canal treatment with Revo-S/One Step Obturator in group 1 (G1, $n = 20$) or GTX/GTX Obturator in group 2 (G2, $n = 23$). The irrigation protocol was standardized and consisted of rinses with 5.25% sodium hypochlorite. Clinical assessment and preliminary radiographic examination were performed at baseline and repeated after 6 months. Radiographic healing scores based on a previously described scale were attributed by two blind examiners. Comparability of baseline clinical data between the groups was tested with an independent samples *t*-test (age, apical gauging) and chi-square test (positivity to percussion and palpation). The comparison of the two groups in terms of radiographic scores and clinical data registered at recall were compared with a Mann–Whitney test and chi-square test, respectively ($\alpha = 0.05$).

Results The groups had similar baseline parameters. No drop-outs occurred during the observation time. The radiographic assessment revealed total healing, partial healing and failure in 45.0%, 55.0% and 0% of cases in G1 and in 52.2%, 43.5% and 4.3% of cases in G2. One patient per group was still reporting symptoms at the six-month recall. No significant differences between groups were found.

Conclusions The two integrated endodontic systems allowed for high and comparable 6-month success rate in central incisors with necrotic pulps. Cases presenting incomplete healing will be further followed even in the absence of symptoms.

R200

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The antibacterial effect of Nd:YAG laser irradiation compared to 1% NaOCl irrigation in teeth with apical periodontitis

Aim To evaluate the antibacterial effect of Nd:YAG laser irradiation compared to irrigation with 1% unbuffered sodium hypochlorite (NaOCl) solution in teeth with apical periodontitis.

Methodology A total of 41 patients (45 teeth) with apical periodontitis in single-rooted, endodontically untreated teeth were allocated to laser ($n = 22$) or control ($n = 23$) groups following initial root canal sample and block randomization. All teeth were instrumented with manual stainless steel K-files up to size 20 and then with rotary instruments up to minimum size 30, 04 taper. The last file was required to be at least 3 sizes larger apically than the first apically binding file. Teeth in the laser group were irrigated with a saline solution during instrumentation, dried and lased with Nd:YAG laser (Fotona Fidelis II+, Fotona D. D., Slovenia) according to a standard procedure. Teeth in the control group were irrigated with 1% unbuffered NaOCl-solution and 15% EDTA. At the end of the first treatment session a second root canal sample was obtained. No antibacterial dressing was used and after 2–4 days a third root canal sample was obtained from all teeth. The bacterial samples were blinded and immediately sent for culturing and analysis to the Department of Microbiology and Immunology, Institute of Odontology at The Sahlgrenska Academy, University of Gothenburg, Gothenburg. Chi-square test and Fisher's exact test were used for statistical analysis.

Results Initial infection could be cultured in 20 of 22 in the laser group and 18 of 23 ($P = 0.414$) in the control group. After initial treatment 11 of 21 in the laser group and 13 of 23 ($P = 0.768$) in the control group yielded negative bacterial samples. After 2–4 days with no antibacterial dressing in the root canals, 5 of 22 in the laser group and 9 of 23 ($P = 0.337$) in the control group yielded negative bacterial samples.

Conclusions The results suggest that Nd:YAG laser treatment of infected root canals did not give more negative root canal samples than conventional chemomechanical treatment in combination with NaOCl.

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R201

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Treatment outcomes of quick-setting calcium-silicate cement direct pulp capping: 18-month results

Aim To assess the treatment outcomes of direct pulp capping with Biodentine, a quick-setting calcium-silicate based dental cement.

Methodology This study was conducted with subjects who were recruited from the pool of patients from five dental practices

between 2009 and 2014. Sixty-one asymptomatic permanent teeth with pulp exposures were treated by pulp capping using Biodentine (Septodont, France). The clinical and radiographic evaluations were performed at 18 months after treatment. Teeth with no response to pulp sensibility tests and those exhibiting clinical or radiographic signs and/or symptoms of irreversible pulpitis or pulp necrosis were considered to be failures. The Fisher exact test and the Kaplan–Meier method were used for analysis with a significance level of $P < 0.05$.

Results Forty-two patients with fifty-three teeth attended the follow-up examinations (recall rate = 86.9%). Forty-five (84.9%) were classified as 'success,' and 8 teeth (15.1%) were considered 'failure.' Success was associated more with mechanical exposure than with carious exposure ($P < 0.05$) and more with Class I occlusal restorations than with Class II restorations ($P < 0.05$). Patients' age and gender had no significant effect on the outcome ($P > 0.05$).

Conclusions Direct pulp capping with Biodentine was a successful therapy when the respective indications are employed.

R202

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Clinical susceptibility of bacterial complex to different intracanal medications and correlation with clinical/radiographic features

Aim The aim of the present study was to correlate clinical/radiographic features and bacterial complexes and verify the susceptibility of complexes to different intracanal medication.

Methodology Microbiological samples were taken from 30 root canals with primary endodontic infection. The presence of bacterial complexes were determined by checkerboard DNA-DNA hybridization before and after root canal treatment using calcium hydroxide, calcium hydroxide + 2% chlorhexidine or calcium hydroxide + 20% glycolic ginger extract as intracanal medication. The relationship between clinical features and bacterial complexes was tested with the linear trend test. Correlation of specific bacterial species and clinical features was tested with the Fisher's exact test. Wilcoxon matched pairs test and Mann–Whitney *post hoc* Dunn was used to observe the effects of each intracanal medication ($P < 0.05$).

Results There was a positive association between pain on palpation and purple ($P = 0.017$), green ($P = 0.007$) and orange ($P = 0.018$) complexes. The presence of a sinus tract was associated with the red complex ($P = 0.038$). Presence of *P. gingivalis* ($P = 0.043$) and *T. denticola* ($P = 0.02$) was associated with pain on percussion. Pain on palpation was associated with *T. denticola* ($P = 0.009$) and *E. corrodens* ($P = 0.039$). Sinus tract was associated with *P. gingivalis* ($P = 0.04$), *F. nucleatum* ssp *vincentii* ($P = 0.045$), *T. socranskii* ($P = 0.043$), *V. parvula* ($P = 0.004$) and *P. intermedia* ($P = 0.044$). Periapical bone resorption were associated with *C. ochracea* ($P = 0.042$). All treatments decreased the DNA load of the bacterial complex. However, associations were more effective, especially calcium hydroxide + 2% chlorhexidine.

Conclusions The present study revealed that different bacterial complexes are related with the development of clinical features. Moreover, a combination of bacterial species was involved in endodontic disease. The intracanal medication calcium hydroxide + 2% chlorhexidine was more effective on bacterial complexes.

R203

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Post-operative quality of life following root canal preparation performed by rotary, swaggering or reciprocating instrumentation: a randomized clinical trial

Aim Root canal treatment may be associated with pain and discomfort, which can affect an individual's quality of life (QoL). Reciprocating instruments are suspected to extrude debris beyond the apex, with occurrence of post-operative discomfort. No data exist concerning NiTi rotary instruments with off-set section design and swaggering motion. This randomized controlled clinical trial compared the impact of rotary, swaggering and reciprocating instrumentation on post-operative QoL (POQoL).

Methodology One hundred and twenty patients with a single tooth requiring primary root canal treatment were recruited and randomized between three groups following access cavity and glide path preparation. A total of 102 patients were analyzed: 30 teeth were shaped with ProTaper™ S1-S2-F1-F2 at working length (rotary group); 32 teeth were shaped with ProTaper Next™ X1-X2 at working length (swaggering group); 30 teeth were shaped with WaveOne™ Primary (reciprocating group). Irrigation was performed with 5% NaOCl and 10% EDTA. Root filling was performed with continuous wave or Therafil techniques in single or multiple visits. POQoL indicators were evaluated for 7 days post-treatment. The variation of each indicator over time was compared using ANOVA for repeated measures ($P < 0.05$). The impact of each variable on POQoL was analyzed with a multivariate logistic regression model ($P < 0.05$).

Results Patients' perception of the impact of treatment on POQoL did not differ between groups ($P = 0.094$). Pain curves demonstrated a more favorable time-trend in the rotary and swaggering groups (mean, $P = 0.028$; maximum, $P = 0.042$). Single-visit approach and the presence of pre-existing peri-radicular inflammation were all associated with a moderate decrease in POQoL.

Conclusions Reciprocating instrumentation may adversely affect POQoL to a greater extent than rotary or swaggering instrumentation.

R204

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In vivo evaluation of the success of root canal treatment of teeth restored with glass fibre posts

Aim The aim of this randomized controlled study was to evaluate the success of root canal treatment in teeth restored with glass fibre posts in comparison to cast metal posts.

Methodology This prospective randomized clinical study consisted of 86 adult patients, with a total of 121 teeth, who presented for the first time seeking routine dental care at the Dental School, between 2009 and 2013. Patients returned for clinical and radiographic evaluation every 6 months after completion of the crown restoration up to 4 years follow-up. The teeth were grouped according to the radiographic evaluation of the root filling; apical status was assessed using the periapical index (PAI). Survival probabilities were analyzed using Kaplan-Meier statistics and log-rank test ($P < 0.05$).

Results Of the 121 root filled teeth with posts, 81 (66.9%) were fibre posts and 40 (33.1%) were cast posts. The number of teeth with adequate density of root filling was 114 (94.2%), and the number of teeth with adequate apical limit of filling was 110 (90.9%). Five cases (4.1%) were considered unsuccessful. All cases of failure were observed at 12 months of follow-up. Significant differences between groups according to post type and tooth type were not found ($P > 0.05$). Although the failures occurred only in glass fibre posts, endodontic failures were not related to post type.

Conclusions As long as a strong marginal adaptation of the final restoration is achieved, endodontic treatment success is not related to the type of intraradicular post and tooth type.

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OUTCOME STUDIES

R205

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The association between technical quality of root filling and treatment outcome

Aim To evaluate the technical quality of root canal treatment performed by postgraduate students in Karolinska Institutet, Stockholm, Sweden during the years (2008–2011) and to evaluate the treatment outcome. In addition, possible influencing factors to treatment outcome were analyzed.

Methodology All maxillary first molars that received root canal treatment between 2008 and 2011 were included. Pre, post and follow up radiographs were collected. The apical status (PAI-score), the quality of the root canal treatment and the treatment outcome were assessed. The analysis of the treatment outcome in relation to the quality of root fillings and other possible influencing variables was performed by Chi square or Fisher's Exact test, and the statistical significance was set at $P < 0.05$.

Results A total of 73 teeth were included. Out of these, sixty-three teeth (86.3%) had periapical lesion at the time for RCT, while the rest (13.7%) had no periapical lesion. A primary root canal treatment was performed in 46 teeth (63%) and 27 teeth (36.9%) were retreated. There was no significant association between the outcome and the type of treatment. The majority of the RCTs (58%) had good seal and correct length. In total, the treatment outcome was successful in 59 (81%) of the cases. The success rate was 88% in cases with high quality root fillings, compared to 71% in cases with less good quality. There was a non-significant trend ($P = 0.066$) of an association between RCT quality and treatment outcome.

Conclusions The association between the quality of the root filling and the treatment outcome was not verified with statistical significance, but the results indicate a trend that satisfactory root fillings are associated with a favorable outcome. This study was limited by its retrospective nature and a relatively small sample size.

R206J.N. Lui^{1,*}, G. Krishnaswamy² & N.N. Chen¹¹Department of Restorative Dentistry, National Dental Centre Singapore, Singapore & ²Centre for Quantitative Medicine, DUKE-NUS Graduate Medical School, Singapore**Evaluation of apexification outcomes with calcium hydroxide and mineral trioxide aggregate****Aim** To evaluate the outcome of apexification with calcium hydroxide or mineral trioxide aggregate in patients with immature permanent teeth with a prospective follow-up.**Methodology** All patients who had undergone an apexification procedure on an immature permanent tooth at the National Dental Centre Singapore from 1997 to 2009 were invited to return for a clinical and periapical radiographic examination. Data from each patient record was entered into a data collection form. Radiographic evaluation of healing was performed by 2 investigators blinded to the material used for apexification. Outcomes were categorized into success and failure based on clinical and radiographic parameters. Statistical analysis was performed using SAS version 9.2.**Results** Fifty one patients with 58 teeth were examined. The patient age was 13 ± 7 years with a range of 6–38 years. Twenty-nine teeth had been treated with calcium hydroxide (CaOH) and 16 teeth with mineral trioxide aggregate (MTA). Thirteen teeth were initially treated with calcium hydroxide for several visits, but converted to MTA (Combined). The review time post-treatment completion was 3.6 ± 2 years, ranging from 0.5 to 8 years. Similar successful outcomes in treatment were obtained using CaOH (26 teeth, 90%) versus MTA (12 teeth, 75%) versus Combined (13 teeth, 100%) (Fisher's exact test, $P = 0.13$). A multiple comparison test showed that number of treatment visits was similar for CaOH (4 ± 2.4) and MTA (3 ± 0.62) but significantly more in the Combined group (6 ± 1.9) (ANOVA Scheffe test, $P = 0.0002$). A multivariable logistic regression model (c -statistic = 0.82) revealed that decrease in age and presence of apical barrier were significant predictors of success; they increased the odds of success by 9% and four times respectively.**Conclusions** In immature permanent teeth, use of MTA for apexification produced similar success rates to calcium hydroxide. Predictors of success were patient age and presence of apical barrier post treatment.**R207**

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Evaluation of avulsed and replanted teeth following the 2012 IADT guidelines after 1-year**Aim** The identification of factors involved in the initiation and progression of root resorption after replantation of avulsed teeth has been the focus of numerous studies. The extra alveolar time and the storage medium into which the avulsed tooth is maintained until replantation receive particular importance by the International Association of Dental Traumatology (IADT), being considered determining factors for the success or failure of replantation. The aim of this study was to assess the presence or absence of root resorption in avulsed and replanted teeth that followed the 2012 IADT guidelines for extra-alveolar time and storage medium after 1 year of clinical and radiographic control.**Methodology** One hundred forty-five teeth replanted and treated in the Clinic of Trauma of the Dental School of the Pontifical Catholic University of Paraná were clinically and radiographic assessed at 1 year following replantation. Information on the procedures carried out from the moment of avulsion until replantation were collected and counted.**Results** Of 150 teeth, five (3.33%) were not replanted. Of the 145 (96.67%) replanted, 83 (57.25%) followed the 2012 IADT guidelines. Seventy-six (52.41%) teeth did not have signs of resorption, 28 (19.31%) had resorption in progress and 18 (12.41%) teeth had been lost. Of all the lost teeth, 15 (83.33%) did not follow IADT guidelines. The other 23 (15.86%) teeth had not completed 1 year of follow-up.**Conclusions** The results suggest that following the 2012 IADT guidelines guarantees better results for avulsed and replanted teeth after a 1-year control period.**R208**L.L. Kirkevang^{1,*}, D. Ørstavik¹, G. Bahrami², A. Wenzel³ & M. Væth⁴¹Department of Endodontics, Institute of Clinical Dentistry, Faculty of Dentistry, University of Oslo, Oslo, Norway, ²Section of Prosthetics,³Section of Oral Radiology, Department of Dentistry, Health &⁴Section for Biostatistics, Department of Public Health, Health, Aarhus University, Aarhus, Denmark**Prediction of future periapical status****Aim** To describe and analyze risk factors associated with deterioration of periapical health over time, assessed using the full-scale PAI in a randomly selected population sample.**Methodology** In 1997–98, 616 randomly selected individuals from Aarhus County, Denmark underwent a full-mouth radiographic survey. They were re-invited in 2003–04 and in 2008–09, and 473 and 363 persons, respectively, consented and attended a new radiographic examination. The study population of the present investigation included 330 persons who had participated in all three examinations, and 143 persons who had participated in the first and second examination. The following variables were assessed in the radiographs: at the level of the individual; gender, age and number of teeth, at tooth level; presence of tooth, PAI score, root filling, caries, marginal bone level, restoration, jaw and tooth-group. The dependent variable was the 5-scale PAI supplemented with extraction. Ordered logistic regression analyses were carried out for root filled and non-root filled teeth separately. The Regional Committee of Ethics accepted the study.**Results** For both root filled teeth and non-root filled teeth the initial PAI score was the most important factor related to future periapical status ($P < 0.0001$). Non-root filled teeth had in general a better prognosis than root filled teeth. However, in non-root filled teeth several other factors had a significant influence on the periapical status at follow-up, and the risk estimates were larger and showed a more pronounced variation between the different categories of the risk factors. For root filled teeth few variables, other than initial PAI score, influenced the future periapical status significantly.**Conclusions** The full-scale PAI was the strongest predictive factor for future periapical status even when adjusted for additional confounding factors. The large difference in risk estimates for non-root filled compared to root filled teeth documents the importance of separate studies aimed at identification and quantification of risk factors associated with the development of periapical disease.

R209

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Partial pulpotomy in cariously exposed pulps in permanent teeth of adult patients

Aim The aim of the study was to evaluate the success rate of partial pulpotomy in cariously exposed pulps in permanent teeth of adults.

Methodology Twenty-eight teeth in 25 patients (17–85 years; mean 35.4 years) were treated with partial pulpotomy after the pulp has been exposed during caries removal of deep carious lesions. Involved teeth were molars ($n = 17$), premolars ($n = 7$) and anterior teeth ($n = 4$). All teeth had no clinical or radiographic signs of pulp pathosis before treatment. After isolation with rubber dam, complete caries removal was performed and 2 mm of the exposed pulp removed with a diamond bur. Haemostasis and disinfection were achieved with a cotton pellet soaked in 1% NaOCl solution. MTA white was used as the capping material, which was additionally covered with a light-curing calcium hydroxide liner. Subsequently, all tooth were restored with composite directly after partial pulpotomy.

Results The observation period was at least 1 year (mean 1.9 years, max. 3.5 years) for all teeth. The success rate was 93%. Treatment failure was observed in 2 teeth. One tooth had clinical symptoms of pain 2 weeks after treatment, one had periradicular pathosis after 1 year. Both cases needed root canal treatment. All other teeth did not have any clinical or radiographic signs of failure during the follow-up period.

Conclusions Partial pulpotomy was a suitable therapy for vital and symptom free exposed pulps in permanent teeth of adult patients with deep carious lesions.

R210

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Tooth survival following root canal treatments in Sweden. First report from a Scandinavian Endodontic Research Collaboration network (EndoReCo)

Aim To assess the proportion of tooth survival and identify factors correlated to tooth survival following root canal treatments performed in Sweden during the year 2009 by using data from the Swedish Social Insurance Agency (SSIA).

Methodology A database at SSIA containing information on treatments was used to identify all teeth in Sweden that were root filled during 1st of January–31st of December 2009 as reported by dentists affiliated to the SSIA. The SSIA covers all 9.3 million residents of Sweden, and the database includes all residents 20 years and older. All root filled teeth were followed for a period of 5–6 years and the root filled teeth that were lost during the period were identified by searching the database for codes indicative of extractions.

Results A total of 250 369 root fillings performed in 216 916 individuals were reported to the SSIA during the year 2009. Women constituted 123 002 (56.7%) of the registered individuals, receiving 123 001 (49.1%) of the root fillings. The age of the individuals at the time of the root filling was on average 55 years, ranging from 20 to 102 years. The majority of individ-

uals (88.2%) received one root filling, and 9.4% received two root fillings. The first molar in both the maxilla and mandible was the most frequently root filled tooth. Of all root fillings 167 750 (67.0%) were performed in the private sector and 82 041 (32.8%) within the public dental service sector. Preliminary results indicate that 8.9% of the root filled teeth in 2009 were extracted during the subsequent 5–6 years.

Conclusions Tooth survival following root canal treatments in Sweden appears to be high at 5–6 years.

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R211

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A follow-up study on the outcome of root canal treatments by dental students 2010–2011: a retrospective study based on patient records

Aim To evaluate the quality and outcome of root canal treatments performed by undergraduate dental students in the University of Oulu, Finland.

Methodology Root canal fillings in total of 840 teeth performed by 3rd–5th year students during the years 2010–2011 were evaluated. Data was collected from the patient records and only teeth with complete patient records, including good quality follow-up radiographs, were selected, resulting with 351 teeth. The success of the treatments was evaluated by radiographic findings. Periapical status and the quality of root filling (length and density) were analyzed from pre- and postoperative and follow-up radiographs. The period between the analyzed radiographs was at least 12 months. Additional aspects such as tooth type, preoperative diagnosis, primary or secondary root canal treatment, selected instrumentation method (manual or rotary), materials used for the restoration and possible complications during treatment were collected and recorded.

Results Two thirds of the cases were excluded due to deficiencies in the protocol and recording. The final study group comprised 105 teeth (93 patients). Two-thirds (65.7%) of the root fillings were adequate as for density and length. At baseline 71.4% of the treated teeth had a preoperative periapical lesion. Of these, 87.6% were partially or fully healed according to radiographic findings after the follow-up period. Mandibular teeth, especially premolars had higher healing tendency than maxillary teeth.

Conclusions The outcome of root canal treatments performed by dental students was good. There are major deficiencies in the protocol and recording findings, which should be emphasized in undergraduate endodontic education in the future.

R212

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Treatment outcome of MTA pulpotomy in permanent teeth with vital pulps in cariously exposed pulps: a retrospective study

Aim To illustrate the treatment outcome of MTA pulpotomy in permanent teeth with vital pulps and cariously exposed pulps, and to investigate factors influencing treatment outcomes.

Methodology Data were collected from dental records at the Postgraduate Endodontic Clinic, Faculty of Dentistry, Chulalongkorn University. During 1999–2012, sixty-six permanent teeth with vital and cariously-exposed pulps including teeth with radiolucent periapical lesions were treated with MTA pulpotomy. Patients were recalled and assessed for clinical and radiographic outcome by two calibrated examiners. Relationship between treatment outcome and factors were analyzed by means of univariate analysis and binary logistic regression.

Results Fifty patients (a total of 55 teeth) attended the follow-up examination. The age of the patients ranged from 7 to 68 years old (mean, 29 years old). The observation period following MTA pulpotomy was 8–62 months (median, 41 months). A successful outcome was observed in 48 teeth (87.3%), and 7 teeth (12.7%) were categorized as failures. From 21 teeth with radiolucent periapical lesions, 16 teeth were treated successfully (76%). Eighty-four percent of teeth (21 of 25 teeth) which were diagnosed as symptomatic irreversible pulpitis were associated with successful outcomes. There was a development of pulp canal obliteration in 17 teeth (30%). Dentine bridge formation was observed in 26 teeth (47.3%). All immature teeth (a total of 10 teeth) with cariously exposed pulps were associated with continuing root development after MTA pulpotomy. Defective restoration resulting in leakage and tooth fracture was found in 42% of teeth (3 of 7) which were classified as failure. The relationship between treatment outcome and treatment factors could not be detected statistically.

Conclusions MTA pulpotomy appears to be a promising treatment of permanent teeth with vital and cariously exposed pulps. Factors which may influence treatment outcome deserve further investigation.

R213

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25-year prospective long-term follow-up of primary and secondary root canal treatment: preliminary descriptive data analysis of success and failure

Aim Long-term follow-up data of primary and secondary root canal treatments are scarce and therefore critically needed. The aim of the present work was to provide a preliminary descriptive data analysis of the success rate of root canal treatments carried out between 1990 and 2013 and included in a large prospective database.

Methodology Data were collected systematically at the School of Dentistry of the Université Catholique de Louvain (Brussels, Belgium) since January 1990 in a Filemaker database. All treatments were performed by a single operator specialized in endodontics (JPVNH) in his practice at the University clinics. The study included 1473 patients (92% referred), which corresponds to 2360 teeth and 5233 root canals. A large range of information was recorded for each treatment, notably patient individual data, tooth restorative status, pre-, per- and post-operative radiographs, clinical and subjective symptomatology, clinical procedure (irrigants, medications, instrumentation, canal filling, etc.), assessment of treatment technical difficulty, pre-existing complications, number of visits, etc. Post-treatment evaluation was performed based on radiographic, clinical and subjective criteria. For the present paper, the sole endodontic success rates were calculated. Success was defined as the absence of clinical symptoms AND of pain AND of periapical lesion on radiograph.

Results The follow-up period ranged from 0 to 279 months, with a mean of 68.1 months and a median of 49 months. Complete follow-up: 55.2% of patients, 60.4% of canals and teeth. Of these, 686 were molars, 377 premolars and 363 incisors or canines. 79 teeth were lost during the treatment procedure, and 251 were extracted post-treatment due to non-endodontic reasons (mainly non-restorable fractures); they were therefore not taken into account for success rates. The overall success rate was 78.8%. More specifically, the success rate was 81.4% for primary endodontic treatments, 76.6% for endodontic retreatments, 87.6% for biopulpectomies, 93.8% for teeth with necrotic pulps without periapical lesions, 72.5% for teeth with chronic apical periodontitis, 62.7% for acute apical periodontitis and abscess.

Conclusions In this preliminary descriptive analysis of a very large database, differences in success rates were observed between the various clinical situations encountered. The data is currently being processed for multivariate analysis.

R214

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Outcomes and prognostic factors of apical surgery: a retrospective study

Aim To evaluate outcome and potential prognostic factors of apical surgery using microsurgical principles and root-end filling with MTA.

Methodology A total of 62 patients (90 teeth, only incisors and canines) underwent apical surgery in the Department of Endodontics, Institute of Stomatology between 2009 and 2014. All apical surgical procedures were performed by one operator (A.B.). Surgical procedures were carried out using microscopes, micro instruments, ultrasonic tips for root-end cavity preparation and ProRoot® MTA (Dentsply) to fill the cavity. Follow up time ranged from 11 to 62 month. The outcome was evaluated clinically and radiographically and defined as success (healed and healing) and failure (criteria). Twelve different prognostic factors were analysed in relation to the outcome using IBM SPSS Statistics v22.0 programme. The Pearson's Chi Square test was used to assess the statistical difference between each prognostic factor and outcome. Significance level was set at $P < 0.05$. Unit for analysis was tooth.

Results It was possible to recall 73% (45 patients, 70 teeth), but only 36 patients (58%) and 57 teeth were possible to include in the data analysis. Thirteen teeth had been extracted because of reasons not related to endodontics. In 46 teeth (80.7%) the outcome was successful. Among the prognostic factors only quality of coronal restoration had a significant impact on the outcome ($P = 0.023$). In cases with a follow-up period < 3 years, successful outcome was 90%, whereas in the group with follow-up time more than 3 years only 59% cases were found to be successful.

Conclusions Outcome and healing potential after apical microsurgery may be overestimated after short-term observation. There is need for further research on the long-term outcome of apical microsurgery and the influence of prognostic factors.

R215T.F. Eyuboglu^{1,*}, K. Olcay¹ & M. Ozcan²¹Department of Endodontics, Istanbul Medipol University, Istanbul, Turkey & ²Clinic for Fixed and Removable Prosthodontics and Dental Materials Science, University of Zurich, Zurich, Switzerland**Frequency of periapical complications and survival rate after single-appointment root canal retreatment****Aim** The aim of this study was to assess the association between outcome of single-visit root canal retreatments and preoperative, intraoperative and postoperative factors to evaluate the frequency of periapical complications and survival rate.**Methodology** In total 234 teeth in 173 patients were retreated in a single appointment between November 2012 and December 2013 by one experienced endodontist. 119 teeth were lost to follow-up and 5 teeth were extracted, leaving 110 teeth (47%) for examination by two calibrated examiners for outcome of healed (periapical index score (PAI) ≤ 2 ; no signs or symptoms) or 'diseased' (presence of apical periodontitis (PAI > 2), signs or symptoms). Preoperative, intra-operative and postoperative factors were evaluated for their association with the outcome. The NCSS (Number Cruncher Statistical System) 2007 & PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) program was used for statistical analysis. Fisher's Exact test and Fisher-Freeman Halton test were used for bivariate analysis to identify potential outcome predictors and logistic regression models were used for multivariate analysis to identify significant outcome predictors.**Results** Two to three year follow-up assessment revealed that 100 teeth (90.9%) were healed and 10 teeth (9.1%) were diseased. Age, gender, tooth type, preoperative (pain, periodontal defects, root filling density and length), intraoperative (sealer extrusion) and postoperative (type of coronal restorations) factors had no significant effect on the outcome ($P > 0.05$). Preoperative periradicular lesions with a diameter smaller than 5 mm had significantly better outcome than the larger lesions ($P < 0.05$) (Odds ratio(OD) = 6.00; 95% CI: 1.449–24.853). Logistic regression model for the effect of preoperative periradicular lesions with a diameter larger than 5 mm showed an increased risk of disease (OD: 6.415; 95% CI: 1.51–27.27).**Conclusions** Single appointment root canal retreatments had a high success rate. The only factor that had a significant effect on outcome was preoperative lesion size. Lesions smaller than 5 mm had significantly better outcome.**R216**A.L. Holzner*, K. Höpfel, A.T.A. Koch, J. Ebert & A. Petschelt
Dental Clinic 1 - Operative Dentistry and Periodontology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany**Evaluation of factors associated with vertical root fractures****Aim** Vertical root fracture (VRF) is one of the reasons for tooth loss after root canal treatment. Little is known about the reasons for VRFs. This retrospective study aimed to evaluate the factors associated with VRFs in root filled teeth.**Methodology** A total of 57 root filled teeth from 55 patients were extracted due to VRFs and evaluated. The patients' files and available radiographs were assessed according to age, gender, tooth group, retreatment, type of restoration, luting of posts and clinical symptoms. Furthermore radiological diagnostic findings and quality of root fillings were interpreted using the Periapical Index (PAI) and Santos Index. Serial sections (1 mm) were evalu-

ated under the light optical microscope where possible. Statistical analysis was done using SPSS Version 21.0.

Results The mean age for patient's VRF was 65 years (range 23–92 years). Anterior teeth (19%) were less susceptible to VRFs than molars (42%) and premolars (39%). Mean time between root filling and VRF was 7 years (range 1 month – 26 years). 66% of teeth were associated with pain. 68% of the radiographs taken after VRF had a apical radiolucency. 65% of teeth with VRF had a PAI ≥ 3 . The time until VRF was not correlated with the PAI or Santos Index. The only results showing significances were: older patients had a significantly higher number of filled surfaces (One Way ANOVA; $P = 0.002$) and teeth restored with posts had significantly more time until VRF than those without posts (12 versus 6 years; One Way ANOVA; $P = 0.003$).**Conclusions** Teeth with VRFs are often associated with pain, radiolucency and elevated PAI but VRFs did not depend on the quality of the root filling. Further research with a larger number of cases needs to be done to find out more about the factors influencing VRFs.**R217**C.M. Diegritz*, T. Hammann, K. Heck & R. Hickel
Department for Restorative Dentistry and Periodontology, Ludwig Maximilians University, Munich, Germany**Prevalence of apical periodontitis and quality of root canal treatment in a German subpopulation****Aim** The aim of this retrospective study was to radiographically assess the prevalence of apical periodontitis and the quality of root canal treatment in a subpopulation in Munich, Germany.**Methodology** A total of 2574 panoramic radiographs taken in the year 2010 were collected from the Department of Restorative Dentistry and Periodontology of the LMU Munich. First, they were investigated for the prevalence of apical periodontitis using the PAI score. Second, the number of root filled teeth, the length and density of the root fillings and the quality of the coronal restorations were recorded. The data was analyzed using the chi-square test and logistic regression.**Results** In total 58 808 teeth were evaluated. The overall prevalence of apical periodontitis (PAI score > 2) was 7.39%, however 68.3% of the patients had at least one tooth with a PAI Score > 2 . A total of 4627 root filled teeth were observed and further examined. 72.46% of the root fillings were dense and homogeneous; 41.11% were filled flush (0–1 mm from the radiographic apex); 54.26% were under- and 3.03% were over-filled. Overall 34% of the root filled teeth were adequately treated. The quality of coronal restorations of the root filled teeth was acceptable in 64.64% of the cases. 39% of the root filled teeth had clear signs of apical pathosis (PAI > 2). Apical periodontitis was associated with inhomogeneous and short root fillings (odds ratio 1.3 and 2.8 respectively). Significantly fewer teeth with adequate root fillings and inadequate coronal restorations had signs of apical periodontitis compared to teeth with inadequate root fillings and adequate coronal restorations ($P < 0.001$).**Conclusions** A high prevalence of apical periodontitis and an overall low quality of root canal treatment was observed (34%). However, the results revealed that the quality of the root filling was more important than the quality of the coronal restoration in terms of the outcome of treatment.

CLINICAL DESCRIPTION OF A TECHNIQUE/MATERIAL

R218

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Effectiveness of a silver diamine solution on dentine surfaces – an *in vitro* study

Aim Open dentinal tubules as a cause of dental hypersensitivity is a common phenomenon. The aim of this *in vitro* study was to assess the effect of a silver diamine fluoride solution (Ag(NH₃)₂F) on human dentine samples.

Methodology A total of five fully retained wisdom teeth were selected. The crowns of the teeth were separated from the roots and the enamel surface was cut horizontally. All dentine samples were treated for 30 s with phosphoric acid (20%) and rinsed thoroughly to remove the smear layer. Then the desensitising agent (Riva Star, SDI; 38% Ag(NH₃)₂F) was placed according to the manufacturer's instruction. Three dentine samples were prepared for element analysis using an electron probe micro-analyser (Jeol JXA 8900RL). The Ag concentrations in the dentine samples were measured in depths ranging from 5 to 40 µm. The other two dentine samples were fractured and specially prepared for visualization with SEM (Zeiss DSM).

Results The application of the desensitising agent to the dentine areas demonstrated an increased Ag concentration. On the dentine surface an Ag concentration of 1.4 weight % (±0.7) was measured, but at a depth of 20 µm only 0.5 weight % (±0.1) were detected. In depths >40 µm the Ag concentration was below the detection limit. The SEM images revealed a covering on the dentine layer, and in the dentinal tubules deposits were found to a depth of 20 µm.

Conclusions In this *in vitro* study, the effect of silver diamine fluoride on dentine surfaces was demonstrated. The desensitising agent formed a film on the dentine surface and deposits were detected in some dentinal tubules. These findings can explain a certain desensitising effect, but a direct translation to *in vivo* conditions must be done with caution.

CLINICAL REVIEW

R219

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Platelet concentrates for revitalization of immature teeth with necrotic pulps: a systematic review of the literature

Aim To determine the effectiveness of autologous platelet concentrate (APC) in the treatment of immature teeth with necrotic pulps.

Methodology An electronic search was performed on MEDLINE, Embase, Scopus, SciELO, Lilacs, Cochrane Central Register of Controlled Trials. Only comparative clinical studies were included, in which APC was used in the test group for pulp regeneration and

root development. Selected articles underwent risk-of-bias assessment and data extraction. The outcomes considered were: presence of symptoms; response to cold and electric pulp test; radiographic healing of the periapical lesion; radicular development in terms of apex closure, root lengthening and thickening of the root canal walls, evaluated radiographically. The results were dichotomized for statistical tests (Fisher's exact and chi-square test).

Results Three randomized clinical studies were included. One was a split-mouth pilot study on three patients, the other two had a parallel design. One study was considered at moderate risk of bias and two at high risk. A total of 41 patients aged 7–23 years and 46 immature teeth with necrotic pulps were treated. Two studies had a follow-up of 12 months and one of 18 months. All studies used platelet-rich plasma (PRP) in the test group. After treatment all teeth of both groups remained asymptomatic for the entire study duration. Only one study reported response to cold and electric pulp test, showing a non-significant better outcomes for the test group. Periapical healing was not significantly different between test and control groups ($P = 0.17$). The teeth treated with PRP achieved significantly better apical closure ($P = 0.03$), thickening of the dentine walls ($P = 0.01$), and root lengthening ($P = 0.01$) than teeth of the control groups.

Conclusions Despite the potential effectiveness of APC in promoting root development of immature teeth with necrotic pulps, there is still scarce evidence regarding this subject. In the studies evaluated in this review the use of PRP was associated with promising results that warrant further investigation.

EPIDEMIOLOGY

R220

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Factors associated with prevalence of apical periodontitis in an adult sub-population in Malaysia

Aim To assess the factors associated with prevalence of apical periodontitis (AP) in an adult sub-population in Malaysia

Methodology A random sample of digital panoramic radiographs of patients attending the Dental Clinics at the Universiti Kebangsaan, Malaysia were examined. The periapical status of teeth was assessed radiographically for the presence or absence of AP according to the Periapical Index. Factors associated with prevalence of AP; gender, age group, and tooth type were recorded. Data obtained were analysed using the Chi-Square test, Fisher's exact test and binary logistic regression.

Results A total of 233 radiographs with 6409 teeth were examined. Forty-three of these teeth (0.7%) were root filled. Twenty-five percent of patients had at least one tooth with AP, and <2% ($n = 111$) of all teeth had radiographic evidence of AP. Among the root filled teeth, 16 (37.2%) were associated with presence of AP. AP was significantly more frequent in root filled compared with non-root filled teeth ($P < 0.001$). Teeth with AP were significantly found more in males (2.5%) than females (1.3%) ($\chi^2 = 13.510$, $p < 0.001$, $DF = 1$). The prevalence of AP increased with age ($p < 0.001$, $OR = 1.061$, 95% C.I. = 1.048–1.073). AP was found more frequent in mandibular molars ($n = 39$, 3.6%), followed by maxillary premolars ($n = 24$, 3.2%) and maxillary molars ($n = 21$, 1.9%).

Conclusions Prevalence of AP increased with age. Root filled teeth and males had a significantly higher prevalence of AP in an adult sub-population in Malaysia. The results are similar to previous studies.

R221I. Jersa^{1,*} & R. Kundzina²¹Department of Conservative Dentistry, Riga Stradins University, Riga, Latvia & ²Institute of Clinical Dentistry, Faculty of Health Sciences, UiT the Arctic University of Norway, Tromsø, Norway**Prevalence of apical periodontitis and relating factors in an adult population in Riga. An epidemiological study****Aim** To determine the prevalence of root filled teeth, apical periodontitis (AP) and risk factors relating to AP among Riga (Latvia) adults.**Methodology** The present study was part of an oral health cross sectional investigation where 35–44 year old 350 randomly selected adults from Riga were invited to participate. Participants were examined clinically and radiographically (prevalence of root filled teeth, quality of root filling, prevalence of AP, quality of coronal restoration). A questionnaire was used to collect information about general health, health-related habits and attitudes and social parameters. Associations between AP and relating factors were tested using multiple logistic regression and linear logistic regression analysis using SPSS 20 computer software program. Significance level was set at $P < 0.05$.**Results** Out of 265 individuals who it was possible to contact, 134 (51%) participated in the study. The evaluation revealed that 91% (123 individuals) had root filled teeth, but 70% (94 individuals) had teeth with apical periodontitis. In total 3536 teeth were examined. The number of root filled teeth were 518 (14.6%) and 383 (74%) had incomplete root fillings. A strong significant association ($P = 0.001$) was found only for AP and quality of root filling, and AP and DMFT/DMFS-scores.**Conclusions** The prevalence of root filled teeth and AP is high in an adult population from Riga. Apical periodontitis was highly related to caries and poor quality of root fillings.**R222**M. Garrido^{1,2,*}, C. Díaz¹, M. Baeza¹, F. Solanes², P. Ahumada², T. Sorsa^{3,4}, T. Tervahartiala^{3,4}, J. García-Sesnich², P. Pussinen⁵ & M. Hernández^{2,6}¹Department of Conservative Dentistry, ²Laboratory of Periodontal Biology, Faculty of Dentistry, Universidad de Chile, Santiago, Chile, ³Department of Periodontology, Institute of Dentistry, University of Helsinki, ⁴Department of Oral and Maxillofacial Diseases, ⁵Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland & ⁶Department of Oral Pathology and Medicine, Faculty of Dentistry, Universidad de Chile, Santiago, Chile**Longitudinal study evaluating systemic inflammation and endotoxin levels in asymptomatic apical periodontitis patients with root filled teeth****Aim** To assess serum levels of endotoxin and inflammatory markers in patients with asymptomatic apical periodontitis (AAP) at baseline and after root canal treatment, and healthy volunteers.**Methodology** Patients having at least one tooth with asymptomatic apical periodontitis ($n = 14$) and healthy volunteers ($n = 20$) were included. Patients with marginal periodontal diseases were excluded and all the participants were assessed for classic cardiovascular risk factors. Serum samples from all the participants were taken at baseline, and 1 week and one month after root canal treatment, in the case of AAP patients. Inflammatory markers were determined as follows: endotoxin levels by quantitative limulus amoebocyte lysate assay; MMP-9, IL-6, MPO and the soluble adhesion molecules E-Selectin, ICAM-1 and VCAM-1, by multiplexing assay, whereas MMP-8 was measuredby immunofluorometric assay. According to data distribution, determined by Shapiro Wilk test, baseline data were compared using Mann-Whitney test (bivariate) and multiple logistic regression. Longitudinal data were analyzed with Wilcoxon's test and considered statistically significant if $P < 0.05$.**Results** All markers tended to show higher levels in AAP patients compared to controls at baseline, but only MPO was significant, even after adjusting for age and gender. IL-6 was significantly reduced 1 week after root canal treatment and remained low at one month, whereas MMP-9 and soluble E-selectin were significantly reduced at one month compared to baseline.**Conclusions** Patients with asymptomatic apical periodontitis may show signs of systemic inflammation and root filled teeth may be associated with a reduction of some systemic inflammatory markers.**Acknowledgements** Funding FONDECYT Grant #1120138.**TOOTH RESTORATION****R223**G. Plotino^{1,*}, A. Isufi¹, N.M. Grande¹, G. Di Giorgio¹, R. Pecci², R. Bedini² & G. Gambarini¹¹Department of Endodontics, Sapienza- University of Rome &²Department of Technology and Health, Italian National Institute of Health, Rome, Italy**Fracture resistance of root filled teeth restored with a bulkfill flowable material (SDR) and a resin composite****Aim** To compare the fracture resistance of root filled teeth restored with a bulkfill flowable material (SDR) and a traditional resin composite.**Methodology** Thirty maxillary and 30 mandibular first molars were selected based on similar dimensions. Average tooth size was calculated to minimize the influence of size and shape variations on the results. Specimens were subsequently assigned to 3 subgroups ($n = 10$) for each tooth type: Group A: control group, which included teeth that were left intact, without any cavity preparation, root canal treatment and reconstruction. Group B: after cleaning, shaping and filling of the root canals and adhesive procedures, access cavities were restored with a traditional resin composite (EsthetX; Dentsply-Italia, Italy). Group C: after cleaning, shaping and filling of the root canals and adhesive procedures as in Group B, access cavities were restored with a bulkfill flowable composite (SDR; Dentsply-Italia), except 1.5 mm layer of the occlusal surface that was restored with the same resin composite as Group B. The specimens were held into *ad hoc* grips and placed in a material static-testing machine and subjected to compressive force until fracture occurred. The fracture (breaking) load of the specimens was measured (N) and the data were analyzed statistically with one-way analysis of variance (ANOVA) and Bonferroni tests ($P < 0.05$).**Results** No significant differences were found among groups ($P > 0.05$). Fracture resistance of root filled teeth restored with a traditional resin composite and with a bulkfill flowable composite (SDR) was similar in both maxillary (Group B: 1072 ± 525 N; Group C: 1241 ± 388 N) and mandibular molars (Group B: 1332 ± 318 N; Group C: 1527 ± 449 N). Restored teeth showed no significant decrease in fracture resistance compared to intact specimens in both both maxillary (Group A: 1183 ± 313 N) and mandibular molars (Group A: 1620 ± 170 N).**Conclusions** No significant difference was observed in the mechanical fracture resistance of root filled molars restored with resin composite restorations compared to bulkfill flowable composite (SDR) restorations.

R224

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Effects of applying ascorbic acid and removing the dentine surface on the microtensile bond strength of resin adhesive to NaOCl-treated dentine

Aim It is known that the use of NaOCl lowers the bond strength of adhesive cements but that the decrease in bond strength can be reversed using antioxidants, such as 10% ascorbic acid. The purpose of this study was to compare the effects of an antioxidant (ascorbic acid) and a removal of superficial dentine surfaces on the microtensile bond strength between adhesive cement and sodium hypochlorite-treated dentine.

Methodology Twenty four extracted human third molars were examined. The occlusal surfaces of the teeth are cut horizontally in order to expose the sound dentine and the samples then divided into four groups. The dentine surfaces of teeth in each group are treated as follows: group 1, a control group, irrigated with saline only for 10 min; group 2, irrigated with 5.25% NaOCl for 10 min; group 3, irrigated with 5.25% NaOCl for 10 min and 10% ascorbic acid for 10 min; group 4, irrigated with 5.25% NaOCl for 10 min and removal of the superficial dentine surface up to 0.1 mm using a coarse diamond bur (TR14). The treated teeth in all groups are restored with resin composite (Filtek™ Z250), using the two-step self-etch adhesive (Clearfil SE primer and bond™), as a bonding agent. After storing in water for 24 h, the resin bonded specimens were subjected to a microtensile bond strength test at a crosshead speed of 1.0 mm min⁻¹ (*n* = 30 beams per group). Data was analyzed statistically using one-way ANOVA and the Tukey test (*P* < 0.05).

Results The microtensile bond strength of the NaOCl-treated group (19.44 ± 4.49 MPa) had significantly lower bond strengths than the control group (27.26 ± 5.74 MPa). No significant differences are found in bond strength between superficial dentine surface removed group (26.08 ± 5.25 MPa) and the control group. Application of 10% ascorbic acid on NaOCl-treated dentine (31.96 ± 8.48 MPa) demonstrated significantly higher bond strength than the other groups.

Conclusions 5.25% NaOCl irrigation on dentine surfaces produced a significant reduction in resin-dentine bond strength, but this was reversed by treating with 10% ascorbic acid or removing the superficial dentine surface.

R225

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Periapical status of root filled teeth restored with composite, amalgam or full crown restorations: a cross-sectional study of a Swedish adult population

Aim To compare the periapical status of root filled teeth restored with either laboratory-fabricated crowns, composite or amalgam in a Swedish general population.

Methodology From a randomly selected sample of 1000 adults living in the county of Skåne, Sweden, 440 individuals were examined clinically and radiographically. Caries and the type, material and quality of the restorations were recorded for all root filled teeth. Periapical status, root filling quality, marginal bone loss and type of post were evaluated on panoramic radiographs.

Three calibrated observers performed the observations. The association between the registered variables and the periapical status was analysed using Chi-square tests and regression analysis.

Results Out of 11 876 teeth 660 (6%) were root filled. Apical periodontitis (AP) was observed significantly more often in teeth restored with amalgam (49.2%) compared with laboratory-fabricated crowns (26.4%) and composite (31.3%) (*P* < 0.05) while there was no significant difference in AP between teeth with laboratory-fabricated crowns or composite restorations. When only teeth with adequate restorations were compared, there was no difference in the frequency of AP between the restoration groups. A significantly higher frequency of AP was detected in teeth with restorations of inadequate quality (50%) compared to teeth with restorations of adequate quality (29.7%) (*P* < 0.05). The smallest proportion of AP (13.3%) was detected in teeth having both root filling and coronal restoration of adequate quality. The highest proportion of AP (64.3%) was detected when both root filling quality and coronal restoration were inadequate (*P* < 0.001).

Conclusions There was no difference in the frequency of AP in teeth restored with either laboratory-fabricated crowns, composite or amalgam, provided that the quality of the coronal restoration was adequate. AP was observed more often in teeth with inadequate quality of the coronal restorations.

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Evaluation of shear bond strength of composite to Biodentine with different adhesive systems

Aim Aim of this study was to determine the shear bond strength of resin material to Biodentine with different bonding strategies at several time intervals.

Methodology Acrylic blocks with a hole measuring 4 mm in diameter and 4 mm in height were prepared and filled with Biodentine mixed according to manufacturer's instructions. After setting of the material, specimens were randomly divided into three experimental groups according to the bonding protocols: two-step total-etch (group 1), one-step total-etch (group 2), one-step self-etch strategy (group 3) and control group (group 4). Each group was divided into three subgroups (*n* = 10) depending on time elapsed after placement of Biodentine: 12 min (subgroup A), 7 days (subgroup B) and 28 days (subgroup C). Bonding systems were applied appropriately for respective groups and then covered with resin-based composite material placed into 2-mm high silicone rings and polymerized. Specimens were stored in 37°C and 100% relative humidity. Shear bond strength was measured using universal testing machine Zwick/Roell Z020 and statistical analysis of the data was performed using two-way and one-way analysis of variance (*P* < 0.05).

Results When bonding procedures were performed immediately after Biodentine setting (subgroup A), the highest bond strength was obtained in group 3 (self-etch system; *P* = 0.002) and group 1 (two-step total-etch system; *P* = 0.001), 6.13 MPa and 3.63 MPa respectively. These values were significantly higher than in group 2 (one-step total-etch; 1.21 MPa). Bond strength significantly increased (*P* = 0.001) with time that elapsed after Biodentine setting only in group 2 (1.21 MPa after 12 min, 2.59 MPa-7 days, 4.28 MPa-28 days). There were no significant differences between experimental groups after 7 and 28 days.

Conclusions Shear bond strength of the bonding protocols to Biodentine depended on time elapsed after material placement and bonding strategy. Shear bond strength increased over time for the one-step total-etch group. There was no difference between bonding protocols over time.

R227

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Effect of antioxidant on shear bond strength of bleached enamel

Aim To evaluate the effect of two antioxidants, rosmarinic acid and sodium ascorbate, on shear bond strength of 38% hydrogen peroxide bleached enamel.

Methodology The buccal surfaces of 80 extracted human third molars were vertical cut to expose sound enamel. The teeth were divided into 4 groups. The enamel surfaces of the teeth were treated as follows: group 1, no treatment; group 2–4 bleached with 38% hydrogen peroxide Opalescence Xtra Boost (Ultradent, USA) 2 cycles 10 min per cycle; group 2, no antioxidant application after bleaching; group 3, 10% sodium ascorbate solution is applied for 10 min to the bleached surface; group 4, 100 micromolar rosmarinic acid solution is applied for 10 min to the bleached surface. All treated enamel surfaces were bonded with a 3-step total etch adhesive system Scotchbond™ Multi-purpose (3M ESPE) and restored with a resin composite Filtek™ Z350XT A3 (3M ESPE). All the specimens were stored in distilled water for 24 h, and were subjected to the shear bond test at a crosshead speed of 0.5 mm min⁻¹. Data were analyzed by a one way ANOVA and Tukey's multiple comparison ($P < 0.05$).

Results The bleached group had significantly lower bond strength than the unbleached group ($P < 0.05$). There was no significant difference between bond strength of the group where 10% sodium ascorbate solution was applied for 10 min after bleaching and the group with no antioxidant application ($P > 0.05$). On the other hand, the 100 micromolar rosmarinic acid solution was associated with a significant reversal effect with the same application times ($P < 0.05$).

Conclusions Bleaching with 38% hydrogen peroxide significantly decreased enamel bond strength. The application of 100 micromolar rosmarinic acid for 10 min increased bond strength to bleached enamel.

R228

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Two single-taper post systems luted with self-adhesive cement: a fracture resistance analysis

Aim To assess the resistance to fracture of bonded single-taper fibre posts. These posts can be placed ideally without post space preparation, since they are chosen to match the taper of the prepared canal.

Methodology Sample size was calculated according to previously published data ($\alpha = 0.05$; $\beta = 0.20$; $\delta = 20.0$; $\sigma = 19.8$). Thirty-two single-rooted freshly-extracted teeth were cut 1 mm coronal to the cemento-enamel junction. Canals were shaped with Mtwo rotary instruments up to size 40, .06 taper, irrigated with 5.25%

sodium hypochlorite and filled with the continuous wave of condensation technique. The coronal portion of the canal was left unfilled for 9 mm and served as the post space after having been cleaned with a rotary endodontic brush. The specimens were randomly allocated to two groups corresponding to the post systems, namely SurgiPost Multiconical (G1, $n = 16$) and Tech ES Endoshape (G2, $n = 16$). A self-adhesive cement (RelyX Unicem) was poured into the post space. By following a standardized approach, .06 tapered posts were adapted to the prepared canals, seated and cut 5 mm coronally to the root surface. Samples were loaded to fracture applying a continuous compressive force (45°, 0.75 mm min⁻¹) on the top of the post. The maximum breaking loads of the two groups were compared statistically with a Student's *t*-test.

Results The means (SD) of the failure forces (N) were: G1, 134.29 (17.95); G2, 154.00 (14.34). The difference was significant ($P < 0.005$). The qualitative analysis of the loaded samples revealed bending of the post at the level of the canal orifice and absence of root fractures.

Conclusions In comparison to the data already available, the two single-taper post systems offered high values of resistance to loading without causing catastrophic root fractures. Tech ES Endoshape exhibited greater resistance than SurgiPost Multiconical.

R229

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Biomechanical composites for restoration of root filled teeth

Aim The purpose of this study was to develop and characterize polyethylene-hydroxyapatite (PE-HA) composites tailored to function as biomechanical posts for restoration of structurally compromised root filled teeth.

Methodology A melt extrusion process was used to fabricate composites of low density polyethylene (LDPE) with silanated hydroxyapatite (HA) and zirconium dioxide (ZrO₂) as fillers. The composites were characterized using infrared spectroscopy, and thermal properties determined using differential scanning calorimetry and dynamic mechanical analysis. The flexural strength and modulus of the composites were determined both in dry state and after ageing for 3 months in simulated body fluid (SBF), using an universal testing machine. The water uptake and radiocontrast were also measured and compared with commercial fibre reinforced composite posts. Data was analysed using one-way analysis of variance (ANOVA) and *post hoc* Tukey multiple comparison tests at a level of significance $P < 0.05$.

Results The melting point of the composites were found to range between 135.2 and 136.1°C that would facilitate removal in case retreatment was required using conventional dental heating devices used in the root canal. The incorporation of silanated HA in the composites reduced the damping and shifted the glass transition temperature to higher values. The flexural strength of the composites increased with increasing HA content both under dry and post ageing conditions in SBF ($P < 0.05$) with flexural modulus ranging between 3.0 to 3.3 GPa. The radiopacity of the composites revealed they were sufficiently radiopaque and were acceptable for clinical application. The low water uptake of 0.2–0.3% compared to commercial FRC posts is clearly advantageous as oral fluids that may penetrate within the interfaces can affect the retention of the post within the root canal.

Conclusions Highly biocompatible components, HA & LDPE were used to fabricate the experimental composites, which had properties suitable for potential application as posts for restoration of root filled teeth. Furthermore, the biomechanical behaviour,

hydrolytic stability, radiopacity and retrievability offer significant advantages over current commercial post materials.

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Effect of universal adhesive on shear bond strength of resin composite to feldspathic ceramic

Aim To investigate the shear bond strength between resin composite and feldspathic ceramic using a new adhesive system (Single Bond Universal Adhesive) compared with a conventional technique for ceramic repair.

Methodology Eighteen cylindrical specimens were fabricated with feldspathic ceramic; each specimen had two bond area tests (thirty-six bond areas in all). Specimens were etched with 4% hydrofluoric acid, rinsed and air-dried and then divided randomly into three groups, of six specimens and twelve bond areas each. Group 1 (control group) was treated with silane coupling agent and applied Scotchbond™ Multi-Purpose adhesive. Group 2 was treated with silane coupling agent and applied Single Bond Universal Adhesive. Group 3 applied Single Bond Universal Adhesive. Adhesive layer of all specimens were light cured for 20 s. Each feldspathic ceramic specimen was bond with two direct resin composites; in diameter of 3 mm and 2 mm thick, and light cure for 40 s. All specimens were stored in 37°C distilled water for 24 h. An Universal Testing Machine was used for shear bond strength testing at a cross-head speed of 0.5 mm min⁻¹. Mean shear bond strength was analyzed by One-way ANOVA and Tukey multiple comparison test ($P < 0.05$). The failure surfaces were examined by light stereomicroscope.

Results The highest mean shear bond strength was Group 1 (27.94 ± 6.08 MPa) followed by Group 2 (23.55 ± 8.10 MPa) and Group 3 (19.66 ± 5.26 MPa). No significant differences were found between the groups. Most of the failure were cohesive in the feldspathic ceramics.

Conclusions Using Single Bond Universal Adhesive with or without silane coupling agent and Scotchbond™ Multi-Purpose adhesive with silane coupling agent had no effect on the shear bond strength of resin composite and feldspathic ceramic.

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Push-out bond strength of fibre posts to root canals using self-etch and self-adhesive resin cements

Aim The purpose of this study was to compare mean push-out bond strengths between self-etch adhesive system with resin cement (Multilink® N) and self-adhesive resin cement (RelyX™ Unicem) in coronal, middle and apical root canal regions.

Methodology Twenty single-canal mandibular premolars were sectioned perpendicular to their long axis at the mesial CEJ level. Peeso reamers No. 2 were used to prepare all canal surfaces followed by post reamer No.2 of FibreKleer® set at 10 mm. The specimens were randomly divided into two groups of ten ($n = 10$). The root canals in group 1 fibre posts (FibreKleer® No. 2) were cemented with self-etch adhesive system and resin cement (Multilink® N). Those in group 2 were cemented with

self-adhesive resin cement (RelyX™ Unicem). The samples were fixed in plastic blocks with epoxy resin. Each root was cut into 3 sections of coronal, middle and apical regions using an Isomet® saw, 1.0 ± 0.1 mm. in height. The push-out bond strength was measured with an Instron® Universal Testing Machine with a cross-head speed 0.5 mm min⁻¹ and the data was analyzed using two-way ANOVA and Tukey's test ($P < 0.05$).

Results In the self-etch adhesive system and resin cement group, the mean push-out bond strength in the coronal region was significantly higher than in the apical region. In the self-adhesive resin cement group, there was no regional significant differences in the mean push-out bond strengths. In addition, the mean push-out bond strength of the self-etch adhesive system with resin cement group in the coronal region was significantly higher than the coronal region of the self-adhesive resin cement group ($P < 0.05$).

Conclusions Within the limitation of this study, the self-etch adhesive system with resin cement was a better luting cement for bonding fibre posts to the root canal with better push-out bond strength values than the self-adhesive resin cement.

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The effect of placement lengths on stress distribution of oval and circular fibre posts in a mandibular premolar tooth: a three-dimensional finite element analysis study

Aim The aim of this study was to evaluate the effect of two different anatomic shapes and placement lengths of posts on stress distribution using the Finite Element Method (FEM) on a root filled mandibular premolar restored with a full coverage crown.

Methodology A mandibular premolar was modelled using the ANSYS software. Four separate models were formed. Model 1 (M1): Circular fibre post placed 10-mm length in the canal, M2: Circular fibre post placed 5-mm length in the canal, M3: Oval fibre post placed 10-mm length in the canal, M4: Oval fibre post placed 5-mm length in the canal. An oblique force of 300 N, angled at 45° with respect to the occlusal plane, and oriented towards the buccal side was simulated. von Mises stress evaluations were carried out at three regions for four teeth models. Region 'a': von Mises stresses were measured along the apical extent of the post; region 'b': von Mises stresses were measured combination of the core-root dentine interface from the labial to the palatal side; region 'c': von Mises stresses were measured 3 mm above from the 'b' point from the labial to the palatal side.

Results The results of the finite element analysis revealed that the circular fibre post with two different placing lengths had von Mises stresses greater than the oval fibre post models. Maximum stresses that occurred in the models were: 400.14 MPa for M1 and 362.9 MPa for M2 at 'a' regions; 215.83 MPa for M3 and 293.82 MPa for M4 at 'b' regions.

Conclusions Shortened placement length increased the stresses in oval fibre post models, and decreased the stresses in circular fibre post models. Maximum stresses were concentrated around the post tip in M1 and M2 models; and at the cervical part of the coronal restoration in M3 and M4 models.

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Push-out bond strength of a self-adhesive resin cemented fibre posts to root canal dentine after Nd:YAG laser irradiation and Er:YAG and Er,Cr:YSGG laser activated irrigation

Aim To evaluate the effect of Nd:YAG laser irradiation, Er:YAG and Er,Cr:YSGG laser activated irrigation combined with QMix or saline on the push-out bond strength of fibre posts cemented with a self-adhesive cement to root canal dentine.

Methodology Eighty two single-rooted human permanent teeth were used. Root canals were instrumented with ProTaper Next instruments (Dentsply Maillefer, Switzerland) to an X3 instrument. Final irrigation protocol included 1 mL 2.5% NaOCl, 1 mL 17% EDTA for 1 min and 1 mL saline solution. Fibre-reinforced composite post preparations were created in previously filled root canals. The samples were divided into six experimental groups ($n = 12$ each) according to the final pretreatment of root canal: I: Nd:YAG laser (pulse energy: 100 mJ, repetition rate: 10 Hz, medium power: 1.5 W); II: Er:YAG + saline solution (pulse energy: 20 mJ, repetition rate: 15 Hz, pulse duration: 50 µs); III: Er:YAG + QMix; IV: Er,Cr:YSGG + saline solution (pulse energy: 62.5 mJ, repetition rate: 20 Hz, pulse duration: 140 µs); V: Er,Cr:YSGG + QMix. Ten samples did not receive any pretreatment and served as positive controls. Fibre-reinforced posts were cemented with a self-adhesive cement (SpeedCEM, Ivoclar Vivadent, Liechtenstein) according to the manufacturer's instructions. The samples were cut into 2 mm thick parallel transverse sections and prepared for the push-out bond strength test in an universal testing machine. The results were analysed using the Kruskal–Wallis test. One sample from each group was used to show and evaluate the dentine surface after each pretreatment protocol by scanning electron microscopy.

Results The significantly highest bond strength was recorded in the group Er:YAG laser + QMix (mean 3.401 MPa), followed by Er,Cr:YSGG and Er:YAG laser activated saline solution (mean 1.111 MPa and 1.094 MPa), which did not differ significantly ($p > 0.05$). There were no statistically significant differences between Er,Cr:YSGG (QMix) and Nd:YAG laser ($p > 0.05$). All tested protocols provided higher bond strength than conventional needle irrigation (mean 0.727 MPa, $p < 0.05$).

Conclusions Pretreatment of root canals with Er:YAG laser activated QMix irrigation provided the highest bond strength of a fibre posts cemented with a self-adhesive cement to root canal dentine.

R234

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Fracture resistance of coronoradicular restorations restored with short fibre or polyethylene woven fibre composites

Aim To assess the fracture resistance of root filled maxillary premolars with one remaining cavity wall restored either with short fibre or polyethylene woven fibre composites.

Methodology A total of 48 maxillary premolars were root filled. The teeth were randomly assigned to four groups ($n = 12$). Palatal cusps were removed and standardized cavities (3.5 mm depth and 1.5 mm diameter) were prepared in the palatal canal entrances in the first three study groups. In Group 1: Fibre-reinforced composite (FRC) (GC everX) was used both for the restoration of deep cavities and coronal parts; in Group 2: Gc everX was placed inside the deep cavities and core segment of the restoration and the outer layer was covered using fibre reinforced posterior composite (GC G-aenial); in Group 3: Both deep cavities and coronal parts were built up using only GC G-aenial posterior composite; in Group 4: Post spaces were prepared (10 mm depth). Polyethylene woven fibre (Ribbond) system was placed in post spaces and coronal parts were restored using GC G-aenial posterior composite. The restored teeth were placed into a universal testing machine and the load was applied with a stainless steel ball (4 mm diameter) at a crosshead speed of 1 mm min⁻¹ until fracture occurred. The data were analyzed statistically using non-parametric Kruskal–Wallis test ($\alpha = 0.05$).

Results Although there was no significant difference between the groups ($P > 0.05$), group 1 had the greatest and group 3 had the lowest fracture resistance.

Conclusions Although GC everX gave the best results, no difference was found for the chosen materials and techniques for the fracture resistance of maxillary premolar teeth with only the buccal cusp remaining.

R235

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Comparison of different final irrigant activation techniques on push-out bond strength of fibre posts

Aim To compare the efficacy of different final irrigant activation techniques on the push-out bond strengths of fibre posts to root canal dentine.

Methodology Twenty single-rooted human maxillary central teeth were used. The teeth were decoronated to a standardized length and the root canals were prepared by using the ProTaper system (Dentsply Maillefer, Switzerland) to size F4 and filled with gutta-percha and AH Plus root canal sealer (Dentsply DeTrey GmbH, Germany). After preparation of the post space, the roots were randomly divided into 5 groups ($n = 4$) and the post space was irrigated with 2.5% sodium hypochlorite and 17% EDTA before post placement using the following final irrigant activation techniques in each group: Group 1: conventional syringe irrigation (CSI); Group 2: manual-dynamic activation (MDA); Group 3: EndoVac; Group 4: Passive ultrasonic irrigation (PUI) and Group 5: Er,Cr:YSGG laser. After activation protocols the post spaces were irrigated with distilled water and dried with absorbent paper. Fibre posts were placed in each group with dual curing resin based cement (Panavia F 2.0, Kuraray, Osaka, Japan). The specimens were transversally sectioned from coronal to apical. Three 1-mm-thick dentine slices were obtained from each root region (coronal and apical). A push-out test was used to measure the bond strength of fibre posts. Statistical analyses were performed using two way analysis of variance and *post hoc* Tukey test.

Results The EndoVac, PUI and Er,Cr:YSGG laser had higher push-out bond strength values compared to CSI and MDA ($P < 0.05$). There were no significant differences between CSI and MDA and among EndoVac, PUI and Er,Cr:YSGG laser ($P > 0.05$). The bond strength values mostly decreased in the coronal-apical direction ($P < 0.05$).

Conclusions Final irrigant activation protocols except MDA improved the push-out bond strength of fibre post.

R236

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Cluster and silver nanoparticles absorption in human dentine

Aim Nanoscale materials often have very different properties than those exhibited at the macroscale level, enabling new often unsuspected applications that are not possible with bulk materials. Nanoparticles (2–100 nm) and clusters (0.5–2 nm) have many applications in such diverse fields such as optics, engineering, electronics, nanomedicine, and of course in dentistry. Nanotechnology is already being successfully applied in dentistry in the increasing use of adhesives, cements and resin materials based on nanotechnology. The objective of this study is to evaluate the absorption of clusters and silver nanoparticles by human dentine.

Methodology Human teeth extracted for orthodontic or periodontal reasons, which were individually preserved in sterile saline solution were used. Experiments in solutions with defined concentrations of clusters (NGAP AQC Ag-1104-W) and nanoparticles (Ag-NP NGAP 2103-W) were performed. The technique used to measure the absorption of the particles was ultraviolet-visible spectroscopy (UV-Vis). To obtain information of a solution, the amount of energy absorbed as a result of excitation was measured. The results of this analysis were represented as spectrum (graph of absorbed radiation versus frequency or wavelength). The optical absorbances of the respective solutions at 0 (control), 24, 48, 72 and 96 h were periodically measured.

Results The absorbances of the solutions decrease with time of tooth immersion, indicating absorption of silver clusters from dentine. In absorbance experiments although much of the clusters were absorbed by dentine in each experiment, it was incomplete, with some of the silver remaining in the solution. By contrast, in similar experiments with nanoparticles, results unequivocally revealed that nanoparticles were not absorbed by dentine, since the absorbances of solutions in which the teeth were immersed did not decrease over time.

Conclusions Silver clusters but not nanoparticles were absorbed in human dentine.

R237

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Influence of drying time of contemporary adhesives on dentine bond strength after the application of endodontic irrigants

Aim To evaluate the effect of various solvent evaporation times on shear bond strength of two adhesives when dentine is treated with 2.5% sodium hypochlorite and 2% chlorhexidine gluconate.

Methodology One hundred and ninety-two caries free extracted third molars were collected. Flat dentine surfaces were created on mid-coronal dentine using a slow speed diamond saw (Isomet, USA) and polished with wet 600-grit silicon carbide paper to create standardized surfaces. Teeth were randomly assigned to two groups and irrigated with one of the endodontic irrigation solution ($n = 96$) for 30 s and either Clearfil SE Bond (Kuraray, Japan) or Single Bond Universal (3M ESPE, Germany) was applied to exposed dentine surfaces and subsequently air dried (10, 20 or 30 s). Next, a resin composite (Universal Restorative, 3M ESPE, Germany) was built up on the dentine of each specimen by packing the material into a cylindrically shaped plastic apparatus with

an internal diameter of 2.34 mm and a height of 3 mm and light-cured with an LED (Bluephase, Ivoclar Vivadent) for 20 s. All specimens were stored in a moisture medium at 37°C for 24 h. Bond strength was tested using a Universal Testing Machine (Instron, USA) with a notched blade attached to a compression load travelling at a crosshead speed of 1 mm min⁻¹. The data was calculated as MPa and statistically analyzed using three-way analysis of variance (ANOVA) ($\alpha = 0.05$).

Results No significant differences were noted in shear bond strength of two adhesives ($P > 0.05$). The bond strength was decreased when dentine was treated with 2% chlorhexidine gluconate prior to the bonding ($P < 0.05$). The dentine bond strengths varied according to the air drying time ($P = 0.039$) and increasing solvent evaporation time from 10 to 30 s resulted in significantly higher mean shear bond strength for both adhesives.

Conclusions Irrigants which are used during endodontic treatment procedures may affect bond strengths of adhesive agents. Residual water and/or solvent may also compromise the performance of the adhesive agents, and might be improved by extending the evaporation times.

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RETREATMENT

R238

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Success rate of root canal retreatment of teeth with persisting apical pathosis following apicectomy: a retrospective study

Aim To evaluate the success rate of root canal retreatment of teeth with persisting apical pathosis following apicectomy.

Methodology Eighty-six consecutive cases of failed apicectomy in terms of persisting apical lesions or pain in 75 patients were treated in a Private Dental Practice using modern technology (DOM, ultrasonics, MTA). All teeth received root canal retreatment without a second surgical intervention. The root-end filling (16 cases) could be removed only in 1 case. Ninety-one non-apicected teeth (61 patients) receiving primary root canal treatment and 86 teeth in 65 patients receiving root canal retreatment served as controls. All treatments were performed by an experienced endodontist (G.T.). The assessment of radiographs was independently performed by two other dentists (A.Z., M.H.). Statistical analysis was performed using Pearson's Chi-square test ($P = 0.05$).

Results Fifty-nine of 86 cases (68.6%) could be recalled after at least 11 months (median 20 months). Root canal retreatment after failed apicectomy was rated successful in 69.5% of the cases. The presence of a root-end filling or medication with calcium hydroxide did not significantly influence the outcome. The success rate for primary treatment (control group I) in the same dental clinic was 75.8% (recall rate 72.5%) and for retreatment (control group II) 77.6% (recall rate 57%).

Conclusions Root canal retreatment can be considered as a feasible treatment option but with a slightly lower success rate after failing apical surgery even for teeth with root-end fillings.

R239

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*Department of Endodontology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden***Long-term tooth survival after endodontic retreatment and the relation to root canal posts - results of a randomised clinical trial****Aim** To evaluate the long-term survival of teeth after surgical or nonsurgical retreatment in relation to presence of root canal retained posts and the post dimensions.**Methodology** In a randomised clinical trial, 92 consecutive patients (95 teeth) were assigned to surgical or nonsurgical retreatment. 48 teeth were allocated to nonsurgical retreatment, including post removal in 37 (77%). 47 teeth were allocated to surgical retreatment. In these, a post was present in 39 teeth (83%). Follow-up was attempted until (i) the patient was not available, (ii) the tooth was extracted or (iii) at least 10 years had passed since retreatment. Outcome was evaluated as tooth survival. The relative post dimensions (post width and length) were measured on radiographs exposed prior to retreatment. Fisher's exact test was used to analyse differences between groups.**Results** The mean follow-up time was 8.9 years (range 0.0–15.6 years). At the end of the data collection, 72 teeth (76%) survived. Eleven (23%) nonsurgical and twelve (26%) surgical retreated teeth had been extracted. Median follow-up time until extraction was 7.1 years (Range 0.0–15.4 years). Teeth without posts survived in 84% and teeth with posts in 74%. The difference was not significant ($P = 0.55$). Teeth with posts, in which the distance from the apical terminus of the post to the radiological apex was <4 mm were lost in 57%, while teeth where this distance was ≥ 4 mm were lost in 20%. The difference was significant ($P = 0.047$).**Conclusions** Long-term survival of teeth randomly allocated to surgical or nonsurgical retreatment was 76%. No significant difference was found between retreatment methods. Neither did the analyses disclose any significant difference between teeth with or without posts. A short distance (<4 mm) between the apical terminus of the post and the radiological apex was found to be a prognostic factor for tooth loss ($P = 0.047$).**R240**A. Yilmaz^{1,*}, D. Helvacioğlu-Yigit², C. Gur², H. Ersev¹, G. Kiziltas-Sendur³, E. Avcu⁴, C. Baydemir⁵ & P.V. Abbott⁶¹Department of Endodontics, Faculty of Dentistry, Istanbul University, Istanbul, ²Department of Endodontics, Faculty of Dentistry, Kocaeli University, Kocaeli, ³Department of Mechatronics Engineering, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, ⁴Ford Otosan İhsaniye Automotive Vocational School, Kocaeli University, Kocaeli, ⁵Department of Biostatistics, Kocaeli University, Kocaeli, Turkey & ⁶School of Dentistry, University of Western Australia, Perth, Australia**Micro-CT evaluation of dentine defect formation during re-treatment with hand and rotary instruments****Aim** The purpose of this study was to compare the incidence and length of dentine defects (fractures, partial cracks and craze lines) after removal of gutta-percha (GP) with hand and rotary instruments using micro-CT analysis.**Methodology** Twenty mandibular incisors with single, straight root canals were selected and mounted in resin blocks with simulated periodontal ligaments, and with the apex exposed. The root canals were instrumented with nickel-titanium K-Flex files to size 35 used with the balanced force technique. The teeth were scanned in a high-resolution micro-CT system at 19.9 μ m resolu-tion. The root canals were then filled using the lateral compaction technique with GP and AH26 cement. The root fillings were subsequently removed with ProTaper Universal re-treatment rotary instruments (Group 1) or hand instruments (Group 2). The teeth were then re-scanned in the micro-CT scanner. The image sets of the roots after initial instrumentation and after removal of GP were co-registered by computer software. Then, the cross-section images of the apical, middle and coronal portions of the roots ($n = 24/120$) were screened by two pre-calibrated examiners. First the images of roots after removal of GP were analyzed and the number and type of the defects for each cross-section were recorded. Also, the sections presenting the start and finish of the defect was recorded and the length calculated. Subsequently, the first scans were examined to verify the changes. Kolmogorov–Smirnov, Wilcoxon T, Mann–Whitney U, Fisher's Exact and Monte Carlo chi square Tests were used.**Results** Dentine defects were observed in 36.90% ($n = 44/51$) of the cross-sections from both groups. The defects ($n=73$) were, 87.67% ($n=64$), craze lines, 2.73% ($n = 2$) partial cracks and 9.58% ($n = 7$) fractures. In all groups, the apical and middle portions of the roots had more dentine defects than the coronal portions. Dentine defects of three teeth in group 1 increased in length and new defect formation was detected in only one tooth of each group. There was no significant difference between groups in terms of increase in number of dentine defects.**Conclusions** Within the limitations of this study, hand and rotary instrumentation techniques caused similar dentine defect formation during root canal re-treatment.**R241**

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*Department of Operative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo, Bauru, Brazil***Influence of ultrasonic agitation of solvents in the removal of an epoxy resin sealer from the walls and grooves of a root canal: a SEM analysis****Aim** The study aimed to evaluate the influence of ultrasonic agitation of two solvents (Xylol and Endosolv R) in the removal of AH Plus sealer from the simulated grooves and walls of root canals.**Methodology** Forty roots of bovine incisors were selected and sectioned in two parts. A groove was created in the root canal wall of one section and AHplus was placed on the wall and into the groove. The roots were divided into four groups ($n = 10$): G1 – Xylol for 5 min; G2 – Xylol and ultrasonic agitation for 1 + 4 min with the solvent without agitation; G3 – Endosolv R for 5 min. G4 – Endosolv R and ultrasonic agitation for 1 + 4 min with the solvent without agitation. Next, the root canals were irrigated with 10 mL of saline solution. The sections of the root were scanned in an electron microscope and the images before and after treatments were analyzed by scores from 0 to 4, according to the cleanliness of the walls and the grooves. The data were statistically compared by the Kruskal–Wallis, Dunn's and Mann–Whitney tests ($P < 0.05$).**Results** When comparing the solvents, independent of the ultrasonic agitation, there were no significant differences for the removal of sealer from the wall and groove ($P > 0.05$). When comparing the effect of ultrasonic agitation, independent of the solvent, the ultrasound improved the effectiveness of the removal of the sealer ($P < 0.05$). Comparing the groups, significant differences were found ($P < 0.05$) for sealer removal in the apical, middle and cervical thirds.

Conclusions Ultrasonic agitation of solvents improved removal of AH Plus sealer from dentinal tubules. However, when associated with Endosolv R solvent, ultrasound improved the removal of sealer in the simulated groove.

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R242

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Radiographic evaluation of filling material remaining in root canal retreatment using nickel titanium rotary systems

Aim The aim of this study was to evaluate radiographically the effectiveness of ProTaper Retreatment and Mtwo Retreatment instruments in removing filling material (gutta-percha and cement) during root canal retreatment and complemented with further root canal preparation using instruments from the same system.

Methodology Forty-eight premolars with fully formed apices were used, measuring between 18 and 22 mm in length with a straight canal and no evidence of calcification and resorption. After root canal shaping manually and filling using lateral condensation of gutta-percha the teeth were stored at 37°C and 100% relative humidity for 15 days. The specimens were then divided randomly into four groups ($n = 12$) according to the instruments used for retreatment: PTF1: ProTaper Retreatment and F1 instrument, PTF4: ProTaper Retreatment and instruments until F4, MT20: Mtwo Retreatment instruments and instrument size 20, 0.06 taper, MT40: Mtwo Retreatment instruments and instruments until size 40, 0.04 taper. Subsequently, the specimens were radiographed in a mesial-distal direction and the radiographs scanned to check the amount of remaining filling material in the root canal. The images were analyzed by the computer Image Tool program. The data were subjected to statistical tests of Anova and Tukey HSD ($P < 0.05$).

Results The results indicate a significant difference between the groups PTF4 (4.24 mm^2) and MT20 ($11.62 \text{ mm}^2 - P = 0.005$) and PTF1 ($11.23 \text{ mm}^2 - P = 0.009$) but not significantly different with MT40 ($6.43 \text{ mm}^2 - P = 0.722$). Regarding the time there was a significant difference between the groups that were completely re-instrumented and the sub-instrumented groups and PTF4 were faster ($P = 0.0000$).

Conclusions The ProTaper and Mtwo Retreatment instruments when associated with their respective instruments for further root canal preparation resulted in a greater removal of filling material.

R243

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Effectiveness of various retreatment approaches in canals filled with several gutta-percha techniques

Aim To test the effectiveness of various retreatment approaches in canals filled with several gutta-percha techniques.

Methodology One hundred and eighty human single-rooted teeth were used. Root canals were shaped with Ni-Ti rotaries and teeth were divided into 4 groups ($n = 45$). Canals were filled with

warm vertical condensation, carrier-based gutta-percha system, cold lateral condensation or single cones: AH Plus was used as the sealer. The teeth were stored in a 37°C incubator under 100% humidity for 2 weeks. Then, groups were divided into 3 subgroups of 15 teeth in each. Root filling materials in each subgroups were removed using either the ProTaper Universal Retreatment System, Mtwo Retreatment System or Hedström files+Gates Glidden burs. All teeth were split into 2 halves, canal surfaces were examined under $\times 15$ magnification and photographed. AutoCAD software was used to calculate the remaining filling material. Data were analyzed with Kolmogorov-Smirnov, Kruskal-Wallis and Mann-Whitney U tests. The significance level was set at $P < 0.05$.

Results The area of remaining filling material in the single cone group was significantly lower than the warm vertical and lateral condensation groups ($P < 0.05$). No significant difference was observed between warm vertical and lateral condensation groups ($P > 0.05$). The percentage of remaining filling material in the Hedström files+Gates Glidden bur group was significantly higher compared to Mtwo and ProTaper techniques ($P < 0.01$). No significant difference was found between Mtwo and ProTaper groups ($P > 0.05$).

Conclusions No retreatment technique was able to remove the root filling material completely. Ni-Ti rotaries were more effective in the removal of root filling materials. Less filling material remained in canals filled with the single-cone technique.

R244

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Evaluation of the effectiveness of ProTaper, GPX and K3XF files for gutta-percha removal

Aim The aim of this study was to compare the amount of the residual gutta-percha and sealer in root canals after retreatment using ProTaper retreatment files (Dentsply Maillefer), GPX files (Brasseler) and K3XF files (SybronEndo).

Methodology A total of 30 mesial root canals of mandibular molars were cleaned and filled with gutta-percha and AH Plus sealer. The samples were randomly assigned to 1 of 3 groups ($n = 10$). The coronal third gutta-percha was initially removed using Gates Glidden drills. The remainder was removed using ProTaper retreatment files (group 1), GPX files (group 2) or K3XF (group 3). The samples were split longitudinally and observed using scanning electron microscopy (100X) at 3 different levels: coronal, middle and apical. The presence of residual gutta-percha, sealer and smear layer were evaluated in each image. The data were analyzed with Kruskal-Wallis test.

Results Groups 1 and 3 had similar amounts of remanent gutta-percha in the coronal third, less than group 2. Group 3 had less gutta-percha in the middle and apical third. However, there was no significant difference between the techniques in the amount of the filling remnants ($P > 0.05$). Remaining filling material was observed in all specimens.

Conclusions None of the retreatment techniques were able to completely remove all gutta-percha and sealer. The complete removal of gutta-percha from apical regions of curved canals remains a challenge.

R245

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Retreatability of a bioceramic root canal sealer with Reciproc and ProTaper retreatment systems: a confocal laser scanning microscopy study

Aim To evaluate the efficacy of Reciproc and ProTaper D instruments to remove three endodontic sealers when used in combination with a single gutta-percha point.

Methodology Palatal canals of 36 maxillary molars were sectioned to 15 mm length and shaped with the ProTaper Universal System until F2, and then filled with a single gutta-percha point with one or other of three different sealers: TotalFill BC Sealer, MTA Fillapex and Top Seal. Sealers were mixed with 0.1% Rhodamine. Afterwards, retreatment was undertaken with Reciproc R25 and Protaper D instruments. Confocal laser scanning microscopy was used to observe the remaining filling material which was measured with Adobe Photoshop CS5. Working time was determined for each instrument and material. Statistical analysis: mean working time and amount of remaining material over the canal walls were compared using the non parametric Kruskal–Wallis test; two by two comparisons were done with Duncan's test; significant level was done for $P < 0.05$.

Results Filling material could not be removed completely from the root canals independently of the sealer type and retreatment technique. Patency after retreatment was achieved in 100% of the samples. ProTaper D, when used to eliminate MTA Fillapex, was associated with significantly less remaining material than the other groups ($P < 0.05$). Top Seal had the greatest amount of remaining material with both instruments. The shortest working time was achieved with Reciproc when used to eliminate MTA Fillapex and Top Seal, with significant differences with ProTaper D when used to remove Top Seal and TotalFill ($P < 0.05$).

Conclusions MTA Fillapex was better removed from root canal walls. TotalFill needed more time to be removed. ProTaper D removed greater amounts of material from root canals, but the Reciproc system was quicker.

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R246

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Assessment of various kinematics for removal of root canal filling material

Aim To assess the effectiveness of various kinematics (150° clockwise [CW]–30° counter-clockwise [CCW], 270° CW–30° CCW, and continuous rotation) on filling material removal.

Methodology Forty mandibular premolars were instrumented using K-files and Gates Glidden drills up to size 35, and filled using cold lateral compaction. After, the specimens were stored at 37°C and 100% humidity for 2 months, retreatment was then performed using one of the following techniques ($n = 10$): hand file (control group), 150° CW–30° CCW reciprocating motion, 270° CW–30° CCW reciprocating motion, and continuous rotation. ProTaper Universal Retreatment System was used in the

machine-assisted groups and final apical preparation was performed with a size 40 H-file. The teeth were sectioned and photographed at 15× magnification using a stereomicroscope. Images were transferred to a computer, and residual filling material was quantified using image analyzing software. The time to retreatment was also recorded. The data were analysed with one way analysis of variance and LSD *post-hoc* tests at the 95% confidence level ($P = 0.05$).

Results Continuous rotation and 270° CW–30° CCW reciprocating motion left less filling material than the control group ($P < 0.05$). However, there was no significant difference between the control group and the 150° CW–30° CCW reciprocating motion. Continuous rotation and 270° CW–30° CCW reciprocating motion were faster than the control group and 150° CW–30° CCW reciprocating motion in retreatment ($P < 0.05$).

Conclusions Continuous rotation and 270° CW–30° CCW reciprocating motion was advantageous in retreatment over the hand file and 150° CW–30° CCW reciprocating motion.

R247

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The effectiveness of several instrumentation technique for removing gutta-percha during root canal retreatment – SEM analyses

Aim The aim of this *in vitro* study was to compare the effectiveness of three rotary and two hand instruments when removing gutta-percha and AH Plus® sealer, during root canal retreatment using scanning electron microscopy (SEM).

Methodology The root canals of sixty extracted single-rooted straight premolars were prepared using a crown-down technique and enlarged to a size 40 apical file, before filling with laterally condensed gutta-percha and AH Plus® sealer. The samples were stored for 3 weeks in saline at 37°C in an incubator, before being divided randomly into 5 groups of 12 teeth each with regards to the instrument used: K-files, Hedström files, D-RaCe, ProTaper Universal Retreatment System (PTUS) and ProFile. Following retreatment the roots were split longitudinally and three different areas (coronal, middle and apical thirds) of the root canal were evaluated using SEM. The assessment and comparisons of three different parameters: smear layer, debris with remaining filling material, irregularities of surface profile were made using a predefined scale. Statistical analyses were performed using the Kruskal–Wallis test with Bonferroni *post-hoc* test.

Results The D-RaCe system was the most effective in removing both smear layer and removing filling material, while PTUS was the least aggressive on surface profile. No significant difference was found between the instruments concerning removing of smear layer and irregularities of surface profile. When comparing effectiveness in removing debris and gutta-percha/sealer, a significant difference was found in the apical third ($P = 0.007$). Apically, D-RaCe ($P = 0.004$), PTUS ($P = 0.0055$) and Hedström ($P = 0.0073$) instruments were more effective than the ProFile system. Effectiveness of instruments in all of three evaluating parameters was the best in the coronal third and worst in apical third of root canals ($P < 0.05$).

Conclusions In the apical third of root canals, the instrumentation technique had an influence on removal of debris and filling material. ProFile instruments were significantly less effective. Instrumentation technique had no influence on removal of smear layer or on surface profile irregularities. The apical third had the greatest amount of smear layer, debris and dentine irregularities, compared to coronal and middle thirds.

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*Department of Endodontics, Faculty of Dentistry, Hacettepe University, Ankara, Turkey***The efficiency of three single file systems for removing root filling material: a micro-computed tomography study****Aim** To evaluate the efficacy of three single file systems when removing root filling material using micro-computed tomography.**Methodology** Thirty extracted human mandibular incisor teeth with a single canal were used. The canals were prepared using the ProTaper Universal System up to F2 according to the manufacturer's instructions and filled with gutta-percha and sealer. Samples were scanned preoperatively using micro-CT with a voxel size of 21 µm. The teeth were distributed into three experimental groups ($n = 10$) and removal of the filling material was achieved in group 1 using the OneShape system, group 2 using WaveOne and group 3 using Reciproc. The retreatment time was recorded for each tooth. After removing the root filling material, teeth were scanned postoperatively using micro-CT imaging. Percentage of residual root canal filling material and time required to remove filling material were calculated. The results were analysed with Kruskal–Wallis test ($P < 0.05$).**Results** Residual filling material was observed in all groups. There was no significant difference between the groups according to the percentages of filling remnants in the canal ($P > 0.05$). There was no significant difference among the groups in terms of the time required for removing of canal filling ($P > 0.05$).**Conclusions** All the specimens had some filling remnants on the root canal wall.**R249**I. Ferreira¹, S. Soares², J. Correia-Sousa³, J. Barros³, M.A. Lopes² & I. Pina-Vaz^{3,*}¹Faculty of Dental Medicine of University of Porto, ²Department of Metallurgical and Materials Engineering, Faculty of Engineering, University of Porto & ³Department of Endodontics, Faculty of Dental Medicine of University of Porto, Porto, Portugal**New insights into endodontic resin sealer removal****Aim** To determine the best solvent for removal of an epoxy-resin sealer (AH Plus).**Methodology** The solubility of epoxy-resin sealer AH Plus (Dentsply DeTrey, Germany) was assessed in eucalyptol, chloroform, 85% phosphoric acid, xylene, methyl ethyl ketone (MEK) and ethyl acetate following ISO 6876/2012 specifications. Standardized samples of AH Plus ($n = 6$) were immersed in the solvents, for 2 and 5 min. The control group was tested by immersion in distilled water. Pre and post-weight measurements were recorded by percentage weight loss. The samples were also analysed using scanning electronic microscopy (SEM). Statistical analysis was performed using the Mann–Whitney test.**Results** Chloroform, Ethyl acetate and MEK had significantly different solvent profiles from the control group ($P < 0.005$). Chloroform was the best solvent (2 min: $13.42\% \pm 2.22$; 5 min: $15.29\% \pm 2.62$). However, eucalyptol, one of the most common endodontic solvents, had the worst results (2 min: 0 ± 0 ; 5 min: 0 ± 0). Ethyl acetate showed promising results (2 min: $9.51\% \pm 1.65$; 5 min: $10.55\% \pm 2.70$) and MEK achieved similar efficacy after 5 min (2 min: $3.03\% \pm 1.41$; 5 min: $10.67\% \pm 2.18$). Xylene and phosphoric acid were not effective, with solubility values near zero. SEM images supported

the results showing the greatest surface topographic changes in samples immersed in chloroform.

Conclusions Ethyl acetate, a substance not commonly used in Dentistry, was comparable to chloroform, without the potential hazards. Chloroform, well known as a good gutta-percha solvent, had the best sealer solvent profile. MEK, another new substance for this purpose, also showed good efficacy at 5 min. Ethyl acetate and MEK, presented as new solvents, had markedly superior dissolution ability in the resin-based sealer than eucalyptol. These results suggest that both ethyl acetate and MEK might be universally used as an effective solvent for sealer removal during root canal retreatment.**R250**

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*Endodontic Unit, Melbourne Dental School, Melbourne, Australia***Efficiency of endodontic retreatment: GuttaCore versus lateral compaction****Aim** To assess the efficiency (treatment time) of root canal retreatment between GuttaCore (GC) and conventional lateral compaction (LC)**Methodology** Two-hundred and fifty six extracted teeth comprised of incisors, canines and premolars were set-up in a simulated clinical environment. Canals were prepared using Mtwo rotary Niti instrument with a crowdown technique and then root filled by dental students with either GC or LC. Teeth were screened and excluded from the study if there were fractured instruments, perforations, apical extrusion of filling material and severely curved canals ($>30^\circ$). Remaining specimens were stratified in to four experimental groups: anterior teeth with LC ($n = 47$), anterior teeth with GC ($n = 69$), premolars with LC ($n = 22$) and premolars GC ($n = 28$). A canal of single-rooted teeth or lingual canal of multi-rooted teeth was subsequently retreated by endodontic postgraduate students. The time taken to reach the working length (T1) and time taken to complete instrumentation (T2) was recorded. Retreatment efficiency was defined as the sum of T1 and T2. The Mann–Whitney U test was used for statistical analysis.**Results** One hundred and forty nine canals were retreated. The mean retreatment time was significantly shorter for GC compared to LC in both tooth types. For anterior teeth, the mean total time was 116 ± 18 s for GC and 236 ± 37 s for LC ($P < 0.001$). For premolars, mean total time was 77 ± 12 s for GC and 220 ± 44 s for LC ($P < 0.05$).**Conclusions** For straight and moderately curved anterior and premolar teeth, GC was significantly more efficient to retreat compared to LC. However, decision to adopt a new obturation technique clinically should not depend solely on retreatment efficiency. Treatment effectiveness of initial treatment as well as other factors such as adverse effects, cost, time and operator perception should also be considered.

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Effectiveness of two rotary NiTi systems in filling material removal during root canal retreatment: evaluation by CBCT and diffusion capacity of calcium hydroxide through dentine

Aim Root canal retreatment is necessary when failure of the primary treatment has occurred. Rotary instruments for retreatment are effective, but they leave root filling residue in the canal. The aim of this study was to evaluate the efficacy of gutta-percha removal with ProTaper and MTwo retreatment files, with and without supplementary files, by measuring the area of the residual material and pH levels as well as calcium hydroxide ion release.

Methodology The root canals of 120 single-rooted mandibular human teeth were instrumented and divided into six groups. Four groups were filled with gutta-percha and AH-Plus sealer and retreated after three months, by two nickel titanium retreatment files, ProTaper and Mtwo, with and without supplementary instruments. Two groups served as positive and negative controls.

Therefore, the following groups were obtained: G1-Mtwo size 20, .06 taper (MT20); G2- Mtwo size 40, .04 taper (MT40); G3- ProTaper F1 (PTF1); G4- ProTaper F4 (PTF4); Negative Control (N) and Positive Control (P). The residual filling material was measured using high resolution micro-computed tomography. The groups were filled with calcium hydroxide, except in group N, and all teeth were placed in individual vials with deionized water. The pH was measured at 7, 21, 45 and 60 days. Data were compared by Kolmogorov-Smirnov and Shapiro Wilk statistical tests to verify the normality and the Tukey HSD test for multiple comparisons at 5% significance.

Results Groups with supplementary files, MT40 and PTF4, were significantly more effective in removing gutta-percha and endodontic sealer, but no statistical difference was found between them ($P > 0.05$). As regards pH levels, PTF1 group hindered calcium hydroxide spread (2.07 ± 0.41) differing significantly from groups MT40 (2.55 ± 0.43) and P (2.49 ± 0.43) ($P < 0.05$).

Conclusions Neither retreatment files completely removed filling material from the root canal. There were no difference between ProTaper and MTwo retreatment files, but the use of supplementary files was effective. The change in pH was greater when the root canal walls had less root filling residue. It suggests that this methodology can be used for evaluating root canal cleaning in endodontic retreatments.