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ESE Wladimir Adlivankine Research Prize, Education Prize and Original Research Abstracts

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Contents

ESE Wladimir Adlivankine Research Prize  
RP1 3

ESE Education Prize  
EP1 - 2 4

Original Scientific Abstracts

Session 1: Thursday 14th September

Anatomy and Access  
R001 – R004 5

Imaging  
R005 – R009 6

Microbiology  
R010 – R023 8

Treatment planning  
R024 12

Irrigants/disinfection: Materials  
R025 – R029 13

Irrigants/disinfection: Antimicrobial activity  
R030 – R032 14

Irrigants/disinfection: Canal cleaning  
R033 – R041 15

Irrigants/disinfection: Irrigant agents  
R042 – R046 18

Education  
R047 – R058 20

Session 2: Friday 15th September

Apex locators/working length  
R059 – R063 25

Preparation: Instrument Fracture  
R064 – R069 26

Preparation: Apical Extrusion  
R070 – R073 28

Preparation: Cleaning ability  
R074 – R075 30

Preparation: Shaping ability  
R076 – R079 30

Filling: MTA  
R080 – R085 32

Filling: Sealers  
R086 – R090 34

Filling: Canal  
R091 – R093 36

Restoration of root filled tooth  
R094 – R102 36

Surgery  
R103 – R104 39

Retreatment  
R105 – R112 40

Root Fracture  
R113 – R121 43

Session 3: Saturday 16th September

Basic science pulp  
R122 – R125 46

Biocompatibility  
R126 – R130 47

Clinical Trial  
R131 – R136 49

Epidemiology  
R137 – R150 51

Histopathology periapical lesions  
R151 – R152 55

Modern and new technology  
R153 – R155 56

Outcome studies  
R156 – R163 57

Trauma/Regeneration  
R164 – R174 60

Other  
R175 – R179 63

Author Index  66
Efficacy of irrigant activation techniques in removing intracanal smear layer and debris from mature permanent teeth: a systematic review and meta-analysis

**Aim** To establish whether Irrigant Activation Techniques (IAT) result in greater intracanal smear layer and debris removal than Conventional Needle Irrigation (CNI).

**Methodology** Six electronic databases (PubMed, EMBASE, Cochrane Library, Science Direct, Web of Science and Google Scholar) and supplemental sources were searched by two reviewers from 01.2000 to 11.2016 to identify scanning electron microscopy studies evaluating smear layer and/or debris removal following use of Manual Dynamic Activation (MDA), Passive Ultrasonic Irrigation (PUI), Sonic Irrigation (SI) or Apical Negative Pressure (ANP) IATs in mature permanent teeth. Meta-analyses were performed for each canal segment (coronal, middle, apical and apical 1 mm) in addition to subgroup analyses for individual IATs with respect to CNI. Outcomes are presented as Standardised Mean Differences (SMD) alongside 95%-Confidence Intervals (CI), to allow direct comparisons between studies that used semi-quantitative scoring systems to evaluate intracanal cleanliness.

**Results** From 252 citations, 16 studies were included in the qualitative analysis and 12 in the meta-analysis. Nine studies investigated smear layer, 1 debris and 12 examined both with ANP (n = 10) and PUI (n = 10) being most commonly tested followed by SI (n = 7) and MDA (n = 6). The meta-analysis demonstrated significant improvements in the coronal (SMD: 1.15/CI: 0.72–1.57, SMD: 0.54/CI: 0.29–0.80), middle (SMD: 1.30/CI: 0.59–2.53, SMD: 0.8/CI: 0.58–1.13) and apical thirds (SMD: 1.22/CI: 0.83–1.62, SMD: 1.86/CI: 0.76–2.96) for smear layer and debris removal respectively. In the apical 1 mm, IATs improved cleanliness; however, the differences were insignificant (SMD: 1.15/CI: −0.47–2.77). The most effective IATs in the coronal third were SI (SMD: 1.29/CI: 0.76–3.41) for smear layer and ANP (SMD: 0.62/CI: 0.12–1.12) for debris, and for the middle third were SI again (SMD: 2.21/CI: 1.20–3.22) and PUI (SMD: 1.26/CI: 0.77–1.74). In the apical segment MDA removed the greatest quantity of both smear layer (SMD: 1.50/CI: 0.62–2.37) and debris (SMD: 2.22/CI: 1.19–3.26).

**Conclusions** Within limitations of this study, IATs were found to significantly improve intracanal cleanliness across a substantial portion of the canal. Therefore their use is encouraged during routine root canal treatment. No single technique produced the highest impact across all regions hence a combination of machine and hand assisted IATs is postulated to result in greater efficacy. Based on this review and surrounding literature SI in combination with MDA, the latter being equally effective as ANP at the apex, is likely to lead to the greatest smear layer and debris removal throughout the canal. Further in vivo experiments are required to understand the impact this would have on periapical healing.
ESE EDUCATION PRIZE

EP1

A. Baaij* & A.R. Özok
Department of Endodontontology, Academic Centre for Dentistry Amsterdam, Amsterdam, Netherlands

Influence of method of teaching endodontology on the self-efficacy and self-perceived competence of undergraduate students

Aim To assess whether self-efficacy and the self-perceived competence of students were influenced by methods of teaching endodontology.

Methodology Methods of teaching Endodontology at our institution were revised. Changes included: an increased number of tutorials, the method of clinical training, the method of summative assessment, the number of endodontic treatments required, and the supervision while performing endodontic treatment. An intermediate cohort comprised students who were exposed to all or some of the former methods and students who were exposed to all or some of the revised methods of teaching. Twenty-four students participated; their self-efficacy and self-perceived competence were assessed close to graduation with a questionnaire. Additionally, their performance in carrying out endodontic treatments was assessed according to predetermined criteria. Data were analyzed using Cohen’s Kappa, Cronbach’s Alpha, Mann-Whitney and T-tests.

Results Neither the method of clinical training, nor the method of summative assessment influenced the self-efficacy or self-perceived competence of students. The larger number of tutorials increased students’ self-perceived competence, but did not influence their self-efficacy. Not the entire number, but the number of endodontic treatments performed under supervision of endodontists was associated with an increase in students’ self-efficacy and self-perceived competence. Students’ self-efficacy and self-perceived competence were not influenced by their performance in carrying out endodontic treatments.

Conclusions Among the teaching methods assessed, only the number of tutorials and the number of endodontic treatments performed under supervision of endodontists influenced the self-efficacy and the self-perceived competence of students.

EP2

M.R. Reymus* & C.D. Diegritz
Department of Operative Dentistry, University of Munich, Munich, Germany

Self-printed artificial teeth for endodontic education

Aim To assess the feasibility of creating artificial teeth for endodontic training. The workflow was aimed to be simple, time and cost effective as well as transferable to other educational institutions. The final goal was to create an alternative to extracted human teeth and commercial artificial ones.

Methodology Suitable extracted human teeth were selected according to their degree of development and destruction, the volume of the pulp chamber and root canals on radiographs as well as to the estimated difficulty for root canal treatment. A three-dimensional radiograph of the selected tooth was taken using a cone-beam CT with a small field-of-view (Kodak 950). The generated data were processed with several software applications (InVesalius, Brazil; Meshmixer, USA; PreForm, USA) to generate a printable STL file. This file was printed with a stereolithographic printer using a resin mixed with barium sulphate for radiopacity (Print 2, Formlabs, USA).

Results The self-production of artificial teeth for endodontic training was feasible. The workflow was kept as simple as possible thus reducing the number of processing steps to a minimum. The software applications were user-friendly, easy to learn and free for educational purposes. The quality achieved by the cone beam CT was sufficient, thus being transferable to other institutions possessing this equipment. The search and selection of suitable teeth was the most time-consuming step. The process of transferring these real teeth to printed artificial copies takes approximately 7 h for 40 molars. Material costs for one tooth amount to approximately 0.31 €.

Conclusions The use of extracted human teeth has serious drawbacks which have come to the fore in recent years. Commercial artificial teeth have been promoted as a possible alternative, yet they are too expensive to be purchased in large quantities for sufficient training in the preclinical settings. The workflow presented shows a time and cost effective way to produce artificial teeth, which are suitable for endodontic training and exceed commercial ones in various criteria. They are less expensive, can have unlimited variations in anatomy, present radiopacity and are available at the right time in sufficient numbers.
ORIGI NAL SCIENTIFIC ABSTRACTS

SESSION 1: THURSDAY 14TH SEPTEMBER

ANATOMY AND ACCESS

R001
K. Oleczak* & H. Pawlicka
Department of Endodontics, Medical University of Lodz, Poland

Morphology of maxillary first and second molars analyzed by cone-beam computed tomography in a Polish population

Aim To evaluate the root and canal morphology of permanent maxillary first and second molars in a Polish population using cone-beam computed tomography.

Methodology The sample included 112 cone-beam computed tomography (CBCT) images representing 185 maxillary first and 207 maxillary second molars. The number of roots and root canals, and the frequency of additional canals (MB2) in the mesiobuccal roots were determined. The results were subjected to statistical analysis using chi-square tests with Yates’ correction.

Results All maxillary first molars had three roots (100%). The majority of maxillary second molars had three roots (91.8%), 5.8% had two roots and 2.4% had one root. A significant difference was observed between maxillary first and second molars for number of roots (P < 0.01). A significant difference was also found in the distribution of the number of canals in the maxillary first and second molars (P < 0.001). The majority of maxillary first molars had four root canals (59.5%), while only 40.5% had three root canals. Most maxillary second molars had three root canals (70%). In other maxillary second molars, four canals (23.2%), two canals (3.9%), and one canal (1%) or C-shaped canals (1.9%) were observed. Additional canals (MB2) in the mesiobuccal roots were detected significantly more frequently in the maxillary first molars than the second molars (P = 0.000). In turn, the presence of three root canals was significantly more common in the second than in the first maxillary molars (P = 0.000).

Conclusions There are differences in the number and configuration of roots and root canals between maxillary first and second molars in this Polish population. This data may facilitate successful root canal treatment. More attention should be given to the detection of additional canals (e.g. MB2) during root canal treatment of maxillary first molars. CBCT scanning is an effective method for studying dental morphology.

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R002
A. Nowicka1*, K. Kot2, J. Kolecki1, M. Sroczyk-Jaszczynska1, G. Wilk1 & M. Lipski2
1Department of Conservative Dentistry and Endodontology, 2Department of Preclinical Conservative Dentistry and Preclinical Endodontology & General and Dental Radiology, Pomeranian Medical University in Szczecin, Szczecin, Poland

A study of anatomic patterns of root canals in mandibular anterior teeth using cone-beam computed tomographic imaging

Aim To investigate various anatomic configurations of root canals in mandibular anterior teeth using cone-beam computed tomographic (CBCT) imaging.

Methodology A total of 376 CBCT images of mandibular anterior teeth were collected from 69 patients who accepted CBCT examinations as part of their dental diagnosis. Axial, sagittal, and cross-sectional slices with a thickness of 0.2 mm were used. The following parameters were recorded and evaluated: tooth position, root number, canal number, and root canal configuration. The number of root canals and internal patterns were classified according to Vertucci’s criteria. Data were analysed using the Mann-Whitney U test. Differences were considered significant at P < 0.05.

Results All of the incisors in this study had 1 root, and 7.69% of the canines had 2 roots. Two canals were more frequent in the central and lateral incisors (37.6% and 38.89%, respectively). The largest proportion of central (60.80%) and lateral (58.73%) incisors, and canines (83.46%) had type 1 root canal configurations. Type III was found in 23.20%, 23.02% and 4.72% of the central incisors, lateral incisors, and canines, respectively. The prevalence of the other configuration types was type V and II. Types IV, VI, VII, and 2-1-2-1 were also found with relatively less frequency. There were fewer variations in mandibular canines than in incisors.

Conclusions This study provides detailed information about the root canal morphology of mandibular anterior teeth. There was a high prevalence of 2 root canals in the mandibular incisors. In endodontic practice, clinicians should be aware of possible root canal anatomic differences.

R003
G. Rover1*, F.G. Belladonna2, E.A. Bortoluzza1, C.S. Teixeira1 & E.J.N.L. Silva1
1Department of Dentistry, Federal University of Santa Catarina, Florianopolis, 2Department of Endodontics, Fluminense Federal University, Rio de Janeiro & 3Department of Endodontics, Grande Rio University, Rio de Janeiro, Brazil

Influence of cavity access design on root canal detection, instrumentation outcomes and fracture resistance in maxillary molars

Aim To assess the influence of conservative endodontic cavities (CECs) on root canal detection, instrumentation efficacy and fracture resistance of maxillary molars.

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Thirty extracted intact maxillary first molars were assigned to CEC or traditional endodontic cavities (TEC) groups (n = 15/group), and accessed accordingly. Root canal detection was carried out in three stages: 1) no magnification; 2) under an operating microscope (OM); 3) under OM and ultrasonic preparation. After root canal preparation with Reciproc files, samples were scanned again. Untouched canal walls, debris accumulation, canal transportation and centring ratio were analysed. After root canal filling and cavity restoration, the samples were submitted to a fracture resistance test (FR). Data were analysed by the Fisher exact, Shapiro-Wilk and t-tests (α=0.05).

**Results** In the TEC group it was possible to locate more root canals in stages 1 and 2 (P < 0.05). After preparation, the proportion of untouched canal walls and accumulated debris were similar between the groups. Canal transportation was significantly greater in palatal canals at 7 mm from the apical end (P < 0.05). The preparation of the root canal was more centralized with the TEC in the palatal canal at 5 and 7 mm from the apical end (P < 0.05), and with the CEC in the disto-buccal canal at 5 mm from the apical end (P < 0.05). There was no difference in FR between the groups TEC (937.55 ± 347.25N) and CEC (996.30 ± 490.78N) (P > 0.05).

**Conclusions** CEC compromised the detection of root canals in maxillary molars when no ultrasonic preparation with associated OM was used. This cavity design had a negative influence on the instrumentation of the palatal canal and did not contribute to an increase of fracture resistance of the molars evaluated.

**R004**

C. Keskin* & A. Keleş
Department of Endodontics, Faculty of Dentistry, Ondokuz Mayıs University, Samsun, Turkey

**Quantitative evaluation of apical delta morphology in the mesial roots of mandibular first molar teeth: a micro-CT study**

**Aim** To analyze apical delta morphology quantitatively using micro-computed tomography (micro-CT) in the mesial roots of mandibular first molars.

**Methodology** Two hundred and sixty-nine mesial roots of mandibular first molar teeth were scanned by micro-CT. Twenty-two specimens with apical delta ramifications were selected from reconstructed micro-CT images. The number and vertical extension of apical ramifications were recorded. Major diameter, minor diameter and roundness values in the most apical slices of apical delta ramifications and at the slice where ramifications merged to form the main canal were calculated. Data was analyzed using descriptive statistics.

**Results** The incidence of apical deltas was 13.01%. The mean number of apical delta ramifications was 4.45 and the mean vertical extension was 0.95 mm. The majority of the apical delta ramifications had noncircular cross sectional shape.

**Conclusions** Complex morphological properties of apical deltas might complicate the three-dimensional shaping and filling of root canal systems.

**Acknowledgements** This study was supported by the Scientific and Technological Research Council of Turkey-TUBITAK (grant no. 114S002). Authors deny any conflict of interest.
Circumstances behind the use of Cone Beam Computed Tomography (CBCT) for endodontic reasons in Sweden from the perspective of the referring dentist

**Aim** To study the circumstances preceding the CBCT examination referral for endodontic reasons in Sweden.

**Methodology** Fourteen dentists (8 female) 33–58 years of age (mean =44), practicing in Sweden were strategically selected. Ten of the dentists were specialists in endodontics. The absolute inclusion criterion was experience of referring patients for CBCT for endodontic reasons. The included dentists provided a variation concerning gender, age, work experience, education background, location of practice, service affiliation and accessibility to CBCT. Data was obtained through thematic, semi-structured interviews exposing the context of their last self-reported three referrals. Dentists were encouraged to describe their experiences of the circumstances in their own words, aided by the interviewer’s open-ended questions. The interviews were audio recorded and transcribed verbatim. Qualitative content analysis was used to analyze the text.

**Results** The preliminary results may indicate that high clinical diagnostic standards, clinical common sense and a willingness of helping the patient with minimal harm may restrict the use of CBCT to address complex diagnostic judgements or therapeutic decisions, which comply with existing European guidelines on the use of CBCT in endodontics. Knowledge of guidelines was however limited among the interviewed dentists.

**Conclusions** Common sense and high professional standards seem to lead dentists in Sweden to comply with current European guidelines for the use of CBCT in endodontics even when the dentists lack knowledge of the guidelines.

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Pulp canal obliteration of anterior teeth: canal detection using various radiographic methods

**Aim** To determine the ability of CBCT to detect canals in teeth with pulpal obliteration compared to conventional periapical radiographs. A second aim was to determine root canal morphology and dimensions using μCT in anterior teeth.

**Methodology** Teeth were screened from a human tissue bank and 48 teeth with pulpal obliteration perceived on standard PA radiography were selected. Teeth were then scanned with μCT at 20 μm resolution. The data was reconstructed and dimensional analysis was performed in Materialise software. The use of a soft tissue phantom was developed to mimic beam attenuation and improve contrast using a material of similar radiographic density developed as a substitute for soft tissue. The teeth were the scanned in a radiographic phantom using a Morita Accuitomo 170 as well as straight on and angled periapical views.

**Results** Canals were present in all teeth, however, selected samples with canals measuring >100 μm with μCT were not perceptible on conventional radiographs. Some samples showed no detectable canal at 20 μm in the coronal third of the root. CBCT results revealed varying effects of kV, mA, scan time and rotation on canal detection, as well as soft tissue factors, canal morphology and reconstruction algorithms.

**Conclusions** Contrary to previous research that a canal lumen is always present and is never less than 100 μm, some teeth had regions of the root with no canal detectable at 20 μm resolution. Optimisation of CBCT to detect root canals is a complex interplay between spatial resolution, contrast and noise.

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Living oral bacteria detected on pathologically changed heart valves using molecular-microbiological techniques

Aim To detect living bacteria of oral origin on pathologically changed heart valves and thus show the importance of oral health status.

Methodology Patients with pathologically changed heart valves with the diagnosis of aortic stenosis were included. Patients with the diagnosis of infective endocarditis were excluded. Heart valves removed during cardiovascular surgery with heart valve replacement were transported in a container for microbiological examination. The tissue was cut into two parts and homogenized. One aliquot was cultivated aerobically and anaerobically. DNA from living bacteria was extracted using Ultra-Deep Microbiome Prep (Molzym GmbH, Bremen, Germany). 16S rRNA gene specific for bacteria was amplified by PCR using Mastermix 16S Complete kit (Molzym GmbH) and sequenced in positive samples. DNA sequences were interpreted by an experienced microbiologist by using BLAST software (http://www.ncbi.nlm.nih/BLAST).

Results Currently, twenty samples have been processed. Bacterial DNA was detected in 15 samples. The following bacteria were identified: Propionibacterium acnes (n = 14), Staphylococcus species (n = 5), Streptococcus sanguinis (n = 3), Streptococcus oralis (n = 1), Streptococcus species (species not identified based on poor sequence quality) (n = 1) and Carnobacterium divergens (n = 1). In 11 samples, more than one bacterium was found.

Conclusions Preliminary results reveal a significant appearance of living oral bacteria in pathologically changed heart valves. More samples will be analyzed to determine an influence of oral health on the pathology of heart valves in future.

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Antifungal effect of various intracanal medicaments in Candida albicans-infected dentine model

Aim To assess the antifungal efficacy of a synthetic human β-defensin-3-C15 (HBD3-C15, 15 amino acids in length) peptide using C. albicans-infected human root dentine blocks

Methodology Forty-five human root dentine blocks were infected to induce biofilm formation and tubular penetration of C. albicans. Calcium hydroxide (CH), 2% chlorhexidine (CHX), and HBD3-C15 were placed into canal lumens as intracanal medicaments. Saline (S) and non-functional HBD3 peptide gel (NFP) served as controls. After one week of intracanal disinfection, the dentinal debris at the depth of 200 and 400 μm were collected from the root canal lumen. Antifungal efficacy was assessed by measuring colony-forming unit (CFU) of C. albicans after 72 h incubation at 37°C. Collected data were analyzed statistically with one way ANOVA and paired t tests.

Results All medicaments were associated with significantly lower CFUs than controls (P < 0.05), and their efficacy was not affected by tubular depth (P > 0.05). Intracanal HBD3-C15 medication lowered CFU values significantly more than CH and NFP at both depths (P < 0.05), and its antifungal efficacy was similar to that of CHX at both depths (P > 0.05). There was no significant difference in the CFU values between NFP and CH groups (P > 0.05), but they were significantly lower than that of the control at both depths (P < 0.05).

Conclusions Synthetic HBD3-C15 had antifungal effect on C. albicans-infected human root dentine, which were similar to CHX and much greater than CH intracanal medicament.

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Morphological changes induced by Streptococcus dentisani in bacteria involved in endodontic infections

Aim To analyze the morphological changes produced by the action of S. dentisani supernatant on a selection of bacteria involved in root canal infections

Methodology A scanning electron microscopy (SEM) was used to directly observe the effect of the S. dentisani supernatant on the cell surfaces of the following bacteria: F. nucleatum, P. intermedia, E. corrodens, E. faecalis, and P. micra. Briefly, 160 μL of the bacterial cultures in exponential phase (O.D.610 = 0.8–1) were mixed with 40 μL of the supernatants (assay concentration of 2X), and incubated for 60 min at 37°C. The suspension was centrifuged at 4000 rpm 10 min and the supernatant discarded. The pellets were fixed in Karnovsky solution, washed twice with PBS buffer and exposed to 1% osmium tetroxide in PBS buffer for 1 h. The samples were rinsed with PBS buffer and moved through a gradual process of dehydration, starting with 30% ethanol and ending with absolute ethanol. Finally, the samples were mounted on scanning electron micrograph stubs, sputter coated with gold, and viewed on a Hitachi S-4800 scanning electron microscope.

Results SEM images showed that exposure of the bacteria to the supernatant of S. dentisani induced structural changes in the membrane. F. nucleatum suffered a dramatic lysis and even the release of cellular contents was observed. P. intermedia showed small vesicles in the surface and disruption of the cellular wall. E. corrodens showed agglutination of their cells. The cell membrane of E. faecalis was unstructured, acquiring a sticky appearance. Finally, as expected from previous inhibition assays, no structural change was observed in P. micra.
Conclusions S. dentisani supernatant induced morphological changes in the cellular wall of the majority of evaluated bacteria, consistent with the action mode of antimicrobial peptides.

R013
A. Adl-3, M. Ghoreishi1, F. Moazzami1 & A. Bazargani2
1Department of Endodontics, Shiraz University of Medical Sciences & 2Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran

Prevalence, antibiotic susceptibility and antibiotic resistance genes of Enterococcus faecalis isolated from secondary endodontic infections

Aim To isolate Enterococcus faecalis from secondary endodontic infections and determine the antibiotic susceptibility of the bacteria in addition to the presence of antibiotic resistance genes.

Methodology Under sterile conditions, clinical samples were obtained from 51 patients undergoing nonsurgical root canal retreatment of teeth with persistent periapical lesions. Isolates that were identified as E. faecalis by cultivation-based 16S rRNA sequencing were further analyzed for antibiotic susceptibility to the commonly used antibiotics in dental practice using E-test method. The antibiotics were penicillin G, amoxicillin, co-amoxiclav, tetracycline, and erythromycin. Polymerase chain reaction (PCR) was used to distinguish the presence of some genes encoding resistance to antibiotics (blaTem, TetM, tetW, cfxA, and ermC).

Results Of the 51 teeth included, one case was excluded because of contamination of the tooth crown as revealed by sterility controls. Out of 50 root canal specimens, 5 isolates were identified as E. faecalis (10%). All isolates were sensitive to penicillin G, amoxicillin and co-amoxiclav. Only one isolate was resistant to tetracycline and erythromycin. Four isolates showed intermediate resistance to erythromycin. All samples of E. faecalis harbored tetracycline resistance genes (tetM and tetW). The genes encoding beta-lactamase, blaTem and cfxA, were detected in 100% and 40% of isolates, respectively. The ermC gene, responsible for resistance to erythromycin, was not detected in any isolate.

Conclusions The prevalence of E. faecalis was low in secondary endodontic infections. The results of this study indicated that despite antimicrobial susceptibility of E. faecalis strains to most tested antimicrobial agents, antibiotic resistance genes are frequent in this bacterium which may increase the potential threat of its antibiotic resistance in the future.

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R014
R.C.D. Swinberghe1, T. Coenye2, R.J.G. De Moor3 & M.A. Meire1
1Department of Restorative Dentistry & Endodontology, Dental School & 2Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium

Biofilm model systems for root canal disinfection: a literature review

Aim To present an overview of in vitro root canal biofilm model systems described in the literature, and to discuss their advantages and drawbacks.

Methodology The electronic databases MEDLINE, Web of Science and EMBASE were searched up to and including August 2016, using the following MeSH terms in various combinations: biofilm, root canal, in vitro, root canal infection model, sessile bacteria, attached bacteria. Growth of the biofilm within a root canal configuration and sampling of the biofilm inside the canal were important inclusion criteria. The following data were extracted from the identified studies: bacterial composition, substrate, growth conditions, validation and quantification of the biofilm.

Results A total of eighty studies were included. A biofilm was grown on human dentine as substrate in 89% of the studies. Only six studies utilized bovine teeth and in three reports the model consisted of non-biological, synthetic material. In the majority (86%) of the publications, a mono-species biofilm was cultured. In two studies a dual-species was grown; others cultivated a multispecies biofilm, containing at least three species. Enterococcus faecalis was the most frequent test species (in 86% of all studies, 91% of the mono-species studies). Four studies used an inoculum derived directly from the oral cavity. Incubation times differed considerably, ranging from one to seventy days. Root canal samples were taken using paper points (62%), by collecting dentine shavings (38%) or rinsing fluid (11%). The most common quantification method (in 82% of the studies) was bacterial culturing. Microscopy techniques, such as SEM and CLSM, were rather used to confirm the presence of a biofilm.

Conclusions The variation in in vitro root canal biofilm model systems is notable. Because of substantial variation in experimental parameters including species, incubation time, quantification method, it is difficult to compare results between studies. More so, a consensus on a standardized endodontic biofilm model is advisable.

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R015
V. Sakhaei Manesh1, P. Giacomini2 & R. Stoll1
1College of Medicine and Dentistry, James Cook University, Cairns, Australia & 2Centre for Biodiscovery and Molecular Development of Therapeutics, Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia

Quantitative comparison of biofilm formation on rough and smooth root canal surfaces using flow cytometry

Aim To compare biofilm formation of Enterococcus faecalis on rough and smooth surfaces.

Methodology Five roots were cut vertically. The ten root half surfaces were each ground to a flat surface. One half of each tooth was finished with a rough disc and the other half was finished up to a fine disc. The prepared root halves were sterilized and placed in a standardized solution inoculated with the same E. faecalis and kept in a shaking incubator for 48 h. Control specimens were prepared using the same method but without the incubation stage (n = 7). A dentine block (1 x 1 x 0.8 mm) was cut out of each prepared surface using a precision saw. The specimen was vortexed to detach the biofilm and the total number of bacteria in each solution was counted by flow cytometry. One-way ANOVA and Tukey post-hoc tests were carried out to compare groups (P<0.05).

Results The rough surface group had a significantly higher bacteria concentration (Mean= 5.156 x 10^5 ± 2.431 x 10^4 bacteria/ml) compared to the smooth (Mean=1.897 x 10^5 ± 0.906 x 10^5 bacteria/ml) and control (Mean=0.554 x 10^5 ± 0.349 x 10^5 bacteria/ml) groups (P < 0.001).

Conclusions Achieving a final smooth surface in root canal treatment reduces the chance of bacterial biofilm formation. Considering the wide range of instrument designs and functions, these results indicate the necessity for further investigations into their effect on the quality of the treated canal surface.

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Abstracts

R017

A. Logani1,*, S. Ranjit1 & T.S. Roy2
1Department of Conservative Dentistry and Endodontics, CDER, All India Institute Of Medical Sciences & 2Department of Anatomy, All India Institute Of Medical Sciences, New Delhi, India

Association between the radiographic size of a periapical lesion and bacterial invasion of apical dentine in teeth with asymptomatic apical periodontitis

Aim To investigate the association of the radiographic size of a periapical lesion with the penetration depth and the degree of invasion of bacteria into apical dentinal tubules of teeth with asymptomatic apical periodontitis

Methodology Ethical clearance was obtained. Eighty six non vital, non restorable mandibular/ maxillary anterior teeth of subjects between the age group of 18–35 years with radiographic evidence of periapical pathosis were extracted and decalcified. Based on the radiographic size of the periapical lesion (less than 5 mm and between 5–10 mm) the experimental teeth were divided into Group A (n = 43) and Group B (n = 43) respectively, A control. Group C (n = 10) comprised of disease free mandibular premolars that were intended for extraction as a part of an orthodontic treatment plan. Standard histo-technical procedures were performed. Four transverse sections (5–7 µm) were obtained 1 and 3 mm from the root apex. Each section was stained with Brown and Brenn stain and observed under light microscope at 400x magnification. A high resolution photograph was taken. The depth of bacterial penetration was measured using Image J basics (version 1.38). Extent of invasion was expressed as tubule invasion index (TI). Data was subjected to parametric Independent ‘T’ test. Level of significance was fixed at 5% and P-value was predetermined at 0.05.

Results The mean depth of bacterial penetration in group A and B was 171.9 and 145.3 µm respectively. No significant difference (P = 0.107) was observed. Both the groups predominantly exhibited a TI score in the range of 0.5–1, indicating mild invasion (P = 0.471).

Conclusions No direct association could be established between the radiographic size of a periapical lesion and bacterial invasion of apical dentine in teeth with asymptomatic apical periodontitis. Hence it can be postulated that apical preparation, three times larger than the initial binding file would be sufficient for periapical healing of lesions of endodontic origin irrespective of their size.

R018

F. Khaled1,*, R.S. Abiad1, K. Abed Galil2 & E. Osman3
1Division of Endodontics, Department of Restorative Sciences, Faculty of Dentistry, 2Pharmaceutical Microbiology, Faculty of Pharmacy & 3Department of Restorative Sciences, Faculty of Dentistry, Beirut Arab University, Beirut, Lebanon

Incidence of Enterococcus faecalis isolates in endodontic retreatments of teeth with apical periodontitis: in vivo study

Aim To detect the incidence of the microbial strains of Enterococcus faecalis isolates in selected clinical cases of failed root canal treatment with apical periodontitis.

Methodology Completely formed single rooted human mandibular first or second premolars (n = 42) requiring root canal retreatments were chosen. Following isolation of the experimental tooth with a rubber dam, the field was disinfected with 30% H2O2 and then 5% tincture of iodine, followed by 2.625% NaOCl. Caries and or existing restorations if present was removed, and then the disinfection sequence was repeated, making sure that the fluids did not seep into the chamber. The pulp chamber was then accessed with a new sterile bur. Removal of previous root fillings was achieved using sterile Protaper Universal instruments for retreatment (Protaper D, Dentsply Sirona, Ballaigues, Switzerland). After working length determination, a sterile K-file size 20 (Dentsply Sirona) was placed 1 mm shorter than working length and pumped 5 times with minimal reaming action to accumulate dentine shavings and intra canal debris. Then three successive paper points (size 20) were placed in the canal to the full working length for 2 min. The file and the three successive sterile paper points were immediately transferred to a vial containing 1 mL of Liquid Dental Transport (LDT). All samples were transported and processed in the microbiology lab within 2 h of sample collection. Polymerase chain reaction (PCR) analysis was performed to identify the existence of Enterococcus faecalis. Two genes were used in this PCR analysis (Universal 16 S rDNA, and Enterococcus faecalis).

Results Seventeen cases out of 42 sampled (40.47%) revealed the presence of the microbial strain of Enterococcus faecalis isolates.

Conclusions E. faecalis was involved in a very high percentage of failed root canal treatments. Efforts for eradication of this species from the root canal should be made in attempt to improve treatment results in endodontics.

R019

Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba, Brazil

Analysis of bacterial content of asymptomatic and symptomatic endodontic infections and susceptibility of specific bacteria to antimicrobial agents

Aim (a) To study the microbiota of 3 specific sites: (1) root canals of necrotic teeth with symptomatology; (2) abscesses associated with such canals; and (3) root canals of necrotic but asymptomatic teeth; (b) to correlate the clinical findings with the microbial data; and (c) to determine the antimicrobial susceptibility of anaerobic black-pigmented bacteria (BPB) against the antimicrobials most frequently used in dental clinics

Methodology Patients in need of endodontic intervention due to the presence of necrotic pulps, with or without symptoms were selected. Microbiological samples were collected from 20 root canals (10 symptomatic and 10 asymptomatic) and the associated periapical abscesses (n = 10). Part of the samples had the DNA extracted and subjected to the checkerboard technique using probes for 40 different bacterial species. Another part was diluted, plated and incubated for isolation BPB, whose colonies were further identified by genetic sequencing. The antimicrobial susceptibility of BPB was determined by the E-test method using the following antibiotics: benzylpenicillin, amoxicillin, amoxicillin + clavulanic acid, erythromycin, azithromycin, clindamycin and metronidazole. Data were tabulated and statistical analysis was performed using SPSS for Windows software. Pearson’s Chi-square test or Fisher’s exact test, when appropriate, was used to test the null hypothesis that there is no association between
The microbiota present in the 3 sites investigated were predominantly composed of anaerobic Gram-negative rods in positive and negative associations. There was no significant difference in the association between bacterial species and clinical features in all groups ($P > 0.05$). Amoxicillin+ clavulanic acid, metronidazole, amoxicillin and clindamycin were the most effective antibiotics, while azithromycin was the least.

Conclusions The microbiota of the 3 sites investigated revealed a wide diversity of species, presenting positive and negative associations. Most of the BPB revealed some resistance to all antimicrobial agents tested.

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R020
M. Barbosa-Ribeiro*, R. Arruda-Vasconcelos, E.C. Bicégio-Pereira & B.P. Gomes
Department of Restorative Dentistry, Endodontic Division, Piracicaba Dental School, State University of Campinas, Piracicaba/SP, Brazil

Immuno-microbiological profile of the post-treatment apical periodontitis

Aim (a) To characterize the Gram-positive microorganisms (MO) and to determine the prevalence of Enterococcus faecalis (EF) during endodontic retreatment; (b) to monitor the levels of MO, lipopolysaccharide (LTA) and matrix metalloproteinases (MMP) before (C1) and after (C2) chemomechanical preparation and after intracanal medication (ICM) (C3); also (c) to correlate these findings with the clinical/radiographic features.

Methodology Samples were taken from RC (MO and LTA) and periapical region [(PR), (MMP -2, -3, -8, -9, -13)] of twenty-four single-rooted teeth with endodontic treatment failure. They were divided into two groups according to the chemical substance used: 6% NaOCl (G1) and 2% Chlorhexidine gel (G2). MO were quantified by the bacterial culture (CFU/mL) and identified by biochemical tests (BT), Nested-PCR (PCR) and genetic sequencing (SEQ). LTA and MMP were measured by ELISA kits (pg/mL). Paired t-tests and repeated measure analysis of variance were also applied for intra-group analysis at the different phases of endodontic therapy. All tests were performed at significance of 5%.

Results Eighty-two Gram-positive species were isolated (C1: 62, C2: 4, C3: 16). EF was the most frequently found MO (BT: 19/82; PCR: 20/20; SEQ: 42/82). MO (101.2), LTA (94.1) and MMP (-2: 803.7, -3: 453.9, -9: 129.4 and -13: 70.8) were present in C1. Overall reductions in C2 were: MO (94.4%), LTA (60.8%) and MMP (-2: 7.8%, -3: 30.3%, -8: 6.9%, -9: 6% and -13: 13.9%) ($P < 0.05$). Regarding ICM, there was a reduction for MO (16.7%), LTA (39%) ($P < 0.05$) and MMP8 (2.4%) and increase in MMP (-2, -3, -9, -13) in C3. Regarding the groups, ICM was effective in reducing LTA (G1): MO and MMP (-3 and -8) in G2, while all MMPs were increased in G1. Positive correlation was found between the presence of LTA with pain on percussion and increased periapical lesion size.

Conclusions E. faecalis was the most prevalent microorganism in teeth with endodontic treatment failure. Chemomechanical preparation was effective in reducing the infectious/inflammatory contents (MO, LTA and MMP) of root canals with endodontic treatment failure. LTA is associated with clinical/radiographic features.

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R021
F.B. Andrade1,*, R.Z. Midenha1, T.J. Dionisio2, A.C. Morandini1, M.R.C. Cuéllar1, C.F. Santos3 & R.N. Stupp4
1Department of Endodontics, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil, 2Department of Pharmacology, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil, 3Department of Anatomy, University of Pacific, San Francisco, USA & 4Department of Microbiology, Piracicaba School of Dentistry, Campinas State University, Piracicaba, SP, Brazil

Gene expression of adhesion factors and biofilm formation by Fusobacterium nucleatum strains isolated from root canals

Aim To investigate several adhesion factors of F. nucleatum, one of the main species found in root canals, and correlate it with its structural behaviour in biofilms, by means of real time PCR and confocal microscope (CLSM) volumetry.

Methodology Four clinical isolates of F. nucleatum were collected from patients, isolated and cultivated in anaerobic chambers, identified and stored. ATCC strain and isolates were cultivated in planktonic and biofilm forms and their RNA purification and transcription to cDNA done. Real-time PCRs of genes FomA (adhesion to other Gram-negative species) and RadD (adhesion to Gram-positive) were performed. Seven-day biofilms produced from the strains were also evaluated by CLSM, comparing their total volume by the software Bioimagel. v2-1. The percentage of life and dead bacteria was also measured, by means of emitted fluorescence from the Life &Dead dye at the biofilms. Kruskal-Wallis test followed by Dunn were performed for statistical analysis.

Results The expressions of both adhesion genes were similar between each other. When in a planktonic form, the ATCC strain expressed these genes more than isolates. When in a biofilm form, the strain number 12 expressed the genes more than strains 13 and 20. The most voluminous biofilm was produced by the ATCC strain.

Conclusions The gene expressions varied when the strain was a clinical isolate, compared to the ATCC strain. When cultivated in a planktonic form, the ATCC produced more transcripts and when in a biofilm form, strain number 12 expressed more genes when compared to other strains. The ATCC strain produced the biggest one-species biofilm. The supposition is that F. nucleatum needs more interactions with other species in vivo and it is probably why the isolates required associations and produced smaller biofilms when in a pure culture.

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R022
P.A. Francisco1,*, M.G. Delboni2, A.R. Lima1, A.J. Soares3, J.F.A. Almeida1, A.A. Zaatla4, C.C.R. Ferraz2, M. Feres5 & B.P.F.A. Gomes1
1Department of Restorative Dentistry, Endodontics Division, Piracicaba Dental School - State University of Campinas (UNICAMP), Piracicaba, 2College of Dentistry, Facid DeVry University, Teresina & 3Department of Periodontology, Guarulhos University, Guarulhos, Brazil

Similarity of microorganisms from saliva, pulp chamber and root canal in teeth with post-treatment apical periodontitis

Aim (a) To study the microbiota composition of saliva (S), pulp chamber (PC) and root canals (RC) of teeth with endodontic failure, by means of the checkerboard technique, b) to correlate the
bacteria found between these sites and with the patient clinical features. **Methodology** Twenty teeth with periapical lesions and need for root canal retreatment, were selected. Samples of endodontic contents were collected from saliva, pulp chamber and root canal. The extracted DNA was subjected to the checkerboard method using probes for 40 different species. Pearson’s Chi-square test or Fisher’s exact test, when appropriate, was used to test the null hypothesis that there is no association between the bacteria in the sites and between specific bacteria and clinical features. **Results** Bacteria were detected in all samples, both Gram-positive and Gram-negative, facultative and strict anaerobes, with an average number of species of 35, 20, and 29, for S, CP and CR, respectively. Simultaneous bacteria most found, at the 3 studied sites, were: *E. faecium*, *P. mira*, *F. nucleatum* (sp. Nucleatum), *E. faecalis*, *E. saburreum* and *C. ochracea*. A positive statistical association (*P < 0.05*) between the bacteria in the sites of the teeth showing signs of microleakage (11/20) was found between *S. oralis* from *S. oralis* from RC (*P = 0.015* and ODOS = 7). In the teeth that did not show signs of microleakage (9/20) a negative association was found between *C. showae* of PC and *C. showae* from RC (*P = 0.045* and ODOS = 0.250). Three significant negative associations (*P < 0.05*) were found between percussion and *D. pneumoniae*, *F. periodonticum* and *E. corrodens*. **Conclusions** The microbiota of root canals associated with secondary or persistent infection was heterogeneous. Furthermore, the similarity and association of the microorganisms present in saliva, pulp chamber and root canals of the same patient suggest a communication route among these three sites. **Acknowledgements** Funding: FAPESP: 2015/19225-2 and 2015/23479-5, CNPq 308162/2014-5, CAPES & FAPEX.

**R024**

K. Croft1,*, S. Kervanto-Seppälä2, L. Stangvaltaitė3 & E. Kerosuo2

1Department of Oral and Maxillofacial Diseases, Institute of Dentistry, University of Helsinki, Helsinki, Finland & 2Faculty of Health Sciences, Department of Clinical Dentistry, UiT The Arctic University of Norway, Tromso, Norway

**Management of deep carious lesions and carious pulpal exposures in adults: a questionnaire study among dentists in Finland**

**Aim** To find out which treatment methods are preferred by dentists in Finland when managing a deep carious lesion or a carious pulpal exposure in an adult patient.

**Methodology** An electronic questionnaire was sent to 1000 dentists (22% of dentists in Finland) who were randomly sampled from the register of the Finnish Dental Association, 323 (32%) responded. The participants were asked to indicate their preferred treatment method for two clinical cases: i) a deep carious lesion in a permanent molar and ii) a carious exposure in the same tooth. Both cases had three scenarios: asymptomatic, or symptomatic referring to either reversible or irreversible pulpitis. Binary multivariable logistic regression analysis was used to study the background characteristics of the respondents related to the treatment preferences.

**Results** Less invasive excavation strategies (stepwise excavation or indirect pulp capping) were preferred by 64.1% for an asymptomatic deep carious lesion, while 34.4% opted for total caries excavation. In the presence of an asymptomatic carious exposure vital pulp therapy was chosen by 70.5% and root canal treatment by 26.4%. Mineral trioxide aggregate (MTA) was chosen as a material for direct pulp capping (DPC) by 39.0% while calcium hydroxide -based materials were preferred by 40.3%. The preference of less invasive treatment strategies for a deep carious lesion was significantly associated with working at the public versus private sector (odds ratio (OR) 2.7, confidence interval (CI) 1.6–4.7) and having clinical guidelines at the practice versus no guidelines (OR 3.5, CI 1.4–8.8). Graduation in year 1986 or after favoured the use of MTA for DPC over other materials (OR 4.5, CI 1.9–10.5), as did graduation from the University of Helsinki or Turku versus other universities (OR 2.9, CI 1.4–6.2).

**Conclusions** Less invasive treatment strategies have been adopted into the clinical practice by the majority of Finnish dentists.

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IRRIGANTS/DISINFECTION:
MATERIALS

R025
S. Van Damme1, R. De Moor, M. De Bruyne, W. Jacquet & M. Meire
Department of Endodontics, Ghent University, Ghent, Belgium

**Effects of irrigation on the push-out bond strength of three bioactive materials to root dentine**

**Aim** To evaluate the push-out bond strength of two calcium-silicate cements (Biodentine and ProRoot MTA) and a conventionally setting glass ionomer cement (Fuji IX), in comparison with gutta-percha/resin sealer after various irrigating protocols within wide root canals.

**Methodology** A total of 180 freshly extracted mandibular single-rooted bovine incisors were decorated and a mid-root section of 1 cm was prepared to an apical size 200, .01 taper (surrounding dentine after preparation at least 1 mm thick). The teeth were divided in three groups according to the irrigation protocol: (A) NaOCl 3%, (B) NaOCl 3% - EDTA 17% and (C) NaOCl 3% - EDTA 17% - NaOCl 3%. All irrigants were ultrasonically activated. Roots within each group were divided in four subgroups according to the root filling material: (1) gutta-percha/AH Plus, (2) ProRoot MTA, (3) Biodentine and (4) Fuji IX. After setting, 2 slices of 1.6 ± 0.2 mm were obtained 3 and 6 mm from the decoronated surface. Push-out bond strength (POBS) values were measured using a universal testing device. The means and standard deviations of the POBS were calculated for each group and the data were analyzed statistically using the three-way ANOVA test.

**Results** Push-out bond strength was significantly influenced by the root filling material and by the irrigation protocol (P < 0.05). The lowest push-out bond strengths were found within the gutta-percha group, and this was not influenced by the irrigation protocol. The highest push-out bond strengths were found in the NaOCl/Biodentine group. When comparing MTA with Biodentine, MTA had the lower push-out bond strengths, but this was only significant for the NaOCl-EDTA irrigation protocol.

**Conclusions** Biodentine and MTA yielded the highest push-out bond strengths whilst that of gutta-percha/resin sealer was the lowest. In the case of Fuji IX, MTA and Biodentine, irrigation protocols had a significant influence on push-out bond strengths. For Biodentine and MTA, maintaining the smear layer created a stronger bond.

R026
A.U. Eldeniz & S. Karagollu1
Department of Endodontics, Selcuk University, Konya, Turkey

**Effect of contemporary irrigants on microhardness of various perforation repair materials**

**Aim** To evaluate the effect of contemporary endodontic irrigants on microhardness of various perforation repair materials.

**Methodology** Thirty discs of each material were made in Teflon moulds as follows; group 1: Biodentine (Septodont), group 2: Freedom Compomer (Freedom, SDI) group 3: Amalgam (Cavex Avallory), group 4: IRM (Dentply), group 5: SDR (Dentsply) and polished with #1000–2000 grit abrasive papers to create flat surfaces. Baseline microhardness values were obtained from the surfaces using Vickers microhardness tester (Matsuzawa) and specimens were then divided into 3 subgroups and either immersed in 2.5% NaOCl (Çağlayan Kimya, Konya), 2% CHX (Klorhex, Drogsan) or saline solution for 15 min after the materials had set. All indentations were made with 50 g loading for 20 s contact time after 12 h. After 7 days, specimens were again immersed into solutions for 15 min and indented for surface microhardness again. Paired t-tests were used to evaluate the differences within the groups towards the baseline values (P = 0.05).

**Results** No significant reduction was detected in the microhardness of Biodentine, Freedom Compomer, IRM and SDR materials when various irrigants were applied to these materials (P > 0.05). Amalgam demonstrated a significant reduction in microhardness when exposed to sodium hypochlorite solution for 15 min after 12 h setting (P = 0.025). IRM cement was associated with a significant reduction in surface microhardness when exposed to serum solution after 12 h setting (P = 0.042).

**Conclusions** Biodentine, Compomer, IRM cement and SDR might be better perforation repair materials when compared with amalgam as they were less vulnerable to endodontic irrigants that might affect their microhardness.

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R027
S. Kucukkaya Eren1,2, E. Uzunoglu1, B. Sezer1, Z. Yilmaz1 & I.H. Boyaci1
1Department of Endodontics, Faculty of Dentistry & 2Department of Food Engineering, Hacettepe University, Ankara, Turkey

**A new method for mineral content analysis of root canal dentine**

**Aim** To introduce a new method for assessing mineral content of root canal dentine ex vivo and to compare the effects of irrigation solutions on the mineral content change of root canal dentine with a well-established standard method.

**Methodology** Forty single-rooted extracted human teeth with a single root canal were decorated and sectioned longitudinally to expose the root canals. The root halves were placed in an ultrasonic bath containing distilled water for 10 min and divided into four groups by allocating each root half belonging to the same root to the same group (n = 20). Twenty root halves served as the control group and did not receive any further treatment. The remaining root halves were then immersed in solutions as follows; Group 1: 5.25% sodium hypochlorite (NaOCl) for 1 h, Group 2: 17% ethylenediaminetetraacetic acid (EDTA) for 2 min, Group 3: 5.25% NaOCl for 1 h and 17% EDTA for 2 min. Final irrigation was performed with 5 mL of distilled water. Each root half belonging to the same root was evaluated for mineral content change of root canal dentine exposed to serum solution after 12 h setting (P = 0.001).

**Results** In groups 1 and 3, the Ca level decreased while the Na level increased compared with the other groups (P < 0.05). The P level was significantly higher in group 1 than group 2 (P < 0.05). The Mg level changes were not significantly different among the groups (P > 0.05). A significant positive correlation was found between the results of LIBS and SEM/EDS analyses (r = 0.84, P < 0.001).

**Conclusions** The irrigants altered the mineral level of root canal dentine. The LIBS method proved to be reliable while providing data for the elemental composition of root canal dentine.
The effect of sodium hypochlorite and EDTA on the push-out bond strength of Mineral Trioxide Aggregate

Aim To evaluate the effect of sodium hypochlorite (NaOCl) and EDTA on the push-out bond strength of Mineral Trioxide Aggregate (MTA).

Methodology One millimetre thick dentine slices of maxillary anterior teeth were prepared using a size 5 Gates-Glidden bur to obtain standardised cavities 1.3-mm in diameter. ProRoot MTA was placed inside the root slices (n = 30) and allowed to set for 10 min at 37°C with 100% humidity. Then the specimens were randomly allocated into 2 groups (n = 10) according to the irrigation solution applied. The specimens were immersed either in NaOCl (5.25%) or EDTA (17%) for 30 min. All samples were then rinsed with distilled water. No immersion was applied for the control group. A wet cotton pellet was placed on each specimen and the specimens were incubated for 48 h. The push-out bond strength was evaluated using a universal testing machine. Data were analysed by Kruskal-Wallis test at a significance level of P < 0.05.

Results No significant difference was recorded between groups (P > 0.05). The lowest push out bond strength value was recorded in the EDTA group (10.34 MPa). The mean push-out bond strength of NaOCl and control groups was 11.37 MPa and 16.95 MPa, respectively.

Conclusions The solutions did not affect the push-out bond strength of ProRoot MTA. The results suggest that the clinicians may irrigate canals with NaOCl and EDTA following a repair using MTA without causing a significant effect on the material’s push-out bond strength.

Structural effects of 5.25% sodium hypochlorite and 13.8% chlorine dioxide solutions on gutta-percha cones: an atomic force microscopy study

Aim To investigate with atomic force microscopy the effects of 5.25% NaOCl and 13.8% ClO2 on the surface properties of gutta-percha cones.

Methodology Standardized gutta-percha cones (size 60) were cut 3 mm from their tip and immersed in the solutions (5.25% NaOCl and 13.8% ClO2) at 1, 5, 10, 20, 30 min time intervals. Then, each cone was attached to a glass base with a double-sided tape. Analysis of the surface topography was performed on three different points located between 1 and 2 mm from the tip using an atomic force microscope. One cone without any treatment was used as a control. AFM images of gutta-percha samples were recorded in the semicontact mode operation on a NT-MDT Nегра Solaris Atomic Force Microscope under ambient condition. The root mean square (RMS) parameters for semicontact mode imaging were measured. The differences between root mean square values were tested by two-way ANOVA and Tukey HSD.

Results According to the RMS values obtained from AFM evaluation, there was no significant difference between the mean RMS values of gutta-percha cones treated with both 5.25% NaOCl and 13.8% ClO2 from the control at time intervals of 1, 5, 10, 20 and 30 min (P > 0.05). In addition, no significant change was observed in the both 5.25% NaOCl and 13.8% ClO2 when compared to the control (P > 0.05).

Conclusions Both 5.25% NaOCl and 13.8% ClO2 solutions did not produce changes to the gutta-percha structure following up to 30 min exposure. Both solutions might be safely used for disinfection of gutta-percha cones.

Antimicrobial efficacy of silver nanoparticles with and without different antimicrobials against E. faecalis and Candida albicans: ex vivo study

Aim To assess the ex vivo antimicrobial efficacy of silver nanoparticles with and without different antimicrobials against E. faecalis and Candida albicans.

Methodology A total of 252 recently extracted single-rooted human teeth were contaminated with E. faecalis and Candida albicans. The teeth were randomly divided into 5 experimental (n = 21) and 1 control group (n = 21). Each subgroup was then exposed to different antimicrobials namely Calcium hydroxide (group 1), 2% Chlorhexidine (CHX) (group 2), Silver nanoparticles (SNP) (group 3), SNP with Ca(OH)2 (group 4), SNP with 2% CHX (group 5) and saline as a control group (group 6). Cultures were made from each group after 24 h, 7 days and 14 days and colony forming units were counted. The Kruskal-Wallis test was used to compare the study parameters among the groups at 24 h, 7 days and 14 days.

Results A significant difference was found in the antimicrobial efficacy of different intracanal medicaments against E. faecalis and Candida albicans after 24 h, 7 days and 14 days. 2% CHX was found to be most effective medicament at 24 h, 7 days and 14 days against E. faecalis and Candida albicans. Combination of SNP with 2% CHX and Ca(OH)2 and SNP alone ranked second in their antimicrobial efficacy against E. faecalis and Candida albicans at 24 h, 7 days and 14 days respectively.

Conclusions 2% CHX was more effective as an intracanal medicament against E. faecalis and Candida albicans biofilm in both short and long term duration, i.e. at 24 h, 7 days and 14 days.

Effectiveness of HICA and alpha-mangostin against endodontopathogenic microorganisms in a multi-species bacterial-fungal biofilm model

Aim To determine the activity of HICA and alpha-mangostin on preformed bacterial-fungal multi-species biofilms in vitro, and to ascertain their impact on biofilm structure.
Methodology Minimal inhibitory concentrations (MICs) for HICA and alpha-mangostin against planktonic Candida albicans, Enterococcus faecalis, Lactobacillus rhamnosus, and Streptococcus gordonii were determined using a standard microdilution method. Single and multi-species (all species 1:1:1:1) biofilms were grown on polystyrene coverslips in RPMI for 48 h. The biofilms were then exposed to 5% HICA or 0.2% alpha-mangostin for 24 h. These concentrations were selected based on pilot experiments and solubility of these compounds. 2% CHX and 2.5% NaOCl were used as positive controls and RPMI as the negative control. The metabolic activity of the biofilms after exposure was measured using XTT assay, and the biofilms were visualised using fluorescent BacLight® LIVE/DEAD staining.

Results 50 mg/mL of HICA was cidal against planktonic bacteria and Candida. 0.008 mg/mL of alpha-mangostin was cidal against planktonic bacteria and 1 mg/mL for Candida. Both HICA and alpha-mangostin were most active against L. rhamnosus biofilms (98% and 99% inhibition of metabolism, respectively) and least active against Candida biofilms (42% and 78% inhibition, respectively). Alpha-mangostin demonstrated better activity against multi-species biofilms than HICA (93% inhibition versus 46% inhibition). NaOCl inhibited the metabolic activity of single and multi-species biofilms by at least 98%. HICA and alpha-mangostin exposure reduced the number of cells in the C. albicans biofilms and no hyphae were observed. Exposure to HICA or alpha-mangostin reduced the number of viable cells in the biofilms as assessed by the BacLight® LIVE/DEAD staining.

Conclusions Both HICA and alpha-mangostin effectively inhibited the metabolic activity of bacterial-fungal biofilms. The anti-biofilm activity of alpha-mangostin was comparable to that of highly active but toxic NaOCl and thus has potential as a novel agent for endodontic therapy. HICA was less active against the biofilms than alpha-mangostin but due to its high biocompatibility it has potential in the treatment of fractured roots or perforated root canals.

R032
B.P. Gomes1,*, V.B. Berber1, A.C. Marinho1, M.R. Passini1, V.G. Pecorari2, A. de-Jesus-Soures1, A.A. Zaia1, J.F. Almeida1, C.C. Ferraz1, M.A. Marciano1 & B.J. Paster1
1Department of Restorative Dentistry, Endodontics Division, Piracicaba Dental School, State University of Campinas - UNICAMP, Piracicaba, Brazil
2Department of Statistics, Universidade Paulista, Sao Paulo & Department of Microbiology, The Forsyth Institute, Cambridge, USA

Effect of chemomechanical preparation on bacterial, LPS and LTA levels in combined endo-periodontal sites

Aim To investigate the levels of cultivable bacteria, endotoxins (LPS) and lipoteichoic acid (LTA) from teeth with combined endodontic-periodontal lesions (EPL), comparing both sites: root canal (RC) and their associated periodontal pockets (PP), before (s1) and after (s2) chemomechanical preparation (CMP) using 2% chlorhexidine (CHX). The anti-biofilm activity of alpha-mangostin was comparable to that of highly active but toxic NaOCl and thus has potential as a novel agent for endodontic therapy. HICA was less active against the biofilms than alpha-mangostin but due to its high biocompatibility it has potential in the treatment of fractured roots or perforated root canals.

Methodology Clinical samples were taken from 10 root canals with pulp necrosis and apical periodontitis and their associated periodontal pockets (PP): before (s1) and after CMP (s2) in 10 root canals (RC/PP). Measurements were performed for bacterial count [colony-forming units (CFU)/mL], and for quantification of LPS (Turbidimetric Limulus Amebocyte Lysate assay) and LTA (Human Lipoteichoic Acid ELISA kit) levels. Paired t-test and repeated measures (ANOVA) were performed for statistical analysis ($P < 0.05$).

Results At s1, bacteria were isolated in 10/10 of the sites investigated (RC: 3.75x10³; PP: 3.99x10⁷ CFU/mL). Prevotella, Gemella, Streptococcus, and Fusobacterium were frequently detected in RC, while Gemella, Parvimonas, Fusobacterium, Porphyromonas and Streptococcus in PP. LPS were 5.5 times higher in PP (148.85 EU/mL) compared to the RC (27.08 EU/mL). LTA was detected in 10/10 samples (RC: 33.38; PP: 386.26 pg/mL). At s2, a significant decrease was found in the RC for: cultivable bacteria (99.4%) and LPS (96.57%), except for LTA (45.14%). In PP, lower percentage reduction was found for cultivable bacteria (31.07%); LPS (45.62%), and LTA (8.16%).

Conclusions A similarity was found between the microbial communities in combined EPL. CMP with 2% CHX was effective in reducing the microbial load and the LPS levels, however, only a small reduction on the LTA levels was found in the RC and PP.

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IRRIGANTS/DISINFECTION: CANAL CLEANING

R033
K. Dervenis* & T. Labrianidis
Department of Endodontology, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

Removal efficacy of Ledermix from the root canal: an ex vivo study

Aim To compare the effectiveness of syringe irrigation (SI) and passive ultrasonic irrigation (PUI) with NaOCl, EDTA and normal saline when removing Ledermix from the root canal of extracted mandibular premolars.

Methodology Seventy-four single-rooted extracted mandibular premolars were used. Following decoronation and root canal preparation, Ledermix was placed in the canals, and the specimens were stored at $37 \pm 1$ °C and 100% relative humidity for 2 weeks. Two teeth served as positive control, two teeth served as negative control, and the remaining 70 teeth were randomly assigned to 7 groups based on the removal method applied: SI with 10 mL NaOCl 2.5% or 10 mL EDTA 17% or 10 mL normal saline, PUI x 3 with 10 mL NaOCl 2.5%, and PUI x 1 with 10 mL NaOCl 2.5% or 10 mL EDTA 17% or 10 mL normal saline. The teeth were split into mesial and distal halves, and each pair of root halves was photographed. The quantity of residual Ledermix on each root canal third was evaluated double-blindly and independently using a scoring system (0 - the root canal completely empty, 1 - less than half of root canal filled with Ledermix, 2 - more than half of root canal filled with Ledermix, and 3 - root canal completely filled with Ledermix). Data were subjected to statistical analysis at 95% confidence level ($P < 0.05$).

Results Remnants of Ledermix were found in all experimental groups. When examining the root canal as a whole, Ledermix removal was more efficient in the PUI groups. When examining the root canal per third, removal efficacy in PUI groups was increased in the cervical third only. Comparison between the cervical, middle and apical third inside each group revealed no differences. PUI application once or three times were equally effective.

Conclusions Differences in chemical and mechanical properties between NaOCl, EDTA, and normal saline did not result in differences in Ledermix removal. Regardless of whether the irritant was delivered by syringe, additional application of PUI improved Ledermix removal, especially from the cervical canal third.
R034
A.C. Hergt*, A. Reus & M. Hülsmann
Department of Preventive Dentistry, Periodontology and Cardiology,
University of Göttingen, Göttingen, Germany

Removal of calcium hydroxide paste from root canals using four different irrigation techniques

Aim To compare the removal of calcium hydroxide from straight root canals using four different root canal irrigation techniques (EDDY, passive ultrasonic irrigation (PUI), Endo Activator and syringe irrigation).

Methodology The root canals of seventy human single-rooted teeth were enlarged to size 40, .04 taper using Mtwo rotary NiTi instruments. Each tooth was split longitudinally and two standardized grooves were prepared in the apical and in the coronal part of each specimen. Grooves were filled with Ca(OH)2, photographed and the root halves were reassembled. The root canals were also filled with Ca(OH)2 and radiographs were taken to confirm the quality of the filling. All teeth were randomly divided into 4 groups (n = 15): I. EndoActivator, II. PUI, III. EDDY, IV. syringe irrigation. Group V (n = 10) served as positive control (no irrigation). Irrigation was performed for 3x20 s with 2 mL irrigant applied 2 mm short of working length. Final irrigation was performed with 2 mL de-ionised water. Cleanliness of root canal halves was evaluated using reflecting microscopy with a 4-grade scoring system. Kappa values were calculated for intra- and inter-observer agreement. Statistical analysis was performed with the Kruskal-Wallis test and the Mann-Whitney-U test (P < 0.05).

Results None of the tested methods could clean the grooves completely. There were no significant differences between coronal and apical grooves (P = 0.190). Syringe irrigation and Endo Activator performed equal (P = 0.186) with no significant difference to the control group (P = 0.643). (P = 0.171). PUI and EDDY removed significantly more Ca(OH)2 than syringe irrigation and Endo Activator (P < 0.001), with no difference between them (P = 0.464).

Conclusions EDDY and PUI were more effective in removing Ca(OH)2 from coronal and apical grooves within root canals than syringe irrigation and Endo Activator.

R035
F. Haupt*, M. Meinel & M. Hülsmann
Department of Preventive Dentistry, Periodontology and Cardiology,
University Medical Center Göttingen, Göttingen, Germany

Efficacy of sonic and ultrasonic activated irrigation on debris and smear layer removal in curved root canals

Aim To evaluate the efficacy of four different irrigation techniques on removal of debris and smear layer.

Methodology Ninety extracted human mandibular molars with an angle of curvature between 20° and 40° were shortened to a length of 19 mm, resulting in a working length of the mesiobuccal root canal of 18 mm. The teeth were embedded in clear casting resin using a muffle system and were randomly divided into 5 groups: Syringe irrigation (SI, n = 20), EndoActivator (EA, n = 20), passive ultrasonic irrigation (PUI, n = 20), EDDY (n = 20), negative control (CO, n = 10). Root canals were accesssed and prepared with BioRaCe instruments to size 40, .04 taper (FKG Dentaire, La Chaux-de-Fonds, Switzerland). After each file, irrigation was performed according to the respective irrigation technique with a total volume of 8 mL NaOCl (3%). Subsequently, final irrigation was carried out with 6 mL NaOCl with x 20 s activation time with the corresponding technique. Teeth were split longitudinally and canal walls were subjected to scanning electron microscopy. The presence of debris and smear layer at coronal and apical levels was evaluated using a 5-point scoring system.

Results Irrespective of the region, cleanliness of the root canal was significantly superior for activation with EA, PUI and EDDY compared to the control (P < 0.05). Regarding the coronal region, there were no significant differences between the groups for both smear layer and debris removal. In the apical half, significant differences were detected. Compared to the control, EA removed significantly more debris and PUI significantly more smear layer.

Conclusions None of the activation methods resulted in complete removal of debris and smear layer. Nevertheless, EA, PUI and EDDY were associated with significantly better results than the control.

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R036
Conservative Dentistry, Complutense University of Madrid, Madrid, Spain

Analysis of irrigant penetration in lateral canals in a semi-closed environment

Aim To determine the penetration capacity of irrigant solutions in lateral canals when using different activation systems.

Methodology Sixty transparent artificial maxillary molars were used whose design includes a lateral canal in the apical third of the palatal root. In all samples the palatal canal was instrumented to a working length of 21 mm using ProTaper Next system up to the X4 file and subsequently the lateral canal were permeabilized with a size 10 K-file. Teeth were immersed in a clear vessel filled with 0.2% agarose gel to create a semi-closed environment. Later, teeth were randomly divided into 4 groups (n = 15) according to the irrigation method: LVN group (closed-ended, lateral vent needle 2 mm from working length), EA group (sonic activation with EndoActivator), EV group (negative apical pressure with EndoVac) and PUI group (passive ultrasonic irrigation with Irrisafe 25.00). After the application of different irrigation protocols (using 5.25% sodium hypochlorite with Indian ink as irritant), standardized photographs were taken and the percentage of the lateral canal filled by the irritant solution was measured using Imagej software. The results were analyzed statistically using the Kruskal Wallis test.

Results Percentages of irrigant penetration in lateral canals were: 63.5% for the LVN group, 66.3% for the EA, 36.1% for the EV and 92.3% for the PUI. Significant differences (P < 0.05) were observed between the EV and PUI groups.

Conclusions PUI had greater penetration in lateral canals than EV.
R037
A. Ismail
Department of Operative Dentistry and Periodontology, University of Mainz, Mainz, Germany

Effectiveness of different activation systems to remove dentine debris from simulated grooves and depressions within root canal walls

Aim To evaluate ex vivo the effectiveness of several activation systems to remove debris and smear layer from simulated irregularities in prepared root canal walls.

Methodology Straight root canals of 48 fresh extracted central and lateral maxillary incisors were prepared to size 40, .06 taper and split longitudinally into 2 halves. Thereafter, simulated irregularities (grooves and depressions) were prepared and filled with dentine debris mix as described by Lee et al. 2004 and photographs were taken. After reassembling the 2 halves, samples were divided into 4 groups (n = 12). Group A: manual irrigation with 3% NaOCl, group B: passive ultrasonic activation (PUI) and irrigation with 3% NaOCl, group C: EndoActivator (Dentsply Sirona, Ballaigues, Switzerland) and irrigation with 3% NaOCl, group D: sonic activation system EDDY (VDW, Munich, Germany) and irrigation with 3% NaOCl. Thereafter, postoperative images from the root canal walls were taken under the microscope. Grooves and depressions were evaluated using a scoring system between 0–3. Data analysis was performed using Mann-Whitney U-test.

Results All additional activation systems reduced significantly the debris score in comparison to manual irrigation. However, EDDY and PUI had the greatest effect and both were significantly more effective than EndoActivator (P = 0.01).

Conclusions Activation with EDDY and PUI had the greatest effect in removal of dentine debris in comparison to manual irrigation and EndoActivator.

R038
B. Mattos, A.T.G. Cruz, L. Piasecki, E. Carneiro, V.P.D. Westphalen, L.F. Fariiniuk & U.X. Silva Neto*
Department of Endodontics, Pontifical Catholic University of Parana, Curitiba, Brazil

Effect of different final irrigation protocols on hard tissue debris removal

Aim To compare ex vivo the hard-tissue debris removal of four final irrigation systems using micro–computed tomography.

Methodology Forty mesial roots of mandibular molar with isthmus connecting the mesiobuccal and mesiolingual canals were used. The specimens were scanned in a micro-CT scanner (SkyScan 1172; Bruker micro-CT, Kontich, Belgium) before and after root canal instrumentation and after final irrigation at a resolution of 12.89 μm. The root canals were prepared with Wave One Gold primary instruments (Dentsply Sirona) and divided into four groups (n = 10) according to the final irrigation system used: passive ultrasonic irrigation, EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA), EasyClean (Easy Equipamentos Odontológicos, Belo Horizonte, Brazil) and XP Endo Finisher (FKG Dentaire, La Chaux de Fonds, Switzerland). The final irrigation procedures were performed as followed: 2 mL of 5% NaOCl in continuous flow were activated by each device for 1 min per canal. The same procedure was repeated with 17% EDTA. The mean percentage reduction of accumulated hard-tissue debris was compared statistically, using one-way ANOVA and post hoc Tukey tests with a significance level set at 5%.

Results XP Endo finisher had higher mean scores (72.74%) for debris removal although, there were no differences among groups, except when XP Endo Finisher and EasyClean were compared (P<0.05).

Conclusions All systems produced some hard-tissue debris reduction. XP Endo Finisher was better than EasyClean for this purpose.

R039
A. Nikitovic*, I. Melih, D. Pestic, V. Kolak, M. Lalovic & A. Jakovljevic
Department of Dental Pathology and Endodontics, School of Dental Medicine, Pancevo, Serbia

Effectiveness of three different methods for smear layer removal in the apical region

Aim To compare three methods for smear layer removal: 17% EDTA, Er:YAG laser and Nd:YAG laser.

Methodology Sixty five extracted human mandibular premolar teeth were used. Their root canals were prepared using nickel-titanium rotary instruments (ProTaper) in sequential crown down technique with 1% NaOCl irrigation. Sixty teeth were divided equally in three groups, according to the smear layer removing technique. In the first group smear layer was removed with 5 mL 17% EDTA. In the second group with Er:YAG laser (AT Fidelis, Fotona, Slovenia) with the following parameters: wavelength of 2940 nm; output of 1.8W; frequency 15 Hz, over a 40 s time interval. In the third group with Nd:YAG laser (Fotona) with the following parameters: wavelength 1064 nm; energy of 1W; frequency rate of 15 Hz, four times at 15 s intervals. The remaining five teeth served as the control group. After treatment root canals were irrigated with distilled water and dried with absorbent paper points. All specimens were split in half longitudinally, and all samples were observed using scanning electron microscope. Statistical analyses were performed using the Kruskal-Wallis and the Mann-Whitney test.

Results Best results were observed in the EDTA group, and the worst in the Nd:YAG group. Based on the analyses there was no significant difference between the EDTA group and the Er:YAG group (P > 0.05). There was a significant difference between Nd: YAG and two other tested groups (P < 0.05).

Conclusions Neither of the tested groups were associated with complete removal of smear layer. EDTA solution and Er:YAG laser had the same efficacy for smear layer removal, while the Nd:YAG laser was the least effective.

R040
Y. Ozbay* & A. Erdemir
Department of Endodontics, Faculty of Dentistry, Kirikkale University, Kirikkale, Turkey

Effect of several laser systems on removal of smear layer with a variety of irritant solutions

Aim To evaluate which laser system is more effective on smear layer removal when distilled water or combination of NaOCl and EDTA are used as irritants.

Methodology Ninety six human mandibular premolar teeth were used. Standard access cavities were prepared and the root canals enlarged using the ProTaper system (Dentsply Sirona, Ballaigues, Switzerland) to size F4 and divided into two equal groups (n = 48) according to final irrigation solution. In group 1, distilled water was used, whilst 2.5% NaOCl and 17% EDTA solutions were used in group 2. Each group was divided into four equal subgroups according to the activation procedure. In subgroups 1A (Control) and 2A, no activation protocol was applied. In other subgroups, Er:YAG laser using photon-induced
The samples irrigated with distilled water had greater smear layer scores when compared with the combination of NaOCl and EDTA ($P < 0.01$). The efficacy of smear layer removal increased when using laser systems ($P < 0.001$). Smear layer was removed more effectively in the coronal and middle levels compared to the apical level ($P < 0.001$).

Conclusions Regardless of the type of irrigation, the use of laser systems enhances smear layer removal. In addition, irrigating root canals with a combination of NaOCl and EDTA was a more efficient when either Er:YAG with PIPSTM, Nd:YAG and Er,Cr:YSGG lasers were applied to activate the irrigation.

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R041
G. Barut1, G. Kutlu1,*, S. Armağan2, P. Bozbuhut2, O. Ünlü2 & F. Haznedaroglu2
1Department of Endodontics, Yeditepe University, Faculty of Dentistry & 2Department of Endodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey

Effect of two sonic agitation techniques on tissue dissolution efficacy of sodium hypochlorite solution at different temperatures and concentrations: EndoActivator versus Eddy

Aim To evaluate and compare ex vivo the tissue-dissolution efficacy of two different sonic agitation techniques under distinct conditions of temperature and concentration of sodium hypochlorite.

Methodology Two sonic agitation techniques, EndoActivator (Dentsply Sirona, Ballaigues, Switzerland) and Eddy (EDDY; VDW, Munich, Germany) were tested at room temperature and 40°C with three different concentration of sodium hypochlorite. The concentrations were 1%, 2.5% and 5.25%. Distilled water was used as the control. Standardized pieces of bovine muscle tissue (50 ± 2 mg) were submersed in 10 mL of each concentration and temperature of sodium hypochlorite for five min. In selected samples, two sonic agitation techniques were performed for 15 s during each minute. The tissue specimens were weighed before and after the exposure. The sample size was 120, with five replications per agitation/testing condition. Independent Samples t-Test, One Way ANOVA and Two Way ANOVA Test were used for statistical analysis ($P < 0.05$).

Results Weight loss was proportionally correlated with the two sonic agitation techniques ($P < 0.05$). The Eddy sonic agitation technique was more effective at organic tissue dissolution than EndoActivator and the no agitation technique. The sodium hypochlorite concentration was related to weight loss. Considering all the experimental conditions 5.25% sodium hypochlorite had the best dissolution results. Also the high temperature of sodium hypochlorite had the best dissolution efficacy ($P < 0.001$).

Conclusions Within the limitations of this study, to maximize the organic tissue dissolution effect of sodium hypochlorite, the following is advised; sonic agitation, higher temperature, greater concentrations.
groups according to the irrigation protocols, as follows: positive control (NaOCl followed by CHX), negative control (distilled water), distilled water as intermediate flush between NaOCl and CHX, sodium thiosulphate irrigation between NaOCl and CHX, and Tween 80 irrigation between CHX and NaOCl. The collected liquid was centrifuged and the precipitate was analyzed using 1H NMR spectra. The data were analyzed with chi-square test at the 95% confidence level ($P = 0.05$).

**Results** PCA was determined for all specimens in the positive control and none in the negative control group. Although distilled water irrigation between NaOCl and CHX did not prevent the formation of PCA, sodium thiosulphate and Tween 80 prevented the formation of PCA between NaOCl-CHX and CHX-NaOCl, respectively.

**Conclusions** The present study revealed that sodium thiosulphate and Tween 80 were beneficial as an intermediate flush to prevent PCA formation.

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**R044**

V.R. Fretes$^1$, P.M. Escobar$^2$ & C.G. Adorno$^{1*}$

$^1$Department of Endodontics and Research Methods, Facultad de Odontología, Universidad Nacional de Asunción, Asunción & $^2$Private Practice, Paraguay

**Apical vapour lock removal using different agitation techniques. An ex vivo radiographic study**

**Aim** To evaluate apical vapour lock removal ex vivo using different agitation techniques in single-canaled mandibular premolars.

**Methodology** The canals of twelve mandibular premolars with slightly curved canals were enlarged to an apical size 40 .04 taper. The canals were filled with a radiopaque dye leaving a 2 mm apical bubbling using a 27G side vented needle. Agitation was performed with either a Canal Brush (600 rpm rotation), Easy Clean (reciprocation), Eddy (5000 – 6000 kHz sonic) or EndoActivator (sonic, power set to high), all placed 0.5 mm short of the working length. Each tooth was evaluated 4 times, once for each agitation technique. A standardized digital radiograph was taken with a proximal incidence before introducing the radiopaque dye (empty canal), before agitation (negative control) and after agitation (experimental images). Additionally, a digital radiograph of the canal completely full was taken (positive control). Two independent evaluators observed the apical portion of the canal and categorized the apical vapour lock removal according to a 4-step score: 1: no difference, 2: less than 50% removed, 3: 50% or more removed, and 4: complete vapour lock removal. The independent variable was agitation technique (nominal), whereas the dependent variable was apical vapour lock removal (ordinal). Therefore, the data was analyzed using the Kruskal-Wallis test with Dunn’s test for multiple comparison.

**Results** All negative and positive controls scored 1 and 4, respectively. The median (minimum-maximum) scores for Canal Brush, Easy Clean, EndoActivator and Eddy were 3 (1–4), 4 (2–4), 3 (1–4) and 4 (4–4), respectively. The Kruskal-Wallis test revealed a significant difference ($P = 0.005$) between agitation techniques and multiple comparisons further revealed that Eddy was significantly different to Canal Brush ($P = 0.0018$) and EndoActivator ($P = 0.0027$).

**Conclusions** All agitation techniques reduced the vapour lock by more than 50% in most cases. The Eddy tips completely removed the apical vapour lock in all cases.

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**R045**

A.U. Eldeniz, G. Kan Hayirci* & S. Karagollu

Department of Endodontics, Selcuk University, Konya, Turkey

**Effect of final irrigation procedures with various chelates on dentine microhardness and roughness**

**Aim** To investigate the effect of various chelates (EDTA, SmearClear, citric acid (10%), maleic acid (7%) and QMix2in1) on dentine microhardness and roughness when they were used as part of final irrigation procedure before sodium hypochlorite, saline, and chlorhexidine gluconate irrigation.

**Methodology** Thirty six extracted human mandibular incisors were sectioned longitudinally into a total of 72 specimens. The specimens were polished with 1000–2000 grit abrasive papers and randomly divided into six groups of 12 specimens each. All groups first irrigated with various chelates as follows: group 1: 5 mL of 17% EDTA for 150 s, group 2: 5 mL of SmearClear (Sybronendo, Orange, CA, USA) for 150 s, group 3: 5 mL of 10% citric acid for 150 s; group 4: 5 mL of 7% maleic acid for 150 s, and group 5: QMix2in1 (Dentsply, Tulsa, Oklahoma, USA) for 150 s. All experimental groups were irrigated consequently with sodium hypochlorite (NaOCl 2.5%), saline and chlorhexidine gluconate (CHX, 2%) solutions. In the control group no chelate was used (group 6). All groups were irrigated for 150 s followed by 2.5% NaOCl, saline and CHX. Surface hardness and surface roughness was tested using Shore A durometer (SHORE, Lever Loader Instrument, MFG Co, New York, USA) and profilometer (Mitutoyo Surf test Analyser, Matsuzawa SEIKI Co. Ltd, Japan). Measurements were taken at 0.5 mm level to root canal wall in apical, middle and cervical regions of the root samples. Microhardness and roughness values were analyzed with one way ANOVA and Tukey HSD tests ($\alpha = 0.05$).

**Results** The QMix2in1 solution did not reduce surface microhardness and demonstrated values comparable to the serum control group ($P < 0.05$). No significant difference was observed between groups 1 to 4, while group 5 was significantly different compared with all other groups except the serum control ($P < 0.05$). Maleic acid, EDTA and citric acid groups increased surface roughness insignificantly, while Qmix2in1 demonstrated values comparable to the serum control.

**Conclusions** EDTA, SmearClear, citric acid, and maleic acid solutions were more destructive chelates than QMix2in1 solution to dentine when they were used as part of the final irrigation procedure.

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**R046**

K. Kot$^{1,2}$, A. Nowicka$^2$, K. Reszka$^2$ & M. Lipski$^1$

$^1$Department of Preclinical Conservative Dentistry and Preclinical Endodontics, $^2$Department of Conservative Dentistry and Endodontics, Pomeranian Medical University, Szczecin & $^3$Department of Technical Physics and Nanotechnology, Koszalin University of Technology, Koszalin, Poland

**Effect of sodium hypochlorite with or without surfactant on root canal cleanliness: a comparative environmental electron microscope study**

**Aim** To examine the cleaning ability of 5.25% sodium hypochlorite with or without surfactant.

**Methodology** Sixteen extracted single-rooted human teeth were used. The crowns were removed at the cementoenamel junction.
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**Results**

There were no significant differences among groups when comparing either debris or smear layer removal. For debris, scores 1 and 2 were recorded in 91.6% in group I and 87.5% in group II. For smear layer, scores 4 and 5 were recorded in 95.8% in group I and 91.6% in group II.

**Conclusions**

Surfactant addition to 5.25% NaOCl did not improve its cleaning effectiveness. There were no significant differences between the two groups with regards to debris and smear layer removal in all three parts of the root canal system.

**Edu**

**R047**

M. Guivarc'h1, A. Gaudin2, D. Seux3 & F. Bukiet4

1Department of Conservative Dentistry and Endodontics, UFR Odontologie, Aix-Marseille Université. Assistance Publique des Hôpitaux de Marseille / UMR 7268-ADÉS Aix-Marseille Universit – EF5-CNRS, Faculté de Médecine de Marseille, Marseille. 2Department of Conservative Dentistry and Endodontics, University of Nantes - Faculty of Odontology Nantes, Nantes. 3Department of Conservative Dentistry and endodontics, Faculty of Odontology - Lyon civil hospitals. Lyon University, Lyon & 4Department of Conservative Dentistry and Endodontics, UFR Odontologie. Aix-Marseille Université. Assistance Publique des Hôpitaux de Marseille. UMR 7287 CNRS, ISM (GIBOC), Marseille, France

**Irrigation trends among dental schools in France: a web-based survey**

**Aim**

To investigate trends in irrigation especially materials and methods among dental schools (DS) and teaching given by endodontic staff (ES) in France.

**Methodology**

An invitation to participate in a web-based survey was e-mailed to the 255 members of the CNEOC (College National des Enseignants en Odontologie Conservatrice) from every dental school in France (n = 16). A filter was applied in order to discriminate answers from DS and ES. Questions related to DS (irrigant concentration, storage method, activation/agitation techniques usage) were only asked to the Head of the Endodontic Department. Questions concerning irrigation instructions (smear layer removal, chlorhexidine (CHX) use) were asked to ES. Additional question regarding dentine removal when treating infected teeth was also investigated.

**Results**

A total of 206 survey invitations were successfully delivered. Positive response rate was 93.7% for DS (n = 15) and 47% for ES (n = 97). Among DS, 86.7% used sodium hypochlorite (NaOCl) between 0.5% and 2.5%, either ‘ready to use’ bottles (66.7%) or prepared extemporaneously (33.3%). Lateral opening needles were the most commonly available (60%). An activation technique was recommended in 66.7% of the schools, manual master cone agitation being the most prevalent (53.3%). Chelator usage was strongly advised by ES in initial therapy (90.7%) and non-surgical retreatment (92.8%). Additional use of CHX was mentioned as necessary for 40% of respondents in case of secondary or persistent infections while 10.3% suggested CHX usage in case of vital teeth treatment. More dentine removal in case of infected teeth was advocated by 19.6% of respondents.

**Conclusions**

In French dental schools, NaOCl is the most commonly used endodontic irrigant. The concept of smear-layer removal is well known. One third of dental schools did not use an activation technique. There is a trend to modify the irrigation and shaping protocols according to the endodontic pathosis and in non-surgical retreatment. This survey provides detailed information about teaching of irrigation protocol and may pave the way for practice harmonization in French endodontic education.

**Acknowledgements**

The authors would like to thank the CNEOC for their assistance in conducting this survey.
R049  
D.M. Ardenghi1*, R. Graziotin-Soares1,2, S.L. Lind3 & D.A. Curtis4  
1College of Dentistry, University of Saskatchewan, Saskatoon, SK, Canada, 2Department of Preventive and Restorative Dental Sciences, School of Dentistry, University of California San Francisco (UCSF), San Francisco, CA, USA & 3School of Economics and Business Administration, Saint Mary’s College of California, Moraga, California, USA

Misconceptions in endodontics and dental implant disciplines

Aim To understand how multiple-choice questions (MCQs) might be used to identify misconceptions scenario questions were compared to knowledge-based questions in endodontic and dental implant disciplines.

Methodology A total of 104 dental students from the University of California San Francisco (UCSF)/USA completed two assessments that included 20 MCQs on endodontics and 20 MCQs on dental implants. At each examination, 10 questions were scenario type (questions requiring interpretation or analysis) and 10 questions were knowledge type (factual based questions, requiring simple recall of information). After students had chosen a response from among 4 alternatives, they indicated their level of confidence for each choice. Misconceptions were defined as the combination: incorrect answer + confident. Incorrect responses and confidence levels by student and subject were recorded to compare the average of misconceptions by question type (statistical T-tests, paired two samples for means).

Results In endodontics, students held a significant higher number of mean misconceptions on scenario questions when compared to knowledge questions (P < 0.0001). However, the difference was not significant for dental implants (P > 0.05). Misconceptions were approximately 75% of incorrect responses for all possible combinations of data, as follows: endodontic scenario (73%); endodontic knowledge (76%); dental implant scenario (72%); dental implant knowledge (74%); endodontic scenario/knowledge combined (74%); dental implant scenario/knowledge combined (73%); and endodontic/dental implant scenario/knowledge combined (73%).

Conclusions MCQs can be used to identify student misconceptions. Students had a consistent rate of overconfidence in their incorrect responses regardless of question type or dental subject. Questions (scenario or knowledge type) that prompted a higher percent of incorrect responses were more likely to detect misconceptions, since students were highly confident in their mistakes, for both assessments.

R050  
E.G. Karova* & V.D. Dogandzhiyska  
Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University, Sofia, Bulgaria

Students assessment of sufficiency of theoretical knowledge and practical training in use of rotary nickel-titanium instruments

Aim To evaluate students’ assessment of sufficiency of theoretical knowledge and practical training four years after the implementation of rotary nickel-titanium instruments in their curriculum.

Methodology The survey was held in Faculty of Dental Medicine, Medical University, Sofia, Bulgaria and 138 fourth-year and 155 fifth-year students participated voluntarily by completing a questionnaire anonymously. The questionnaire, created for the purpose of this cross-sectional study, consisted of 7 multiple-choice questions. Role and sufficiency of university lectures and practical training lessons were estimated, comparing them with non-university theoretical and practical training resources. Self-assessment of the level of students’ experience in use of rotary NiTi instruments in preclinical and clinical course was made using three grades: satisfactory, very good and excellent. Sufficiency of number of clinical endodontic cases shaped with rotary NiTi systems was evaluated by defining it as: insufficient, satisfactory and sufficient.

Results The questionnaire was received by 293 students and 237 of them (80.89%) responded and returned it. Increase of amount/time of practical training was considered necessary by 91.6% of respondents. An extremely high proportion of students (97.5%) appreciated inclusion of more than one rotary NiTi systems in the curriculum. Insufficiency of number of clinical cases was stated (50.4% - insufficient, 38.1% - satisfactory and 11.4% - sufficient). The importance of university teaching program alone or in combination with non-university courses was estimated almost equally – 43% and 49.8% respectively. University practical lessons were considered most influential for acquiring practical experience (74.7%), while non-university practical courses had the lowest importance (6.8%). Practical experience in preclinical course was found ‘satisfactory’ by 57.4% of students and ‘very good’ by 48.1% in clinical course.

Conclusions University teaching programs and practical lessons in use of rotary NiTi instruments were found to be very important but at the same time insufficient. Better knowledge of rotary systems and increase of clinical experience were advocated.

R051  
A. Palatynska-Ulatowska1*, M. Malec2 & H. Pawlicka1  
1Department of Endodontics, Medical University of Lodz & 2Institute of Dentistry, Outpatient Clinic of Endodontics, Independent Public Central Clinical Hospital, Lodz, Poland

Quality of root fillings performed by dental students during undergraduate clinical training

Aim To evaluate radiographically the quality of root fillings in teeth treated by dental students during their undergraduate clinical training.

Methodology Root fillings of 543 root canals: 311 maxillary and 232 mandibular, 263 in single-rooted and 280 in multi-rooted teeth, were independently evaluated by two experienced endodontists on dental RVG images using Kodak Dental Imaging Software 6.11.7.0. The assessment of the length and homogeneity of the fillings was performed. Underfilling, overfilling, overextension and presence of voids or insufficient lateral seal were considered. The results were statistically verified with software StatSoft Statistica 9.1PL using Mann-Whitney and Chi-square independence tests.

Results Overall 78.7% of root canals were filled to the proper length, however, 2.8% of them were underfilled. The sealer, gutta-percha and/or both were present beyond the apex in 10.5%, 7.5% and 0.4% of cases, respectively. Maxillary canines were the most often underfilled teeth (P < 0.01). 82.3% of canals were homogenously filled, nevertheless 17.7% of root fillings were of poor density. The least often homogenously filled canal was in mandibular canines comparing with buccal or palatal canals in mandibular premolars (chi2 = 7.34; P < 0.01) and the distal canal in mandibular molars (chi2 = 7.05; P < 0.01).

Conclusions On the basis of the radiographic database, the root fillings performed during undergraduate training met high standards. For the students, root filling of canines in terms of the density (mandibular) and proper length (maxillary canines) was the biggest challenge.

Acknowledgements This work was supported by grant No 503/2-044-02/503-21-001 from the Medical University of Lodz.
Quality of apical sealing performed by undergraduate dental students: an ex vivo study

Aim To evaluate the quality of apical obturation of the root canal filling performed ex vivo by undergraduate students.

Methodology The study was conducted on 40 extracted single-rooted permanent teeth with fully developed root apices, straight roots, free of cracks, caries, resorptive defects and fractures. The root canals were instrumented to an apical size 4 Hedstrom hand file and then filled with Endomethasone and 0.2 taper gutta-percha with the lateral condensation technique by 4th grade undergraduate students. The access cavities were sealed with glass ionomer cement. Two layers of coloured nail varnish were applied on the surface 2 mm short of the apex. Roots were then suspended in freshly prepared 2% methylene blue for 48 h. Following this, the roots were rinsed for 15 min under running tap water. The teeth were embedded in acrylic blocks and sectioned horizontally at 1 mm (Group A), 2 mm (Group B) and 4 mm (Group C) from the apex. The sections were evaluated under stereomicroscope (102X magnification). The void areas were calculated using Adobe Photoshop CC. The results were analyzed by ANOVA and t-test.

Results Root canals were filled with gutta-percha 58%, 68% and 81% at 1, 2 and 4 mm, respectively. The t-test showed that the results were significant for Group A versus Group B (P < 0.05), and were not significant for Group A versus C and Group B versus C.

Conclusions Although the radiographs can show an adequate root filling, the void areas inspected under magnification are striking. The root filling ability of students needs to be improved.

Infection control assessment of dental students in Endodontic clinics

Aim To assess the infection control level and disseminate awareness among students, nursing staff and clinical instructors at endodontic clinics.

Methodology Three hundred and ten (n = 310) microbiological swabs were taken from students’ endodontic instruments, drawers and working surfaces, as a part of the infection control campaign that was done at the undergraduate (n = 150) and postgraduate (n = 160) clinics. Ten predefined items were selected to take the swabs from, divided into critical and non-critical items. The samples were taken from randomly selected students at the beginning of their treatment session. Sterile wet swabs were used to test the level of microbial contamination on the selected items. The swab was removed from its sealed tube, moistened with phosphate buffered saline (PBS), rubbed and rolled firmly several times across the sampling area, then transferred to 1 ml of PBS (0.85%, pH of 7.4: Oxoid Ltd, UK) and maintained in this buffer for 45 min to standardize storage while in transit to the microbiology laboratory for further processing. Students with positive cultures were informed about their results as well as their assigned instructors and the nursing staff. Those students were visited again to take new swabs from their cubicles to make sure they corrected their performance.

Results Negative cultures within critical items (81.67%) were significantly higher (P = 0.05) than the non-critical shared items such as drawer handles and working surfaces (24.73%). Positive culture incidence have significantly decreased since the beginning of this campaign.

Conclusions Most of the students were applying proper infection control measures for the critical items. As for the non-critical items, extra care should be taken to clean their surfaces properly prior to any dental procedure. Strict instructions were given to the students not to touch, with their contaminated gloves, anything that is not disposable or will not be sterilized. This micro-swab culture campaign was a very effective educational tool in reinforcing the infection control measures.
R055
E. Verheyen1, M.A. Meire, R.J. De Moor & M. De Bruyne
Department of Endodontontology, University of Ghent, Ghent, Belgium

**Performance of Reciproc, ProTaper Next and WaveOne Gold in a pre-clinical student course setting**

**Aim** To evaluate the performance of one NiTi rotary ProTaper Next (PTN) and two NiTi reciprocating single file systems Reciproc (R) and WaveOne Gold (WOG) in a pre-clinical student course setting.

**Methodology** Final-year dental students (n = 25), who had been trained with R (VDW, Germany) two years before and had limited clinical experience in endodontics, were selected. After an introduction to PTN (Dentsply Sirona, Switzerland) and WOG (Dentsply Sirona), they performed canal preparation with each system in the mesiolingual canal of an extracted human mandibular molar according to the manufacturer’s guidelines. The Reciproc system was used for comparison. Standardized radiographs were taken before and after canal instrumentation. The following parameters were evaluated for each system: time of instrumentation, canal transportation, length control, instrument fracture and master apical file. To evaluate their subjective experience, students were provided with a questionnaire after finishing each system. Results were subjected to descriptive and analytical statistics.

**Results** No significant differences were found between the 3 systems for preparation time, canal transportation and length control. One WOG instrument fractured. Concerning the master apical file for R 14 students ended the preparation with R25, 10 students with R40 and 1 student with R50; for PTN 20 students ended with X3 and 5 students with X4; for WOG 6 students ended with the primary file (5 students used the Small file first), 15 with the Medium file and 3 with the Large file. Concerning designation (11/25), controllability (13/25) and the feeling of security (13/25), WOG was the most preferred system, for cutting efficiency PTN (10/25) and for learning curve R (14/25). Overall WOG was preferred by most students (13/25).

**Conclusions** R, PTN and WOG systems had similar behaviour with respect to canal transportation and length control. With the single file systems, instrumentation time was not significantly shorter and in many instances students used more than one file. Most of the undergraduate dental students preferred WOG over PTN and R.

R056
T. Hitij*
Department of Dental Diseases and Dental Morphology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

**Medical acupuncture – basic understanding for students of dental medicine: evaluation sheet**

**Aim** To examine the attitude of undergraduate students of dental medicine towards medical acupuncture after the first ever lecture and the second part tested the knowledge of the students on emergency measures concerning tooth avulsion and tooth crown fractures.

**Methodology** The lecture was a part of the elective course in advanced endodontics in December 2016. After the lecture, an on-line link to the Google Form with 16 questions was sent to all undergraduate students of a Federal University in Brazil, including presentation and question-answer sessions, to promote awareness regarding emergency measures concerning tooth avulsion and tooth crown fractures.

**Results** Fora, Juiz de Fora &

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Abstracts

R057
J.Y. Nigata1*, V.L. Gois1, E.A. Munchow2, M.S. Salas2 & M.T. Albuquerque3
1Department of Dentistry, Federal University of Sergipe, Lagarto, Brazil
2Department of Restorative Dentistry, Federal University of Juiz de Fora, Juiz de Fora a & 3Department of Clinical Dentistry, Federal University of Bahia, Salvador, Brazil

**Influence of dental trauma educational intervention for undergraduate students in Brazil**

**Aim** To investigate the effect of educational intervention among undergraduate students of a Federal University in Brazil, including lecture presentation and question-answer sessions, to promote awareness regarding emergency measures concerning tooth avulsion and tooth crown fractures.

**Methodology** The performance was assessed using a dual-part questionnaire: the first part enquired about demographic data, and the second part tested the knowledge of the students on emergency treatment for tooth crown fractures and tooth avulsion. The questionnaire was administered before (T0) and immediately after (T1) the lecture to a total of 125 undergraduate students including Dentistry (n = 70), Nursing (n = 33) and Speech Therapy (n = 22) courses of a Federal University in Brazil. McNemar’s test with logistic regression was used to compare the responses between the graduation courses before and after the intervention, with the significance level set at 5% (P < 0.05).

**Results** Female gender (78.4%), aged from 18 to 22 years old (73.6%) predominated among all the participants. Dentistry students had a higher percentage (54.3%) of correct answers before the lecture (T0) when compared to Nursing (12%) and Speech Therapy (9%) students concerning storage medium for tooth fragment transportation. Likewise, few Dentistry students (22.9%) and no Nursing and Speech Therapy students knew about the ideal storage medium (milk) for an avulsed tooth when immediate replantation was unavailable. After the educational intervention, a significant improvement was found between the baseline (T0) and post-lecture evaluation (T2) (P < 0.001), for all the courses, mainly regarding tooth avulsion with almost 100% of correct answers. Furthermore, logistic regression demonstrated that Dentistry students had three times more knowledge absorption than Nursing and Speech Therapy ones.
Conclusions The educational intervention demonstrated a significant increase in the knowledge of dental trauma management. Consequently, the guidance of health professionals regarding the appropriate emergency treatment in dental trauma situations is fundamental to help save teeth and minimize the side effects of dental trauma.

R058
A.A. Al Masan*, P.M.H. Dummer, D. Farnell & M.E. Vianna
Dental School, Cardiff University, Cardiff, UK

A questionnaire-based study on antibiotic-prescribing for endodontic therapies amongst general dental practitioners (GDPs) and final-year Bachelor of Dental Surgery (BDS) students

Aim To evaluate the views of Cardiff University final-year BDS students and GDPs within Cardiff, Wales on antibiotic prescribing for endodontic diseases.

Methodology A cross-sectional questionnaire-based survey of 12 questions was distributed to 76 final-year BDS Cardiff University students and 55 dental practices within Cardiff. The data were analyzed using descriptive statistics, and contingency tables, Chi-Square ($\chi^2$) tests, Fisher’s-exact tests, and relative-risk calculations were performed in SPSS software.

Results The response rate was 60% ($n = 79$). All students were aware of the consequences of antibiotic over-use. Approximately 60% were aware of guidelines for antibiotic use in endodontic therapies, and 83% would only use antibiotics for a limited selection of patients (e.g. patients with systemic complications). Student responses to clinical-scenarios indicated overall that they were comparable to the ideal answers except for acute apical abscess (64% believed that antibiotics were indicated). The majority of GDPs were aware of the consequences of antibiotic over-use. Only 28% of GDPs were aware of guidelines for antibiotic use in endodontic therapies. Overall responses showed that antibiotics were indicated: systemic complications (78%), acute apical abscess (72%) and symptomatic apical periodontitis (28%). The GDPs’ responses to the clinical-scenarios showed incompatibility with antibiotic prescribing guidelines for endodontic therapies.

Conclusions Year five students showed awareness of the antibiotic resistance crisis and a third of students were not aware of guidelines for the use of antibiotics in endodontic therapies but majority of responses were more compatible with guidelines. GDPs were less aware of the implications of over-use of antibiotics and the existence of guidelines, and their responses were incompatible with antibiotic-guidelines for endodontic therapies.

Acknowledgements We would like to express our thanks to all participants for their involvement.
SESSION 2: FRIDAY 15TH SEPTEMBER

APEX LOCATORS/WORKING LENGTH

R059
C. Wichniak1,2, A.T. Cruz1, E. Carneiro1, V.P.D. Westphalen1, L.F. Fariniuk1, U.X. Silva Neto3 & L. Fisecski2
1Department of Endodontics, Pontifical Catholic University of Parana, Curitiba, Brazil & 2Department of Endodontics, University of Buffalo, Buffalo, USA

Evaluation of a new rotary motor with integrated apex locator

Aim To evaluate ex vivo the efficacy of both the electronic apex locator (EAL) and auto apical reverse (AAR) functions of the endodontic motor MM Control compared to the Root ZX II.

Methodology The actual length (AL) of 36 single-rooted teeth was obtained using a digital caliper (DC). The EAL measurements at the marks ‘APEX’ and ‘0.5’ of both devices were obtained using an alginate model. The teeth were randomly divided into two groups (n = 18) and root canal preparation was performed with rotary instruments using the AAR function (0.5 mark) of each motor. After preparation, the AL length was obtained (AARL). The electronic lengths (EL) and AARL were compared with the AL, and the difference was assigned as negative when it was less or positive when greater. The means of the absolute values and the percentage of distribution of the electronic measurements between devices were compared.

Results There was no difference between the devices when comparing the means of the EAL measurements or AARL (ANOVA P > 0.05). However, the MM Control was associated with a greater percentage of EL measurements >1.01 mm longer than the AL (chi-square P < 0.01). The AAR function provided an acceptable apical limit in 83.3% of the cases for Root ZX and 77.8% for the MM Control.

Conclusions The AAR function of both MM Control and Root ZX II provided an adequate apical limit of preparation ex vivo. However, the use of only the EAL function of the MM Control resulted in significantly more overextended readings.

R060
D. Arslan, M. Kebudi Benezra*, F. Kaplan, M.K. Benezra & D. Arslan
Department of Endodontics, Bezmialem Vakif University, Istanbul, Turkey

Ex vivo evaluation of the accuracy of two apex locators for determining the working length of endodontically retreated curved mesial roots of maxillary molar teeth

Aim To evaluate the accuracy of PROPEX PIXI (Dentsply Sirona, Switzerland) and Apex ID (Sybron Endo, USA) in determining the working lengths of curved mesial roots of maxillary molars during retreatment procedure.

Methodology Twenty extracted, non-curious, single-rooted human teeth were decoronated at the cementoenamel junction. Visually, the actual canal length to the major foramen was determined by using a size 15 K-file under a stereomicroscope at ×30 magnification. The real working length was determined by subtracting 0.5 mm from the actual length. All teeth were embedded in an alginate mould as the electronic medium. Electronic measurements were performed using a size 20 K-file with the Propex file. Data were analysed using the Wilcoxon Signed Rank and Mann Whitney U test. Statistical significance was accepted at the alpha level of 5% (P < 0.05). The analyses were carried out with MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Belgium).

Results No significant difference was found between direct measurements (DM) and electronic measurements before and during retreatment for PROPEX PIXI (P = 0.135) and Apex ID (P = 0.232). Also there was no significant difference in determination of root canal length between the two apex locaters before treatment (P = 0.108) and during retreatment (P = 0.242).

Conclusions PROPEX PIXI and Apex ID were considered accurate for the root canal length determination during retreatment procedure.

R061
Department of Endodontics, Bezmialem Vakif University, Istanbul, Turkey

Surface active agents do not affect the accuracy of two apex locators for working length determination: an ex vivo study

Aim To evaluate ex vivo the effect of sodium hypochlorite (NaOCl) solutions mixed with various surface active agents on the accuracy of two different electronic apex locators for working length determination.

Methodology Twenty extracted, non-curious, single-rooted human teeth were decoronated at the cementoenamel junction. Visually, the actual canal length to the major foramen was determined by using a size 15 K-file under a stereomicroscope at ×30 magnification. The real working length was determined by subtracting 0.5 mm from the actual length. All teeth were embedded in an alginate mould as the electronic medium. Electronic measurements were performed using a size 20 K-file with the Propex file. Data were analysed using the Wilcoxon Signed Rank and Mann Whitney U test. Statistical significance was accepted at the alpha level of 5% (P < 0.05). The analyses were carried out with MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Belgium).

Results No significant difference was found between direct measurements (DM) and electronic measurements before and during retreatment for PROPEX PIXI (P = 0.135) and Apex ID (P = 0.232). Also there was no significant difference in determination of root canal length between the two apex locaters before treatment (P = 0.108) and during retreatment (P = 0.242).

Conclusions PROPEX PIXI and Apex ID were considered accurate for the root canal length determination during retreatment procedure.
statistically by repeated measures ANOVA and the paired sample t-test. The significant level was set at 0.05.

Results The mean differences between the real working length and electronic length for Propex Pixi were: −0.18 ± 0.21 mm, −0.06 ± 0.23 mm, −0.14 ± 0.21 mm and −0.08 ± 0.2 mm for NaOCl, NaOCl+BAK, NaOCl+TRX and NaOCl+TWN solutions, respectively. In the Raypex6 group, −0.19 ± 0.23 mm, −0.13 ± 0.26 mm, v0.12 ± 0.24 mm and −0.12 ± 0.24 mm for NaOCl, NaOCl+BAK, NaOCl+TRX and NaOCl+TWN solutions, respectively. Among the irrigation solutions in both Propex Pixi and Raypex6 groups, no significant differences were found (P = 0.100 and P = 0.153, respectively). The t test also showed no significant difference between the accuracy of the two apex locators (P > 0.05).

Conclusions NaOCl with added surface active agents did not interfere with electronic working length determination.

The Influence of MTAD and QMIX on the Accuracy of Electronic Apex Locator in Locating Simulated Root Perforations

Aim To assess the accuracy of the Raypex 6 (VDW, Germany) electronic apex locator in detecting artificial root perforations in the root canals of extracted teeth in dry conditions and in the presence of the following irrigation solutions: 0.9% saline solution, Chlorhexidine (CHX), QMIX and MTAD.

Methodology The root canals of twenty-five extracted, single-rooted human teeth were perforated artificially in the middle section of roots. The actual lengths of the canals up to the perforation site were determined by visualization of the tip of a size 20 K-file at the perforation defect under a stereomicroscope (Olympus SZ61, Olympus Corp., Japan) with a magnification of 20 x, and the distance from the rubber stop to the file tip was measured with a caliper. Then the teeth were embedded in an alginate mould. Electronic measurements of the perforations were obtained by electronic apex locator according to the manufacturers’ recommendations in dry conditions and in the presence of 0.9% saline solution, Chlorhexidine (CHX), MTAD and QMIX using a size 20 K-file. Each canal was irrigated with distilled water and then dried with paper points between measurements with the different irrigants. For the Raypex 6 device, a size 20 K-file with a rubber stop was advanced into the canal until an ‘APEX’ reading was obtained. Statistical analyses were performed using the Friedman test and Spearman Rank correlation coefficient to assess the correlations between the measurements of the methods.

Results Dry conditions and 0.9% saline solution had the most (r: 0.932, r: 0.940 respectively) and MTAD (r: 0.697) had the least correlated results with actual lengths according to the correlation analysis. However, there were no significant differences among measurements between groups (P > 0.05).

Conclusions The most accurate measurements of artificial perforations were obtained in dry condition and in the presence of 0.9% saline solution.

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R065
K. Yılmaz1, 4, G. Uslu2 & T. Özüyrek3
1Department of Endodontics, Samsun Oral and Health Care Hospital & 2Department of Endodontics, Ondokuz Mayıs University, Samsun, Turkey

Laboratory comparison of cyclic fatigue resistance of HyFlex EDM, One G and ProGlider Nickel-Titanium glide path instruments in artificial canals with single and double curvature

Aim To compare the cyclic fatigue resistance of ProGlider, One G, and HyFlex EDM nickel-titanium glide path files in single- and double-curved artificial canals.

Methodology Forty ProGlider (size 16, .02 taper), 40 One G (size 14, .03 taper) and 40 HyFlex EDM (size 10, .05 taper) single-file glide path files were included. Sixty files (n: 20/each) were subjected to cyclic fatigue testing using double-curved canal and 60 files (n: 20/each) using single-curved canals. The number of cycles to fracture (NCF) was calculated and the length of the fractured fragment (FL) was determined by a digital micrometer. Twelve pieces of fractured files (n: 2/each) were examined with SEM to determine the fracture mode. The NCF and the FL data were analyzed by using one-way ANOVA and post hoc Tukey tests using SPPS 21 software (P < 0.05).

Results In all groups, NCF values were significantly lower in double-curved canals, when compared to single-curved canals (P < 0.05). For both single- and double-curved canals, NCF values of the HyFlex EDM group in apical and coronal curvatures were significantly higher than the NCF values of ProGlider and One G groups (P < 0.05). Moreover, in both single- and double-curved canals, NCF values of ProGlider were significantly higher than One G group (P < 0.05).

Conclusions HyFlex EDM glide path files had the highest cyclic fatigue resistance in both of single- and double-curved canals.

R066
E. Rijckaert*, M. Wambrebe, N. Tribout, M. Vandendael & P. Bottenberg
Department of Preventive and Conservative Dentistry, Endodontology, Vrije Universiteit Brussel, Brussels, Belgium

A comparative study of 3 reciprocating single-file systems Reciproc, Reciproc Blue and WaveOne Gold: a laboratory study

Aim To compare the fracture resistance of three reciprocating single-file systems.

Methodology Three groups of 8 instruments with tip size of 0.25 mm were tested: Reciproc (VDW, Germany), Reciproc Blue (VDW) and WaveOne Gold (Dentsply Sirona, Switzerland). Resin blocks with an artificial canal (60° curve and 5 mm radius) (VDW) were used. The canals were prepared with the following protocol: glide path to working length (18.5 mm) with a size 10 K-file; irrigation, three pecking motions, rinsing with NaOCl and patency check with size 10 K-file. This was repeated until working length was reached. Preparation was done with VDW Gold motor (VDW) with the appropriate settings. A single operator prepared the canals with one instrument. After each preparation files were cleaned and checked by another operator and defects were documented. All instruments were used on a new block until fracture occurred. A total of 117 blocks were prepared. These data were subjected to a Kruskal-Wallis test followed by a post hoc Dunn’s Multiple Comparison Test.

Results Files fractured after a mean preparation of 6.5 blocks for Reciproc, 5.5 blocks for Reciproc Blue and 2.63 blocks for WaveOne Gold. Both Reciproc and Reciproc Blue prepared a significantly greater number of blocks in comparison to the WaveOne Gold. There was no significant difference between Reciproc and Reciproc Blue. All of the Reciproc and Reciproc Blue files fractured without prior deformation, in contrast to the WaveOne Gold files.

Conclusions Reciproc and Reciproc Blue instruments had a significantly higher fracture resistance than WaveOne Gold when used in simulated canals within resin blocks.

R067
A.L. Messias1, A.R. Cerqueira1, L.D. Tavares1 & L. Roseiro2
1Dentistry Department, Faculty of Medicine of Coimbra University & 2Department of Mechanical Engineering, Institute of Engineering of Coimbra, Coimbra, Portugal

Cyclic fatigue resistance of three rotary system files in dynamic model after immersion in sodium hypochlorite

Aim To evaluate in the laboratory the resistance to cyclic fatigue of three different nickel-titanium rotary file system (ProTaper Next (PN), Hyflex CM (CM) and Hyflex EDM (EDM)), after immersion in 3% sodium hypochlorite solution in a clinical simulation of mechanical model featuring axial movement.

Methodology Thirty instruments of 3 different titanium rotary file systems, PN X2 (size 25, 0.06 taper); CM size 25, 0.06 taper and EDM 25, ~ (variable taper), were randomly divided according to a 3 x 3 factorial design and tested under dynamic immersion in a 3% NaOCl solution for 5 min, 1 min or without immersion, making a total of 9 groups (n = 10). The analysis of resistance to cyclic fatigue was performed on a dynamic device specifically developed for this study and tested in an artificial root canal featuring an apical curvature with 45° angle and 5 mm radius. Statistical analysis was performed using two-way factorial ANOVA to detect main effects and interactions associated to the fixed
Factors file system and immersion protocol with Tukey post-hoc tests, at a significance level of 5%.

**Results** Regardless of the immersion treatment, PN X2 had an average 1200 ± 178 cycles to fracture. CM had 1949 ± 362 and EDM had 5573 ± 83, which was a significantly different (P < 0.01). The immersion protocol promoted a significant reduction in the number of cycles to fracture (P = 0.01), regardless of the duration. This was noticed in the groups CM and EDM, for which the immersion in NaOCl led to a mean reduction of 309 cycles [41 to 576] and 682 [46 to 1319].

**Conclusions** HyFlex EDM instruments performed better to cyclic fatigue followed by HyFlex CM. Immersion in NaOCl decreased the resistance to cyclic fatigue of these instruments. Protaper Next was associated with the least resistance to cyclic fatigue but it was not modified by immersion in NaOCl.

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**Abstracts**

**R068**

J.H. Ha1, S.W. Kwak2, J.K. Lee2, S.K. Kim1 & H.C. Kim2,3
1Department of Conservative Dentistry, Kangdong National University School of Dentistry, Daegu & 2Department of Conservative Dentistry, Pusan National University School of Dentistry, Yangsan, Republic of Korea

**Stress generation during pecking motion of rotary nickel-titanium instruments depending on the different pecking distances**

**Aim** To evaluate the stress generated by screw-in forces during file rotation according to different pecking distances.

**Methodology** Twenty simulated resin blocks which had J-shaped canal curvature were used. Twenty OneG files were assigned for a screw-in test depending on pecking depth in two groups (n = 10). The files were operated at 300 rpm and the up-and-down speed was controlled as 1 mm/sec stroke velocity and 10 msec dwell time using a customized device. AEndoS. The pecking distances during pecking motion were ‘2-mm’ or ‘4-mm’ for each group. During the operation, the positive and negative apical loads were recorded at a rate of 50 Hz using customized software attached to the AEndoS. The maximum negative apical load (screw-in force, SF) was recorded and the total energy during pecking motion until file reaches to the working length (integrated screw-in forces, ISF) was computed. The data were analyzed statistically by using an independent t-test at a significance level of 95%.

**Results** No significant difference in SF was found between two groups of pecking distances. However, the longer pecking distance of 4-mm group was associated with significantly larger ISF than the shorter pecking distance group (P < 0.05).

**Conclusions** The shorter pecking distance may generate the lower stresses for root dentine as well as instruments. Shorter prescribed pecking distances when using NiTi rotary instrument may reduce the risk of root cracks and instrument fracture.

**R069**

S. Bürklein1,*, A. Kühn2, K. Urban3 & E. Schäfer1
1Central Interdisciplinary Ambulance in the School of Dentistry, University of Münster, Münster, 2University of Applied Sciences, Osnabrück & 3Department of Periodontology and Operative Dentistry, School of Dentistry, University of Münster, Münster, Germany

**Removing fractured endodontic NiTi instruments with a tube technique: Influence of pre-treatment with various agents on adhesive forces**

**Aim** To enhance the adhesive force for removing intracanalily fractured nickel-titanium instruments using a modified tube technique with various pre-treatment agents in combination with a light-curing composite.

**Methodology** A total of 120 NiTi-Mtwo instruments (VDW, Germany) were cut at their shaft (outer diameter 1.2 mm) and surfaces were smoothed so that no sized undersizes influenced the adhesive bond. Instruments (6 groups/n = 20) were fixed in a chuck with an overlap of 2 mm and surfaces were treated with different agents: (A) GC Metal Primer (GC, USA); (B) Prime&Bond active (Dentsply DeTrey, Germany); (C) NaOCl (3%); D) Citric acid (15%); (E) Phosphoric acid (37%) and one group (F) underwent no pre-treatment. Light curing SDR (Dentsply DeTrey) was placed into suction cannulas (Transcodent LI16G, Transcodent, Germany) with an inner diameter of 1.3 mm and tips were placed in a standardized approach over the instruments. A glass fibre (Conrad Electronic SE, Germany) with a diameter of 1 mm was inserted into the tube and composite was light cured for 2 min. Pull-out tests were performed with a constant speed of 2 mm/ min; failure load was measured digitally. Data were statistically analysed using ANOVA and Student-Newman-Keuls tests.

**Results** Prime&Bond active was associated with significantly greater pull-out values (30.5N) compared to all other groups (P < 0.001). Metal Primer (18.5N) was significantly superior to the untreated (12.6N) and NaOCl (11.7N) group (P < 0.05). No significant differences (including D = 15.2N and E = 14.2N respectively) were obtained between all other groups (P > 0.05).

**Conclusions** Prime&Bond active significantly increased adhesion of NiTi instruments using a modified tube technique with light curing composite.

**Preparation: Apical Extrusion**

**R070**

O.K. Damlı1,*, K.K. Elif2, K. Ergüçürlü1, A. Hakan1 & Ç. Meltem1
1Department of Endodontics, Akdeniz University, Antalya, 2Department of Endodontics, Usak University, Usak & 3Department of Endodontics, Ataturk University, Erzurum, Turkey

**Influence of several glide path files on apical extrusion of debris during canal preparation using the Wave-one system in curved canals**

**Aim** To evaluate the effect of several glide path files on the amount of apically extruded debris during root canal preparation using the WaveOne system in curved canals.

**Methodology** Seventy five extracted mandibular molar teeth were randomly assigned to five groups (n = 15 for each group) for canal instrumentation. Endodontic access cavities were prepared in each tooth. Group1: Glide path was created with PathFile system and canals were shaped with WaveOne Primary. Group2: Glide path was created with G Files system and canals were shaped with WaveOne Primary. Group3: Glide path was created with ProGlider...
file and canals were shaped with WaveOne Primary. Group 4: Glide path was created with 15 and 20 K-files and canals were shaped with WaveOne Primary. Group 5: Glide path was not performed and canals were shaped with WaveOne Primary only. Debris extruded apically during instrumentation was collected into preweighed Eppendorf tubes. The tubes were then stored in an incubator at 70 °C for 5 days. The weight of the dry extruded debris was established by subtracting the pre-instrumentation and post-instrumentation weight of the Eppendorf tubes for each group. The data obtained were analysed using one-way analysis of variance (ANOVA) and Tukey’s post hoc tests.

**Results**

The PathFile+WaveOne and The ProGlider+WaveOne groups extruded significantly less debris than the K-files+WaveOne group (P<0.05). There were no significant differences between WaveOne, The PathFile+WaveOne, The ProGlider+WaveOne and Gile+WaveOne.

**Conclusions**

All instruments were associated with apical extrusion of debris. Creating a glide path using ProGlider and PathFile was associated with less extruded debris than K-files during instrumentation with the WaveOne System.

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**R071**

Ç. Çetin Canbazoglu, I. Arker* & D. Deniz Sungur

Department of Endodontics, Hacettepe University Faculty of Dentistry, Ankara, Turkey

**Effect of four canal preparation techniques on debris extrusion and vertical root fracture resistance**

**Aim**

To compare the effect of several canal preparation techniques on debris extrusion (DE) and vertical root fracture resistance (VRFR).

**Methodology**

Access cavities in 80 extracted single-rooted mature mandibular premolars were prepared and working lengths were measured. Eppendorf tubes without covers were weighted with an analytical balance. Teeth were inserted into the covers up to cementoenamel junction. A needle equalized air pressure inside and outside of the tube. Samples were randomly assigned into four groups according to the preparation technique as follows (n=20): Group PTU: ProTaper Universal (F2; size 25, .08 taper), group PTN: ProTaper Next (X2; 25, .06), group OS: OneShape (25, .06), group R: Reciproc (R25; 25, .08). The extruded debris and irrigation solutions were collected in Eppendorf tubes and stored in an incubator at 70 °C for 5 days. The dry weight of extruded debris was calculated by subtracting the weight of empty tubes from the weight of tubes with debris. After this step, 20 decoronated but not prepared teeth were added as a control group for VRFR experiments. Teeth were embedded into acrylic resin blocks. Periodontal ligament was simulated by impression material. VRFR was tested with an Instron at a speed of 1 mm/min. The maximum force required to fracture was recorded in Newton (N). The data were analysed with Kruskal-Wallis and Mann Whitney U test with Bonferroni correction (P<0.05).

**Results**

The amount of debris extrusion was: OneShape> ProTaper> ProTaper Next> Reciproc. Only OneShape and Reciproc groups were significantly different (P<0.05). VRFR occurred as follows: control>Reciproc, ProTaper> ProTaper Next> OneShape. The control group had significantly higher VRFR than the other groups (P<0.05) except Reciproc (P>0.05) which was significantly higher than OneShape and ProTaper Next (P<0.05).

**Conclusions**

Reciproc was safer than OneShape in terms of apical debris extrusion and vertical root fracture.

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**R072**

M. Verma* & N. Meena

Department of Conservative Dentistry and Endodontics, V S Dental College, Bangalore, India

**Comparison of apical debris extrusion during root canal preparation using rotary and reciprocating instrumentation techniques – an in vitro study**

**Aim**

To quantify the debris extruded apically from teeth using rotary and reciprocation instrumentation systems.

**Methodology**

Eighty extracted human mandibular premolars with single canals and similar lengths were divided into four groups and instrumented using: ProTaper Universal (size 40, 0.06 taper; Dentsply Sirona, Switzerland), Protaper Next (size 40, 0.06 taper; Dentsply Sirona), WaveOne (size 40, 0.06 taper; Dentsply Sirona) and Reciproc (R40; VDW GmbH, Germany). The experimental model described by Myers and Montgomery was followed. Debris extruded during instrumentation was collected into preweighed Eppendorf tubes, which were then stored in an incubator at 70 °C for 5 days. The final weight of the Eppendorf tubes with extruded debris was calculated after obtaining the mean of three consecutive weights for each tube. The amount of apically extruded debris was calculated by subtracting the initial weight of tube from the final weight. Statistical analysis was performed using SPSS version 16.0 software. The groups were compared using the Kruskal-Wallis test for all variables.

**Results**

There was no significant difference between the groups (P=0.1114). However, the ProTaper Universal group produced more extrusion and Protaper Next produced least debris extrusion amongst the instrument groups (P>0.05).

**Conclusions**

All instrumentation techniques irrespective of the operating principle were associated with extruded debris.

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**R073**

M. Kayar* & O. Genc Sen

Department of Endodontics, Dentistry Faculty, Yuzuncu Yil University, Van, Turkey

**An in vitro comparison of apically extruded debris using several rotary instrument systems**

**Aim**

To evaluate the weight of extruded debris apically from root canals using several Nickel Titanium rotary instruments.

**Methodology**

Sixty extracted human mandibular incisor teeth with similar diameters were divided into 4 groups of 15 teeth each. Root canals were instrumented using ProTaper Next, Reciproc, WaveOne and Typhoon rotary files. Irrigant and debris extruded during instrumentation were collected in pre-weighted Eppendorf tubes. The tubes were stored in an incubator at 70 °C for 5 days and weighed again. Initial weights of the tubes were subtracted from final weights to calculate the weight of dry debris. Data were statistically evaluated using Kruskal-Wallis and independent t tests. Tukey test was used for multiple comparisons.

**Results**

The Typhoon group was associated with significantly more extruded debris than other groups (P<0.05). No significant difference was found among ProTaper Next, Reciproc and WaveOne groups (P>0.05).
Conclusions All file systems were associated with extrusion of debris. Typhoon instruments were associated with the most debris extrusion. Continuous rotary systems were associated with greater debris extrusion compared with reciprocating single-file systems.

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PREPARATION: CLEANING ABILITY

R074
M. Lipski1,*, K. Kot1, A. Drozdzik2 & A. Nowicka3
1Department of Preclinical Conservative Dentistry and Preclinical Endodontics, 2Department of General Dentistry & 3Department of Conservative Dentistry and Endodontics, Pomeranian Medical University, Szczecin, Poland

Comparative evaluation of root canal cleaning ability of eS5 and Gentlefile instrumentation systems in the apical third: a scanning electron microscopic study

Aim To compare the efficacy of root canal wall debridement in the apical third following preparation with a conventional NiTi rotary system (eS5) versus a stainless steel file system (Gentlefile). Methodology A total of 22 root canals (DB root canals from 22 maxillary molars) were instrumented with eS5 Endostar nickel-titanium rotary files powered by the endodontic micromotor (Group 1) and with Gentlefile stainless steel instruments activated using a specially designed handpiece at 6500 rpm (Group 2). Irrigation was performed with 3% NaOCl at each change of instrument; final irrigation was conducted with 17% EDTA. For cleanliness evaluation, roots were split longitudinally, examined under environmental scanning electron microscopy and scored according to Hülsmann et al., for debris and smear layer on the surface of the root canal wall in the apical region. Data were analyzed statistically by Kruskal-Wallis and Mann-Whitney tests.

Results There were no significant differences among groups when comparing either debris remaining or quality of smear layer removal. For debris, eS5 and Gentlefile achieved 45.4% and 54.5% scores of 1 and 2, respectively. For smear layer, eS5 and Gentlefile received these good scores in 36.3% and 45.4% of specimens, respectively.

Conclusions Instrumentation with a novel stainless steel file system was as effective as conventional NiTi rotary instrumentation in removing debris and smear layer in the apical third of root canals.

R075
A. Krokidis1,*, N. Barabanti2, A. Cerutti2 & P. Panopoulos1
1Department of Endodontics, National and Kapodistrian University of Athens, Athens, Greece & 2Department of Restorative Dentistry, University of Brescia, Brescia, Italy

Ability of BT-Race and WaveOne to eliminate bacteria from infected root canals ex vivo

Aim To compare ex vivo the ability of BT-Race rotary files versus WaveOne reciprocating files to eliminate viable Enterococcus faecalis populations from long oval root canals.

Methodology Fifty caries free, single-rooted oval teeth (buccolingual to mesiodistal ratio >2:5:1; at 5 mm from the apex) were cleaned, an access cavity prepared and patency was gained (to size 25). Afterwards, the canals were sterilized, contaminated with Enterococcus faecalis (ATCC 29212) and randomly distributed into two groups (n = 25 each): G.1, BT-Race and G.2, WaveOne. For each tooth, irrigation was performed with a total of 15 mL of 2.5%NaOCl. Smear layer was removed with 5 mL 17% EDTA, followed by 5 mL of 2.5% NaOCl which was then inactivated by 5 mL of 10% NaO3S2, and washed away by distilled water. For each tooth, two microbial samples were taken: S1 after determination of the working length, and S2 at the end of the instrumentation. CFUs were log-transformed for the analysis of variance due to the skewed distribution. The non-parametric Mann-Whitney test was used for the comparison of S1 and S2 between the two study groups. Between-group differences in the changes in CFU were evaluated using repeated measurements analysis of variance (ANOVA). All reported P-values are two-tailed. Statistical significance was set at 0.05.

Results Both groups had significantly fewer CFUs in the S2 samples (P < 0.001). The degree of CFU reduction from the S1 to S2 measurements was 99.95% in the Wave One group (IQR: 99.83–99.99) and 99.98% in the BT Race group (IQR: 99.96–100.0). This difference was significantly greater in the BT Race group (P = 0.010).

Conclusions The multiple file system BT-Race was more efficient in reducing the microbiological load of viable E. faecalis from long oval root canals compared to the single reciprocating file WaveOne.

PREPARATION: SHAPING ABILITY

R076
A. Sakalauskienė*, S. Drukteinis & V. Pečulienė
Institute of Odontology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Root canal transportation and centering ability of rotary, reciprocating and hybrid NiTi endodontic instruments: a micro CT study

Aim To evaluate and compare root canal transportation and centering ability of rotary, reciprocating and hybrid NiTi endodontic instruments in extracted mandibular first molars using microcomputed tomography (MCT).

Methodology A total of forty-eight mesial root canals of mandibular first molars were randomly divided into three groups, according to the instrument system used for root canal preparation (n = 16): ProTaper NEXT (PTN), WaveOne (WO) or Genius (GN). Canals were accessed in a conventional manner and instrumented according to the manufacturer’s protocol. MCT, at an isotropic resolution of 22.8 μm, was used to scan the specimens before and after instrumentation. Root canal transportation and centering ability was assessed on a comparison of the pre- and post-instrumentation MCT scans in the coronal, middle and apical thirds. One-way analysis of variance was performed to determine any significant differences among groups; significance was set at P < 0.05.

Results No significant difference in root canal transportation and centering ability in all thirds was found between PTN, WO and GN groups (P > 0.05). Transportation in the mesial direction was greater than that in distal for all files systems.

Conclusions There is no difference between rotary PTN, reciprocating WO and hybrid GN NiTi endodontic instruments when considering canal transportation and centering ability.
R077
S.A. Scarlatescu¹, D.C. Binzaru Ispas², L. Stoica³
A.C. Didilescu², M. Andrei², P. Perlea¹, I. Suciu¹, B.A. Dimitriu¹
& G.F. Moldoveanu¹
¹Department of Endodontics, ²Department of Implantology,
³Department of Anatomy & ⁴Department of Embryology, ‘Carol Davila’ University of Medicine and Pharmacy, Bucharest, Romania

Evaluation of ProTaper Gold, ProTaper Next and ProTaper Universal on dentinal defect formation in curved canals

Aim To investigate the incidence of dentinal defects after preparation of curved root canals, using three rotary systems.

Methodology Eighty mesial roots from extracted mandibular molars with curvatures between 25–35 degrees and radii between 2–4 mm were collected and randomly divided into three experimental groups (n = 20) and one group control (n = 20). The roots were examined under stereomicroscope (Zeiss Stemi 2000-C, Germany) to exclude defects before the procedure. The teeth in the control group were left unprepared. The mesial roots were separated and shaped by ProTaper Gold (Dentsply Sirona, Switzerland), ProTaper Next (Dentsply Sirona) and ProTaper Universal (Dentsply Sirona) up to size 25, .06 taper. The roots were cut horizontally at 3, 6 and 9 mm from the apex and the slices were examined through a stereomicroscope with 20X magnification. The presence of dentinal defects (cracks, incomplete fractures and craze lines) were investigated by two endodontists. Dentinal defects were scored: 0 for no defect and 1 for defects. The data were analyzed using chi-square and Fisher’s exact tests.

Results No defects were observed in the unprepared group. The ProTaper Gold system caused fewer defects (28%) than the ProTaper Universal system (37%) (P < 0.05). There were no significant differences in crack formation between the ProTaper Next and ProTaper Universal groups (P > 0.05). With the ProTaper Next and ProTaper Gold systems, fewer dentinal defects were formed in the apical third than in the middle and coronal regions (P < 0.05). The ProTaper Universal system caused significantly more defects in the middle region comparative to the coronal third (P < 0.05).

Conclusions Rotary instrumentation induced dentinal defects, but the ProTaper Gold files tended to cause fewer defects than the ProTaper Universal system, especially in the apical part of curved root canals.

R078
J. Felgner*, G. Garte & C. Hannig
Department of Operative and Preventive Dentistry, Charité - Universitätsmedizin Berlin, Berlin, Germany

Evaluation of shaping ability and cleaning efficacy of rotary and hand nickel-titanium instruments

Aim To compare ex vivo the cleaning efficacy and shaping ability of rotary and hand nickel-titanium instruments in severely curved root canals of extracted human teeth.

Methodology Sixty root canals of mandibular and maxillary molars with curvatures ranging between 25° and 40° were embedded in a muffle system. Canals were divided into five groups (n = 12) and prepared using ProTaper Universal, FlexMaster, EasyShape and BioRaCe rotary nickel-titanium instruments according to the manufacturers’ recommendations. Hand nickel-titanium instruments were used in a balanced-forced technique. Pre- and post-operative radiographs were taken to assess canal straightening. Pre- and postoperative root canal cross-sections were evaluated to calculate the percentage of unprepared areas and to analyse post-operative cross-sectional shape. Amount of remaining debris and smear layer was calculated using longitudinal sections of the root canals.

Results ProTaper Universal and FlexMaster were able to maintain the original canal curvature significantly better than hand instrumentation (P < 0.05), whereas the other rotary systems did not differ significantly from hand instrumentation (P > 0.05; ANOVA). There was no significant difference for debris or smear layer removal among groups (P > 0.05; Kruskal-Wallis). Instrumentation using BioRaCe and FlexMaster resulted in significantly more adequate post-operative cross-sectional shapes (P < 0.05; Kruskal-Wallis). The instrumentation system had no significant effect on the calculated percentage of unprepared areas (P > 0.05; Kruskal-Wallis).

Conclusions None of the systems was superior in terms of cleaning efficacy. With respect to cross-sectional shaping ability BioRaCe and FlexMaster appeared to be superior compared to the other systems.

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R079
E.M. Moawad*, K.B. Blundell, A.J.P. Preston & F.D.J. Jarad
Department of Restorative dentistry, School of Dentistry, University of Liverpool, Liverpool, UK

An investigation of the efficacy of instrumentation in mandibular molars using the XP-endo Shaper NiTi rotary file: a micro CT analysis

Aim To investigate the percentage of root canal surface instrumentation achieved by XP-end Shaper rotary NiTi file (FKG Dentaire SA, Switzerland) in mandibular molars, using micro computed tomography (μCT) imaging and three-dimensional analysis.

Methodology Thirty-seven mandibular molars were scanned and reconstructed using μCT scanner at a high resolution. Twelve molars were selected from the pre-preparation scans, taking into account the canal space volume, canal anatomy, degree of curvature and canal dimensions. The molars were scanned with μCT at 20 μm resolution pre-preparation and post preparation with the XP-end Shaper (XPS). A single operator undertook all the preparation. Images were manipulated and reconstructed in three dimensions, to allow superimposition and analysis using image analysis software (Materialise mimic package, Leuven, Belgium). Data were recorded and analysed in SPSS 24 software using Univariate analysis and descriptive statistics.

Results The difference in mean canal space volume between pre and post preparation images was = 414989.17 mm³, SD = 518793.9 (95% CI = 195921.72–634056.6). The mean percentage of root canal instrumentation was 62.61%, SD = 15.97, 95% CI = 55.46–68.95. The difference in root canal space volume was mainly affected by the pre-operative volume of the canal (P < 0.001). All other variables did not show any significant effect. No file fracture or procedural errors were detected with the XPS.

Conclusions XP-end Shaper demonstrated a high percentage of root canal surface instrumentation. The percentage of surface instrumentation was comparable and was shown to exceed that stated previously in the literature. The efficacy of the XPS was affected in some canals by the pre-preparation canal volume.
FILLING: MTA

R080
M.A. Marciano1, J. Camilleti2, R.L. Lacatehi1, M.A. Matsumoto4, B.M. Guimaraes1 & M.A.H. Duarte3
1Department of Restorative Dentistry, Piracicaba School of Dentistry, State University of Piracicaba (UNICAMP), Piracicaba, SP, Brazil, 2Department of Restorative Dentistry, Faculty of Dental Surgery, University of Malta, Msida, Malta, 3Department of Dental Surgery and Periodontology, Dental School of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, 4Department of Morphology, Dental School of Araçatuba, State University of São Paulo (UNESP), Araçatuba, SP & 3Department of Dentistry, Endodontics and Dental Materials, Dental School of Bauru, University of São Paulo (USP), Bauru, SP, Brazil

Addition of aluminium fluoride to inhibit tooth discoloration caused by white MTA

Aim The hypothesis tested was that the addition of aluminium fluoride to white MTA could inhibit tooth discoloration.

Methodology MTA Angelus, MTA Angelus with 5%, 15 and 45% aluminium fluoride were tested. The test cements were characterized using a combination of scanning electron microscopy, energy dispersive spectroscopy and X-ray diffraction. Radiopacity and setting time were analysed according to ANSI/ADA and ASTM. Solubility was evaluated using volumetric micro-CT analysis. The pH and calcium and fluoride ion release were assessed after 3 h, 24 h, and 28 d. Tooth discoloration in contact with the cements was assessed after 24 h, 28 d and 90 d. Tissue reaction to subcutaneous implantation in rats was verified after 30 and 60 d. Data were submitted to normality test of D’Agostino & Pearson. Statistical analysis was performed using Kruskal-Wallis and Dunn’s test for radiopacity, setting time, pH, calcium and fluoride ion release and discoloration. The ANOVA/Tukey tests were selected for analysis of solubility and tissue reaction (P < 0.05).

Results The addition of aluminium fluoride altered the structure of MTA, even with small amounts. The addition of 5% aluminium fluoride did not significantly alter radiopacity, setting time and solubility (P > 0.05). pH and calcium ion release were not significantly affected by the addition of aluminium fluoride (P > 0.05). All the tested proportions of aluminium fluoride were effective in preventing tooth darkening verified for MTA Angelus. Aluminium fluoride did not interfere in the inflammatory response of MTA at all periods of analysis, otherwise lower amounts showed less intense inflammatory infiltrate.

Conclusions The addition of low amounts of aluminium fluoride in combination with MTA Angelus resulted in a cement that maintained its chemical, physical and biological properties and prevented tooth discolorisation.

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R081
E. Nagas1,*, A. Eymirli1, E. Uzunoglu1, O. Uyanik1 & Z. Cehreli2
1Department of Endodontics & 2Department of Pediatric Dentistry, Health Science, Ankara, Turkey

The effects of various mixing vehicles on push out bond strength of ProRoot MTA

Aim To assess the effect of various mixing vehicles on the push-out bond strength of ProRoot MTA to root canal dentine.

Methodology Two hundred and ten 1-mm-thick root slices were obtained from extracted single-rooted human teeth. The slices were randomly assigned into 7 groups with respect to the mixing vehicles (n = 30/group): 1: (control group); distilled water (DW); 2: 2% chlorhexidine digluconate (CHX); 3: Articaine; 4: 5.25% sodium hypochlorite (NaOCl); 5: propylene glycol (PG); 6: antiwashout gel; and 7: calcium chloride (CaCl2). In each group, 0.3 mL of test liquid was mixed with 1 g of ProRoot MTA. The push-out bond strength test was performed at a crosshead speed of 1 mm/min, and the bond strength data were analyzed statistically using one-way analysis of variance (ANOVA) and Tukey’s post-hoc tests (P = 0.05). Failure modes were assessed qualitatively under a stereomicroscope.

Results Mean push-out values of CaCl2, antiwashout gel and PG groups were similar (P > 0.05) and significantly greater from those of articaine, CHX and distilled water groups (P < 0.05). NaOCl and PG groups had similar debonding values (P > 0.05). Adhesive failure was the most common type of fracture mode.

Conclusions Viscous mixing vehicles such as calcium chloride, antiwashout gel and propylene glycol significantly increased the push-out bond strength of ProRoot MTA to root canal dentine.

R082
T. Zarra*, T. Lambrianidis, L. Vasiliadis & C. Gogos
Department of Endodontology, Dental School, Aristotle University of Thessaloniki, Thessaloniki, Greece

Effect of curing conditions on setting time, pH and solubility of tricalcium silicate cements

Aim To evaluate setting time, pH and solubility of MTA+ (Cerkamed, Poland) when cured in different environmental conditions in comparison with ProRoot MTA (Dentsply Tulsa Dental, USA).

Methodology Setting time, solubility after 1 and 28 days and pH after 1, 7, 14, 21 and 28 days were evaluated when the cements were cured at 95% humidity or immersed in saline or HBSS at 37°C. Statistical comparisons were employed using one-way ANOVA. The level of significance was set at P = 0.05.

Results Setting time was shorter when the cements were cured at 95% humidity compared with that in saline (P < 0.001); HBSS further retarded the setting time. Setting time of MTA+ was shorter than that of ProRoot MTA (P < 0.001) regardless of the curing conditions. Both materials had an alkaline pH at all conditions. A gradual decrease of pH was observed as evaluation period increased. ProRoot MTA had a higher pH than MTA+ (P < 0.05) at all evaluation periods and in both media of immersion. After both 1 and 28 days of immersion in saline, MTA+ was significantly more soluble than ProRoot MTA (P < 0.001); when immersed in HBSS, no significant difference was found (P = 1.00). Both MTA+ and ProRoot MTA had greater solubility after 1 day compared with that after 28 days regardless of the immersion medium (P < 0.05).

Conclusions The results revealed that saline and HBSS retarded setting time significantly. Setting time of MTA+ was longer than that reported by the manufacturer. MTA+ had a shorter setting
time than that of ProRoot MTA, promoted lower pH values and had higher solubility in saline.

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R083
T. Yuca1,*, M.B. Guneser1, S. Taschieri2, M. Maddalone1, A.N. Dincer1, P. Venino1, V. Zambelli1 & M. Del Fabbro2
1Department of Endodontics, Bezmialem Vakif University, Istanbul, Turkey, 2Department of Biomedical, Surgical and Dental Sciences, Universita degli Studi di Milano, Milan & 3Department of Medicine and Surgery, University of Milano Bicocca, Milan, Italy

Effect of intracanal medicaments on marginal adaptation of ProRoot MTA and Biodentine to coronal dentine: A micro-computed tomographic analysis

Aim To evaluate the effect of three intracanal medicaments on the marginal adaptation of ProRoot MTA and Biodentine to root dentine surfaces using micro-computed tomography.

Methodology Eighty freshly-extracted single-rooted teeth were decoronated, and the root canals instrumented using rotary files. After simulating open apex conditions, Peeso reamers were used to enlarge and standardize the canals. Teeth were randomly assigned into two experimental groups (n = 40), which received a 3 mm coronal barrier of ProRoot MTA (Dentsply Tulsa Dental, USA) and Biodentine (Septodont, France). Before placing barrier materials, the specimens were randomly divided into 4 subgroups (n = 10) that were treated with following intracanal medicaments: a mixture of metronidazole, ciprofloxacin and minocycline (triple antibiotic paste, TAP); a mixture of metronidazole and ciprofloxacin (double antibiotic paste, DAP); a calcium hydroxide powder mixed with distilled water (CH) and a control group (no medicament). After 3 weeks incubation, the medicaments were removed and barrier materials were placed over the blood embedded spongostans by hand condensation. The specimens were then scanned and three-dimensional micro-CT images were constructed using an ex vivo micro-CT scanner (Skyscan 1176, Bruker, Belgium) to analyse the adaptation between the dentine walls and the coronal barrier materials with respect to external void occurrence. The data were analysed statistically using one-way ANOVA and the unpaired Student’s t-test.

Results The percentage of external voids in TAP and DAP treated dentine were greater than control groups in both ProRoot MTA and Biodentine groups (P < 0.05). The occurrence of voids was similar in ProRoot MTA and Biodentine groups when TAP and DAP were used (P > 0.05). A significantly lower percentage of voids were determined in CH-mediated specimens in the MTA group when compared to all test groups (P = 0.04).

Conclusions The application of CH as an intracanal medicament reduced void occurrence between ProRoot MTA and the coronal third of root dentine. However, TAP and DAP medicament decreased the marginal adaptation in both ProRoot MTA and Biodentine when used as coronal barrier material.

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R084
M. Aytore Kosar*, D. Turkaydin & F.B. Basturk
Department of Endodontics, Marmara University, Istanbul, Turkey

Influence of operator-dependent variables on the surface microhardness of Mineral Trioxide Aggregate: a pilot study

Aim To compare the effects of mixing ratio and the operator induced variability on the microhardness of MTA.

Methodology Fifteen specialists, from Endodontics, Paediatric Dentistry and Maxillofacial Surgery, who have been frequently using MTA in clinical practice were selected. They were asked to prepare an MTA sample of a consistency that they considered acceptable for use. Thereafter, operators were asked to prepare another sample by using a pre-weighed amount of MTA. Groups were formed as follows - Unweighed group: The operator manually adjusted the powder/liquid ratio of ProRoot MTA and mixed it with a spatula on a glass slab. Then the operator transferred the mixed slurry to a silicone cylindrical mould with internal dimensions of 6×4 mm. Pre-weighed group: The operators were given a pre-weighed amount of MTA with a ratio of 3:1 powder/liquid, which was in accordance with the manufacturer’s instructions. The clinician manually mixed the MTA and transferred it to the mould. The Mann-Whitney U test was applied to compare the microhardness values of groups at a significance level of P < 0.05.

Results The microhardness values of the unweighed group (mean=50.21) was significantly lower than those of pre-weighed group (mean=66.67) (P < 0.05). The lowest microhardness value was recorded as 39.36 HV in the unweighed group.

Conclusions The mixing ratio of MTA administered by the operators varied from that recommended by the manufacturer. Therefore, the optimum properties of MTA, such as the surface microhardness, may not always be achieved.

R085
H. Aksel1,*, S. Küçükayya Eren1, S. Askerbeyli Örs1 & E. Karaisalıoğlu2
1Department of Endodontics, Hacettepe University, Faculty of Dentistry, Ankara & 2Department of Biostatistics, Kastamonu University, Kastamonu, Turkey

Surface roughness of MTA and Biodentine in several environmental conditions

Aim To identify the surface alterations of MTA and Biodentine after exposure to different environments using a three-dimensional (3D) non-contact optical profilometer.

Methodology Forty-eight cylindrical cavities on acrylic blocks were prepared, randomly divided into two groups and filled with either MTA or Biodentine (n = 24). The samples in each group were then divided into four subgroups according to the storage condition (n = 6): dry; wet (PBS at 7.4 pH); acidic (PBS at 5.0 pH) and blood. The optical micrographs of surface topographies were obtained by profilometry at 45 min, 1, 3 and 28 days of incubation. The surface roughness of the materials was calculated using an image analysis software. The data were analysed using two-way analysis of variance with post-hoc Bonferroni correction.

Results In the dry environment, surface roughness did not change significantly up to 3 days (P > 0.05), while it decreased at 28 days for both materials (P < 0.05). The storage in wet condition caused an initial decrease in the roughness of MTA, while the roughness of Biodentine increased at 1 day (P < 0.05). However, the roughness of both materials in wet condition increased after 1 day up to 28 days (P < 0.05). The acidic environment did...
not cause any significant changes in the surface roughness of both materials (P > 0.05). Exposure to blood caused the highest roughness values for both materials up to 3 days, followed by a prominent decrease at 28 days (P < 0.05). Biodentine had higher roughness values than MTA after storage in wet condition at 1 and 3 days and in blood at 28 days (P < 0.05).

Conclusions Environmental condition and time affect the surface roughness of both materials. 3D optical profilometer is a viable option for determination of surface alterations of materials.

FILLING: SEALERS

An additional application of sealer had very little ex vivo Bacterial suspension with (Suppl. 1), 3–65, 2017 Group 1 had a significantly increased sealer percentage (C20 0.05). To determine the antimicrobial effect of five bioactive Fuller Scholarship, University of Otago Fac-

The efficacy of several protocols for cleaning 10) were cleaned with an air/water syringe.

All sealers reduced the number of bacteria significantly Environmental condition and time affect the sur- 

All cleaning protocols except air/water spray 0.00 - 0.01). To assess the effect of an additional sealer application during 50) were chemomechani-

The least sealer-covered areas were Removal. No method was capable of removing all sealer in 7 s.

Conclusions The most effective method of removing sealer was air/water syringe. The least effective method was air polish-

Methodology After standard access cavity preparation, the canals of extracted human molars (n = 50) were chemomechani-

The efficacy of several protocols for cleaning sealer-contaminated pulp chambers

Aim To evaluate ex vivo the efficacy of various cleaning protocols for sealer-contaminated pulp chambers.

Methodology After standard access cavity preparation, the canals of extracted human molars (n = 50) were chemomechanically prepared and filled with warm vertically condensed gutta-percha and an epoxy resin sealer (AH Plus). The sealer was stained with indigotin dye (0.37 w/w%). Teeth were subjected to the following cleaning protocols (n = 10), all executed for 7 s and followed by air/water spray (3 s); scrubbing with a cotton pellet saturated with alcohol, air polishing, cotton/alkohol + air polishing, and the use of a round bur on the canal walls. Teeth in the control group (n = 10) were cleaned with an air/water syringe. Each tooth was split sagittally and standardized pictures of both pulp chamber halves were taken pre- and postoperatively. The percentage of sealer-covered access cavity area (SCA) in each picture was determined using image analysis software (Sigmascan Pro Image Analysis) and compared pre- and postoperatively and across groups using paired samples T test and one-way ANOVA.

Results The mean SCA before cleaning was 43% and was not significantly different between groups (P > 0.05). The SCA was significantly reduced in all test groups (P < 0.05), but not in the control group. Significant differences in %SCA after cleaning were observed between the control and all test groups. Cleaning with the bur resulted in significantly less %SCA than cleaning with air polishing.

Conclusions All cleaning protocols except air/water spray reduced the amount of sealer. The least sealer-covered areas were observed after use of burs: this method however implies dentine removal. No method was capable of removing all sealer in 7 s.

R087

H. Ahmed, P.R. Cathro & N.P. Chandler Department of Oral Rehabilitation, University of Otago, Dunedin, New Zealand

Effect of an additional application of sealer during continuous wave of condensation obturation

Aim To assess the effect of an additional sealer application during the continuous wave of condensation (CWC) technique by measuring the percentage of sealer and the distance between gutta-percha (GP) and canal wall.

Methodology Palatal roots of 45 extracted human maxillary molars were sectioned and prepared to master apical file size 40, 0.06 taper with Vortex Blue® files. Roots were assigned randomly to three groups (n = 15). Group 1 had a single application of AH Plus™ sealer dyed with Sudan Black B before CWC obturation (Elements™ Free). Group 2 received a second application of dried sealer following the down-pack. Group 3 (control) was obturated without sealer. Roots were sectioned at 2, 3, 4, 6, 7 and 8 mm from the root apex. The obturations were microphotographed (20x) and images measured with ImageJ. The percentage of sealer and distance from GP to canal wall was calculated at each level. Data were analysed using Mood’s median test and the chi-squared test of independence (P < 0.05).

Results Group 1 had a significantly increased sealer percentage when compared to group 2, but only at the 2 mm interval (P = 0.00). Group 2 had a significant increase in the distance between the GP and canal wall when compared to group 1 at the 4 and 6 mm levels (P = 0.02). At 3, 4 and 6 mm, group 1 had increased distance between the GP and canal wall when compared with group 3 (P = 0.00 - 0.05), as did group 2 at 3, 4, 6 and 7 mm (P = 0.00 - 0.01).

Conclusions An additional application of sealer had very little effect on the percentage of sealer present, and increased the distance between the GP and canal wall to a minor degree.

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R088

M. Simunić Munitić1+, I. Bago1 & A. Budimir2

1Dental Polyclinic Split, 2Department of Endodontics and Restorative Dentistry, School of Dental Medicine, University of Zagreb & 3Department of Clinical and Molecular Microbiology, Clinical Hospital Centre Zagreb, Zagreb, Croatia

Antibacterial efficacy of five bioactive root canal sealers against Enterococcus faecalis biofilm

Aim To determine the antimicrobial effect of five bioactive endodontic sealers against 48 h old Enterococcus faecalis biofilms.

Methodology Bacterial suspension with E. faecalis, which had been isolated from root canal with chronic apical periodontitis, was grown on cellulose nitrate membrane filters (Whatman GmbH, Germany) and placed on the Mitis Salivarius agar plates. After the incubation period (48 h), the colonization was confirmed by scanning electron microscopy. Then, the membranes were divided into six experimental groups (n = 8/each) according to the tested sealer: 1: TotalFill Bioceramic Sealer (BC Sealer, FKG, Switzerland); 2. BioRoot RCS (Septodont, Saint-Maur-Des-Fosses, France); 3. MTA Fillapex (Angelus, Londrina, Brasil); 4. MTA Plus (PrevestDenpro, Bradenton, USA); 5. Apexit (Vivadent, Schaan, Liechtenstein); 6. AH Plus (Dentsply, Konstanz, Germany). The sealers were prepared according to the manufacturers’ instructions and approximately 40 μL of each sealer was placed on each membrane. After 30 min of exposure, sealers were removed. The membranes were transferred to tubes containing sterile phosphate buffered saline (PBS) and agitated. After 10-fold serial dilution, the aliquots were plated on Mitis Salivarius agar plates. After 24 h of incubation colony forming units (CFUs) were counted. Positive controls included membranes with E. faecalis and without sealer, and sterile membranes with applied sealer served as negative controls. For the statistical analysis Mann Whitney U and Kruskal Wallis tests were used.

Results All sealers reduced the number of bacteria significantly (p < 0.005). The greatest antibacterial effect was recorded in the Apexit group (p < 0.005). There were no significant differences in antimicrobial efficacy between other sealers (p < 0.001).
Conclusions All tested bioactive endodontic sealers had a significant and similar antimicrobial effect against *E. faecalis*, however, Apexit was the most effective.

R089
E.M. Medioni¹,², S.B. Balbi¹, E.S. Suso³ & C.R. Ricci¹²
¹Micoralis Laboratory, EA 7354, Nice Dental Faculty & ²Nice Dental Faculty, Dental Clinic CHU NICE, Nice, France

**Interfacial adaptation to root dentine of two different root canal sealers used with two different root canal filling techniques**

**Aim** To investigate the ability of the Smart past bio® (bioceramic sealer) and AH Plus® used with a single cone technique C-Point® and the System B® to effectively fill root canals and to determine the adaptation and distribution of sealer inside dentinal tubules.

**Methodology** The root canals of forty-four freshly extracted single-rooted teeth were prepared using a rotary single instrument F360® (size 35, .4 taper; Komet, France). Four groups of ten teeth each were formed: SmartPaste Bio/CPoint, SmartPaste Bio/ SystemB, AH+/CPoint and AH+/SystemB. The two sealers were labelled with 0.1% Rhodamine B fluorescent dye. Four teeth were used as control (sealers without Rhodamine B). The teeth were sectioned horizontally 2, 5 and 9 mm from the apex. The sections were observed under a confocal laser microscope. The maximal penetration depth of sealers inside the dentinal tubules was measured at each level. The percentage of gap-containing region to canal circumference was calculated using the CLSM 2 mm from the apex. Non-parametric statistical tests were achieved to compare both lengths and voids observed in the four groups.

**Results** CPoint/SmartPaste Bio had the greatest penetration length at 5 mm (1528 μm, \( p = 0.0014 \)), and the groups CPoint/SmartPaste Bio and SystemB/SmartPaste Bio at 9 mm (\( p = 0.0001 \)). There was no significant difference at 2 mm. For the four groups and both sealers, the penetration length was significantly better at 9 mm than at 2 mm. At 2 mm from the apex, the groups SystemB/SmartPaste Bio and SystemB/AH Plus had significantly fewer voids than the other groups (2.0% versus 8.8%, \( p = 0.0239 \)).

**Conclusions** The use of a bioceramic sealer with SystemB seems to be the best combination for sealing ability of the root canal.

Further studies are required to verify the stability of the physical, chemical and biologic properties of the sealer with warm compaction techniques.

**R090**
C. Ricci¹,², K. Belhari¹ & E. Medioni²
¹Nice Dental Faculty, Dental Clinic & ²Micoralis Laboratory, EA 7354, Nice Dental Faculty, Chu Nice, Nice, France

**Sealing ability of a single cone root canal filling technique using TotalFill BC sealer® and Bioroot RCS sealer®**

**Aim** To evaluate and compare the interfacial adaptation to root dentine and distribution of sealers inside dentinal tubules of two bioceramic sealers, TotalFill BC® and Bioroot RCS®, used with a single gutta-percha cone filling technique.

**Methodology** The root canals of twenty-two freshly extracted single-rooted teeth were prepared using a rotary single instrument F360® (size 35, .4 taper; Komet, France). Two groups of ten teeth each were formed. The two sealers were labelled with 0.1% Rhodamine B fluorescent dye and the teeth filled using a 0.2 taper gutta-percha cone. Two teeth were used as controls (sealers without Rhodamine B). The teeth were sectioned horizontally at 2, 5 and 9 mm from the apex. The sections were observed under a confocal laser microscope. The maximal penetration depth of sealers inside the dentinal tubules was measured at each level. The percentage of gap-containing region to canal circumference was calculated at 2 and 5 mm from the apex. Non-parametric statistical tests were achieved to compare both lengths and voids observed in the two different groups.

**Results** The penetration length inside the dentinal tubules was not significantly different at the various levels: at 2 mm: \( p = 0.1509 \); at 5 mm: \( p = 0.2265 \), and at 9 mm: \( p = 0.5940 \). No significant difference was observed between the two sealers concerning the percentage of voids at 2 and 5 mm even when the means were lower for the Bioroot RCS sealer: at 2 mm, \( p = 0.1988 \), Bioroot 1.56% voids, TotalFill 8.82% voids, at 5 mm, \( p = 0.6501 \), Bioroot 3.87% voids, TotalFill 10.48% voids.

**Conclusions** The two bioceramic sealers used with a single gutta-percha cone seem to correspond to current criteria of root filling techniques. Clinical studies are needed to confirm the bioactivity of these new sealers.

**R091**
E. Türkel¹, E.O. Onay²,³ & M. Ungör⁴
¹Turkish Ministry of Health, Edirne Dental Hospital, Edirne & ²Department of Endodontics, Baskent University, Faculty of Dentistry, Ankara, Turkey

**The effect of final irrigation activation techniques on dentinal tubule penetration of two different root canal sealers**

**Aim** To evaluate the effect of final irrigation activation techniques on dentinal tubule penetration of two root canal sealers.

**Methodology** The root canals of ninety single-rooted teeth were instrumented using ProTaper rotary instruments up to F4 (size 40) and randomly divided into three study groups based on the final irritant activation technique used: EndoVac (EV) system, photon-induced photosacoustic streaming (PIPS) and conventional syringe irrigation (CSI). Thirty specimens from each experimental group were divided into two subgroups according to the root canal sealer used: AH Plus and TotalFill BC. Root canals were filled using the cold lateral compaction technique. Access cavities were filled with a temporary material and specimens were stored in 100% humidity at room temperature for 1 week to allow the sealers to set completely. The total percentage and maximum depth of sealer penetration were measured at 2, 5, 8 mm from the root apex using confocal laser scanning microscopy. Statistical analysis was carried out by using Student’s t test, Mann–Whitney U test, Friedman Test, Fisher’s exact test, and Chi-square test (\( \alpha = 0.05 \)).

**Results** TotalFill BC use after final irrigation with EV and CSI at 2 mm or PIPS at 5 mm had a significantly greater percentage of sealer penetration than that with AH Plus (\( p < 0.05 \)). For groups in which AH Plus was used, PIPS was associated with a significantly greater depth of sealer penetration than CSI at 2 mm (\( p < 0.05 \)). The maximum depth and total percentage of sealer penetration within each experimental group were observed at 8 mm, followed by 5 mm and 2 mm (\( p < 0.05 \)).

**Conclusions** TotalFill BC had superior tubular penetration compared with AH Plus, whereas PIPS was associated with a greater depth of penetration with AH Plus than CSI at 2 mm.

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FILLING: CANAL

R092
I. Mlehi*, D. Pesic, V. Kolak, A. Nikitovic, M. Lalovic & A. Jakovljevic
Department of Dental Pathology and Endodontics, School of Dental Medicine, Pancevo, Serbia

Scanning electron microscopic evaluation of the adaptation of several root filling materials to root canal walls

Aim To evaluate the adaptation of three materials: AH Plus sealer, AcroSeal sealer with gutta-percha and RealSeal SE system to root canal walls using scanning electron microscopy (SEM).

Methodology The root canals of 120 extracted single-rooted human mandibular premolars were prepared according to a crown-down technique with BioRaCe rotary instruments and enlarged to a size 40 apical file. Each canal was rinsed with 1% NaOCl during and after preparation. The final rinse was performed using EDTA. 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, and filled using cold lateral condensation technique. The teeth were cut longitudinally and the adaptation of materials was evaluated 2 mm from the apex of roots using SEM. The assessment were made using a predefined scale. Statistical analyses were performed using the Kruskal–Wallis test with Bonferroni post-hoc test.

Results There was a significant difference in bond strength (p < 0.05) concerning adaptation to dentine than AcroSeal sealer. AH Plus sealer and RealSeal SE system had better apical sealing ability and adaptation to dentine than AcroSeal sealer.

R093
S.R. Soopen*, F. Albaaj, B. Patel, A.D. Walmsley & P.L. Tomson
Department of Dentistry, University of Birmingham, Birmingham, UK

MicroCT analysis of root canals filled with gutta-percha and BioRoot RCS using three different techniques

Aim To compare the average void percentage using microCT analysis in root canals filled with three different methods: cold lateral condensation (CLC), single cone (SC) and warm vertical condensation (WVC) with a new calcium silicate cement, BioRoot RCS (Septodont, France).

Methodology The root canals of forty single-rooted premolars were prepared with ProTaper Universal using a standard technique. The teeth were divided into 4 groups (n = 10) and root filled using one of three methods – Group 1 LC, Group 2 SC, Group 3 WVC or used a negative control (unfilled). Each group was filled using gutta-percha and BioRoot RCS. Teeth were scanned with high resolution microCT (13.6 μm) before and after root filling then reconstructed for analysis. The pre and post-obtured images were registered using 3D slicer software to ensure alignment. The root canal volume, filling material and remaining voids were calculated for each canal, and for all thirds (cervical, middle and apical) using ImageJ software. Data were compared statistically using the Kruskal–Wallis test and Mann–Whitney Test with the significance level set p < 0.05.

Results The mean void volume percentage was low for each group and ranked as follows (high to low): WVC (0.75% ± 0.18) > SC (0.7% ± 0.19) > LC (0.63% ± 0.13) and no significant differences were determined when compared. The greatest void percentage occurred in the apical third of the root canal and the least occurred in the middle third for each technique. However the difference was only significant in WVC.

Conclusions BioRoot RCS used with the SC technique did not produce a root filling with significantly greater voids than those techniques used to pack gutta-percha. This may suggest that using such a sealer with the SC technique may produce an adequate root filling, which is less time consuming and simpler than other techniques.

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RESTORATION OF ROOT FILLED TEETH

R094
O.V. Must*, J.N. Waddell, P.R. Cathro & K.M. Lyons
Department of Oral Rehabilitation, Faculty of Dentistry and Sir John Walsh Research Institute, University of Otago, Dunedin, New Zealand

Dentine bond strength of a bioactive resin modified glass ionomer cement

Aim To analyse the dentine bond strength of a novel bioactive material using a shear bond strength test, scanning electron microscopy (SEM) and stereo microscopy to evaluate the modes of failure.

Methodology Seventy-five mature bovine teeth stored in distilled water were mounted in dental stone then randomly assigned into five groups (n = 15). Each group was allocated a restorative material: control group 1. Filtek Supreme resin composite (3M), 2. Filtek Bulkfill resin composite (3M), 3. Fuji II LC (GC Corporation Tokyo, Japan) and test groups 4. Activa Bioactive restorative (PulpDent), 5. Activa Bioactive base/liner (PulpDent). The materials were bonded to superficial dentine on the buccal surface of each bovine tooth and the shear bond strength was tested using an Instron machine operated with a crosshead speed of 1 mm/min. Modes of failure were analysed using scanning electron and stereomicroscopy to determine cohesive, adhesive or mixed failure modes within each group. Data for shear bond strength (SBS) were analysed with SPSS using the Kruskal Wallis nonparametric and a post-hoc pairwise test for comparison.

Results There was a significant difference in bond strength between the control group 1 and Activa Bioactive restorative, base/liner in groups 4 and 5 (p < 0.05). Adhesive failure was evident on SEM micrographs for Activa Bioactive restorative material.

Conclusions The shear bond strength and the failure analysis of the bioactive resin material revealed the material is not comparable with conventional resin composites that are used for coronal restoration of root filled teeth. Further investigation into this material is suggested.

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R096
S.R. Olsson11,*, M. Pigg3, P.E. Isberg1 & H. Fransson1
1Department of Endodontics, Odontology, Malmö, 2Department of Orofacial Pain and Jaw Function & Endodontics, Odontology, Malmö & 3Department of Statistics, Lund University School of Economics and Management, Lund, Sweden

Demographic factors in the choice of coronal restoration after root canal treatment in the Swedish adult population

Aim To compare demographic characteristics between individuals choosing an indirect coronal restoration (crown, inlay/onlay) and individuals choosing other restorations after completion of a root filling.

Methodology This was a registry study of a cohort consisting of all root filled maxillary first molars that were reported to the tax-funded Swedish Social Insurance Agency (SSIA) during 2009. After registration of the root filling, any subsequent coronal restorations within two years were identified. The study group consisted of individuals registered with a root filling followed by an indirect coronal restoration and the control group was the remaining individuals with a root filled tooth and a direct coronal restoration or no registration of any coronal restoration. Data on gender, disposable income, age, educational level, civil status and country at birth, were received from Statistics Sweden or SSIA. Chi-square test and t-test compared groups. p < 0.05 was considered statistically significant.

Results Overall 7,806 individuals (21.9%) received an indirect coronal restoration and 27,886 individuals (78.1%) comprised the control group. All demographic variables but gender differed significantly between groups. The mean disposable income and the mean age were higher in the study group receiving an indirect coronal restoration and they also had a higher educational level. A smaller proportion of the individuals in the study group was living on their own or was born outside of Sweden.

Conclusions The identified demographic differences between individuals choosing to restore their newly root filled teeth with an indirect restoration compared to those receiving other restorations indicate that the tax-funded Swedish dental insurance fails to provide dental care on equal terms for Swedish citizens.

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R097
D. Angerame1, M. De Bias1, M. Lenhardt1,6, V. Franco2 & A. Castaldo3
1Clinical Department of Medical Science, Surgery and Health, University of Trieste, Trieste, 2Private Practice, Rome & 3Clinical Department of Medical Science, Surgery and Health, University of Trieste, Trieste, Italy

Endodontic fit of two different fibre post systems: single versus double taper

Aim To evaluate the fit against root canal walls obtained with a single-taper post system, placed without further canal enlargement, and a double-taper post system requiring traditional post space preparation with burs.

Methodology Twenty straight single-rooted teeth were cut to obtain 14 mm-long roots. Canals were shaped with Mtwo rotary files up to size 40, 04 taper and filled with the continuous wave of condensation technique, leaving an unfilled coronal space of 9 mm. Ten roots were randomly assigned to group 1 (G1) and received a SurgiPost Multiconical single-tapered post, which had previously undergone standardised trimming. In the remaining roots (G2), the post space was prepared with the bur of the DT Light-post system to place a double-taper post of corresponding size. The posts were luted with RelyX self-adhesive cement. Cement thickness was measured on sectioned specimens at the scanning electron microscope (120 readings per post third). Parametric statistical tests were used to compare the cement thickness between the two groups and among post thirds (α = 0.05).

Results At the coronal level, cement thickness was minimum and similar in the two groups. The effects of drilling were visible on the canal walls on the apical third of the post in G1, which was characterized by even cement distribution irrespective of the post third. The cement thickness was limited also in G2, but slightly increased at the post tip (p < 0.05).

Conclusions The post systems achieved satisfactory fit in straight single-rooted teeth in the coronal and middle post thirds. At the tip of the post, the post space preparation with drills allowed for excellent fit but required further removal of dentine, while single-taper posts provided an inferior fit at this level due to the standardised methodology needed in the research setting.
Effect of endodontic procedures on gap formation at the tooth-restoration interface assessed by phase-contrast µCT

**Aim** To evaluate gap formation between access cavity walls and bonded composite resin restorations using non-destructive phase contrast (PC) micro-computed tomography (µCT).

**Methodology** Standardized access cavities were prepared in four human maxillary molars. Teeth were subjected to procedures simulating root canal treatment: 1) saline irrigation (control), 2) irrigation with 5.25% NaOCl, followed by 17% EDTA, 3) same irrigation protocol as for group 2, followed by application and removal of Ca(OH)$_2$ on the access cavity walls, 4) same as for group 2 followed by application and removal of root canal sealer on the walls. The access cavities were filled using an etch-and-rinse adhesive and a composite material in a multi-layering technique. The samples were thermocycled for 1000 cycles between 5°C and 55°C. Synchrotron-based µCT imaging was performed obtaining absorption and PC µCT images before and after the immersion of the samples into 50% AgNO$_3$. Using ImageJ computer software PC µCT will be compared to absorption µCT and conventional optical microscopy images.

**Results** PC µCT of samples enabled the best visualization of gaps, while PC µCT with AgNO$_3$ staining led to an overestimation of gap size due to anterograde and retrograde infiltration of AgNO$_3$ into dentinal tubules as well as underestimation of large gaps due to lack of AgNO$_3$ penetration. Gap formation ranged from 10 to 50 μm and was prevalent in places, where Ca(OH)$_2$ or root canal sealer contaminated dentine surfaces and incorporated into the adhesive layer, leading to debonding of the resin composite material. Due to the 3D complexity of gaps further image analysis using advanced segmentation methods will be carried out to visualize and measure their 3D structure.

**Conclusions** Access cavity wall contamination during root canal treatment led to increased gap formation at the tooth-restoration interface best depicted with PC µCT. PC µCT could provide a better alternative for future studies of the tooth-restoration interface compared to absorption µCT or optical microscopy.

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Incidents of gaps and voids in post-endodontic restorations

**Aim** To evaluate and compare the quantitative volume of the gaps formed in endodontically treated teeth with conservative access cavities restored with a bulkfill flowable material (SonicFill) and a traditional resin composite.

**Methodology** Twenty maxillary and 20 mandibular molars were selected based on similar dimensions. After performing conservative access cavities, cleaning, shaping and filling of the root canals and adhesive procedures, specimens were assigned to 2 subgroups for each tooth type (n = 10): Group A: access cavities were restored with a traditional resin composite (EsthetX; Dentsply-Italy, Italy) and Group B: access cavities were restored with a bulkfill composite (SonicFill; Kerr, USA). After restoring the access cavities according to the manufacturer’s instructions the specimens were scanned with CBCT (iCAT). The sliced image data were exported as DICOM files and imported into the MeVisLab framework system for segmentation and volume measurement. After segmentation, volumes of the gaps created at the tooth-composite interface and inside the resin composite of the filling itself were measured. The data were subjected to statistical analyses of variance.

**Results** There were no significant differences between the two groups. The volume of the gaps was similar between teeth restored with traditional resin composite and teeth restored with the bulkfill composite (p > 0.05).

**Conclusions** Endodontically treated teeth with conservative access cavities and restored with bulkfill resin composite had similar gap formation as those restored with gradual light-curing protocols.
Does the activation of resin cement affect the push-out bond strength to root canal dentine?

**Aim** To evaluate the bond strength of self-adhesive resin cement activated with different methods after being placed into the post space.

**Methodology** Thirty six mandibular premolar teeth were selected. Teeth were decoronated 15 mm from the apex. Root canals were prepared and then filled. After 7 days of storage at 37°C and in 100% humidity the root fillings were removed at a 10-mm depth with a number 1 Peeso reamer, and preparation of the post space was completed with a number 1 drill (DT Light-Post System; Bisco Inc). Then specimens were divided into 3 groups according to resin cement activation; Group 1: No activation (control), Group 2: Ultrasonic activation, Group 3: Sonic activation (n:12). After activation of the resin cement (RelyX U200; 3M ESPE) fibre posts were seated and resin cement was polymerized. After 24 h of incubation, the specimens were embedded in autopolymerizing acrylic resin and sectioned horizontally with a water-cooled, low-speed diamond disk. Six slices were obtained, and each slice was approximately 1 mm thick. The first 2 slices were termed coronal, the second 2 middle, and the third 2 apical. A push-out test was applied to slices 2, 4, and 6 at 0.5 mm/min with a 1-mm diameter metallic plunger from the apical to the coronal direction until the post was dislodged. The maximum load required for failure was measured in newtons (N). The maximum failure load was converted to megapascals (MPa) for each slice and adjusted for the total bonding area (mm²) of each segment. Data were analyzed with Kruskal–Wallis and Mann Whitney U test.

**Results** A significant difference was found between activation methods (p < 0.05). Root regions had no significant effect on bond strength (p > 0.05).

**Conclusions** The bond strength of self-adhesive resin cement was negatively affected by ultrasonic and sonic activation of resin cement.

Fracture resistance of endodontically treated teeth with conservative access cavities restored with different materials

**Aim** To determine and compare the fracture resistance of endodontically treated teeth with conservative access cavities restored with two bulkfill materials (SDR and SonicFill) and a traditional resin composite.

**Methodology** Forty maxillary and 40 mandibular molars were selected based on similar dimensions. After cleaning, shaping and filling of the root canals and adhesive procedures, specimens were assigned to 3 subgroups for each tooth type (n = 10): Group A: control group, including intact teeth; Group B: access cavities were restored with a traditional resin composite (EsthetX; Dentsply, Italy); Group C: access cavities were restored with a bulkfill flowable composite (SDR; Dentsply-Italy), except a 1.5 mm region of the occlusal surface that was restored with the same resin composite as Group B. Group D: access cavities were restored with a bulkfill composite (SonicFill; Kerr-California, USA), except a 1.5 mm region of the occlusal surface that was restored with the same resin composite as Group B. The specimens were subjected to compressive force in a material static-testing machine until fracture occurred, the maximum fracture load of the specimens was measured (N) and the type of fracture was recorded as favorable or unfavorable. 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 bulkfill composites (SDR and SonicFill) was similar in both maxillary and mandibular molars with no significant decrease in fracture resistance compared to intact specimens.

**Conclusions** No significant difference was observed in the mechanical fracture resistance of root filled molars restored with traditional resin composite restorations compared to bulkfill composite restorations.

Microleakage and marginal adaptation of three root-end filling materials: a laboratory study

**Aim** To compare microleakage and marginal adaptation of White Mineral Trioxide Aggregate (WMTA), Biocadentine and chemical cured Glass Ionomer Cement (GIC) at root-end filling materials.

**Methodology** Ninety extracted human mandibular premolar teeth were used. The crowns of the teeth were removed at the level of cemento-enamel junction. After canal instrumentation and root filling, the apical 3 mm were resected perpendicular to the long axes of the roots. Root-end cavities were prepared using diamond coated ultrasonic retrofitors. Samples were divided into three experimental groups according to the root-end filling material used (n = 30): White ProRoot MTA, Biocadentine, and EQUIA-Fil GIC. The procedures were performed under the magnification of Dental Operating Microscope (DOM). Samples in each group were subdivided into two subgroups A.B (n = 15). Samples in subgroup A were evaluated for microleakage using a dye extraction method, while those in subgroup B were evaluated for marginal adaptation using a Scanning Electron Microscope (SEM). The results were analyzed statistically using ANOVA and post hoc Tukey tests. The significance level was set at 5% (p ≤ 0.05).

**Results** White ProRoot MTA specimens had the lowest mean dye absorbance value and interfacial gap width followed by Biocadentine and EQUIA-GIC groups with no significant difference between them.

**Conclusions** WMTA, Biocadentine and chemical cured GIC demonstrated comparable microleakage and marginal adaptation as root-end filling materials.
R104
F. Canbolat* & S. Sevimay
Department of Endodontics, Faculty of Dentistry, Ankara University, Yenimahalle, Ankara, Turkey

Assessment of push-out bond strength of three root-end filling materials in retrograde cavities prepared with ultrasonic retrotips

Aim To assess the push-out bond strength of three retro-filling materials in root-end cavities prepared using ultrasonic tips.

Methodology Sixty human freshly extracted central incisors were used. Teeth were randomly divided into three groups of 20 teeth each. Following the preparation of access cavities, root canals were prepared using ProTaper rotary files and filled with ProTaper F3 gutta-percha cones and AH Plus sealer. Apical resection of all specimens was performed using conventional diamond fissure burs and retrograde cavities were prepared by ultrasonic retrotips. In the first group, retrograde cavities were filled with ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA), in the second group with Biodentine (Septodont, Saint-Maur-des-Fosses, France) and the third group with Tech Biosealer RootEnd (Isasan srl, Rovello Porro, Co, Italy). All samples were stored in 100% humidity at 37°C for 7 days to allow complete setting of the materials. Three serial 1 mm thick slices were sectioned from the apex of each root using a low speed saw. Only the slices in the middle were used. Push-out tests were performed at a cross-head speed of 0.5 mm/min using a universal testing machine. Data were analyzed with the Kruskal Wallis statistical test at 0.05 level of significance.

Results The mean values for MTA, Biodentine and Biosealer were respectively 10.07 ± 0.6 Mpa, 10.83 ± 0.62 Mpa and 7.74 ± 0.37 Mpa. Significant differences were established among the groups (p < 0.05). The mean of the Biosealer group was significantly lower than the other groups (p < 0.05). There was no significant differences between MTA and Biodentine (p > 0.05).

Conclusions Tech Biosealer RootEnd had lower bond strength values compared with MTA and Biodentine.

R105
L. Seungwoo*, B. Seungho, L. Wooseeul & Y. Yeonjee
Department of Conservative Dentistry, Dental Hospital, Seoul National University, Seoul, Republic of Korea

Effect of ultrasonic post removal on root surface temperature under different conditions

Aim To evaluate the effect of ultrasonic vibration on root surface temperature during post removal using various ultrasonic tips and cooling methods.

Methodology Eighty extracted single-rooted premolars were decoronated, root lengths standardized and the canals instrumented. Post spaces were prepared, followed by cementation of stainless steel Paraposts. Thermocouples were positioned at 5 and 11 mm from the apex of each root surface. Specimens were embedded in alginate and brought to 37°C in a water bath. Teeth were divided randomly into four test groups according to the cooling method (n = 20): no coolant, air-cooled, water-cooled, stopping instrumentation periodically. Each group was divided into two subgroups according to the ultrasonic tip: CPR tip, Vibrapost. Posts were vibrated ultrasonically for 4 min while continuously measuring temperature. In the periodic stopping group, the stopping time was excluded from the time calculation. Two-way ANOVA and paired t-test compared effects of type of the tip and thermocouple location and environmental condition on temperature change.

Results External root surface temperatures were significantly lower when posts were instrumented using air spray or water coolant irrigation compared with using no coolant or the periodical stopping technique (p < 0.001). Mean root surface temperatures were slightly lower when posts were instrumented using the periodical stopping technique compared with the no coolant group, but higher compared with air-cooled and water-cooled groups. In air-cooled and water-cooled groups, root surface temperatures were significantly lower at the coronal thermocouples compared with the apical thermocouples (p < 0.001). There were no significant differences in mean temperature change between the groups using CPR tip and Vibrapost.

Conclusions Air spray and water coolant irrigation during ultrasonic post removal were effective in inhibiting root surface temperature rise, but ultrasonic instrumentation using periodical stopping technique had a limited effect. CPR tips and Vibrapost similarly affected root surface temperature change.

R106
F. Michellini*, F. Iacono, C. Pirani, M. Rosaria Gatto, M. Giovanna Gandolfi & C. Prati
School of Dentistry, Endodontic Clinical Section, Master in Clinical Endodontology, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

Retreatment of artificial canals filled with Guttaflow Bioseal

Aim To evaluate the possibility to retreat and re-establish apical patency in artificial canals filled with Guttaflow Bioseal, a recently introduced polysiloxane-gutta-percha calcium silicate-bioglass-containing root canal sealer (Celite, Switzerland).

Methodology Twenty-six canals in resin blocks were instrumented with HyFlex EDM sequence (size 25, .12 taper, size 10, .05 taper and EDM OneFile) (Coltèn) and randomly divided in 2 groups according to the root filling method: Group 1 (n = 13): Guttaflow Bioseal with a master gutta-percha cone (Coltèn); Group 2 (n = 13): Guttaflow Bioseal without gutta-percha. The filled canals were temporized with Coltisol (Coltèn) and stored in Hank’s Balance salt solution (HBSS) at 37°C for 10 days. After the removal of the temporary material with ultrasound, all retreatment procedures were performed by a single operator with HyFlex EDM OneFile, without use of chemical solvents. Time needed to reach working length and presence/absence of apical patency were recorded for each sample. The Mann–Whitney U-test was used to compare retreatment time as it had a Gaussian distribution (Shapiro–Wilk test p = 0.0001).

Results No significant difference was observed between the two groups regarding the time needed to reach working length (p = 0.41) with an average time of 21.9 ± 7.9 s for Group 1 and 21.2 ± 9.4 s for Group 2. Apical patency was re-established in 100% of specimens in both groups.

Conclusions The novel calcium silicate-based sealer Guttaflow Bioseal was removed from artificial plastic canals, and apical patency was regained in every sample. The results suggest the possibility to easily remove this filling material during clinical retreatment procedures.
Evaluation of the efficacy of ProTaper Universal, Reciproc and Reciproc Blue in the removal of epoxy-resin based sealer from root canals – a micro-computed tomography study

Aim To compare the efficacy of rotary and two reciprocating techniques during the removal of epoxy resin based sealers from root canals

Methodology Thirty six root canals of extracted single-rooted human maxillary premolars were shaped with ProTaper Next (PTN) up to X3 (Dentsply Sirona, Switzerland) and filled with gutta-percha points and an epoxy-resin based sealer (AH Plus, Dentsply DeTrey, Germany) using the lateral compaction technique. After two weeks, the samples were randomly distributed into three groups (n = 12) according to the retreatment technique used: Group 1: ProTaper Universal retreatment system and additional PTN instruments up to size 40, .06 taper (VDW, Germany); Group 2: Reciproc instrument size 40, .06 taper (VDW, Germany); Group 3: Reciproc Blue instrument size 40, .06 taper (VDW). The retreatment techniques were used according to the manufacturers’ instructions. After the retreatment procedure, the root canals were irrigated with 1 mL 2.5% NaOCl, then filled with 1 mL 15% EDTA, left in canal for 3 min, and finally rinsed with 1 mL 2.5% NaOCl. The samples were scanned in a micro-computed tomographic (micro-CT) device after root canal filling and after the retreatment procedure. The results were analysed with Kruskal–Wallis and post-hoc Mann–Whitney U test at level of significance set at 0.05.

Results There was a significant reduction in the volume of root filling after all retreatment techniques (p < 0.05). The Reciproc technique had the largest reduction in the volume of the filling compared to the ProTaper Universal (p = 0.09) and the Reciproc Blue (p = 0.016). There were no significant difference between ProTaper and Reciproc Blue (p = 0.465).

Conclusions The Reciproc was the most effective technique in the removal of epoxy resin based sealer from root canal compared to Reciproc Blue and the rotary ProTaper technique.

Micro-CT evaluation of CM-wire, M-wire and NiTi instruments in retreatment of curved root canals

Aim To evaluate the removal of filling material when using CM-wire, M-wire and NiTi instruments, under reciprocating and rotary motions.

Methodology Thirty maxillary lateral incisors with apical curvature were used. The teeth were instrumented, filled and divided into three groups (n = 10) according to the filling removal protocol: Group 1: Reciproc R25 followed by Mtwo size 40, .04 taper and ProDesign Logic size 50, .01 taper files; Group 2: ProDesign R size 25, .06 taper followed by ProDesign Logic size 40, .05 taper and ProDesign Logic size 50, .01 taper files and Group 3: Gates-Glidden drills, Hedström files and K-files up to apical size 30, followed by sizes 40 and 50 K-files up to the working length. The samples were scanned before and after each retreatment procedure using a micro-CT system to evaluate filling material removal. The samples were reconstructed, the volumes were recorded and the percentage of remaining filling material at several root canal levels was expressed in terms of percentage of the initial filling material volume. Statistical analysis was performed with Kruskal–Wallis, Friedman and Wilcoxon tests (p < 0.05).

Results All groups had residual filling material after retreatment procedures. No significant difference in filling material removal was found between Groups 1, 2 and 3. The use of Mtwo and ProDesign Logic size 40, .05 taper rotary files did not enhance filling material removal after the use of reciprocating files. The use of ProDesign Logic size 50, .01 taper files significantly reduced the amount of filling material at the apical levels compared with the use of reciprocating files. The apical portion had a large amount of filling material after retreatment procedures in all groups.

Conclusions The combination of reciprocating and rotary files in the retreatment of curved canals, irrespective of the type of the alloy of the instruments, was efficient, but did not remove root canal filling material completely. The use of ProDesign Logic instrument size 50, .01 taper CM-wire is indicated during root canal retreatment, since it significantly reduced the amount of filling material in the apical third without weakening the tooth structure.

Radiographic and microscopic evaluation of ProTaper and Reciproc systems for gutta-percha removal

Aim To compare the efficacy of reciprocating and rotary systems for removing gutta-percha, through radiographic and microscopic evaluation.

Methodology The root canals of sixty mandibular premolars were instrumented using ProTaper Next (Dentsply Sirona) to a final size of X3 and filled by cold lateral condensation and AH Plus (Dentsply, DeTrey). They were randomly divided into two experimental groups (n = 30) according to the instrument used for retreatment: Group 1, Reciproc R25 and R40; Group 2, ProTaper Universal Retreatment (Dentsply Sirona) followed by F4. Remnants of filling material on canal walls were evaluated as a percentage by measuring the amount of radiopaque material in the canal using AutoCAD software. The teeth were then split longitudinally into halves for stereomicroscopic evaluation under 20× magnification and scored 0 (<5% of the root canal area covered by remnants), 1 (6–20% of the split canal) and 2 (>21% of the split canal). The areas of material remnants, as well as similarities or differences between radiographic and microscopic scores were assessed. The data were analyzed using Pearson chi-square test.

Results After radiographic evaluation, Group 2 had the smallest mean value for remaining filling material (p <0.05). Microscopic evaluation revealed more residual filling material than radiographic evaluation, with significant differences in Group 2 (p <0.05). Differences between radiographs and microscopic evaluation were found in 26 of 60 teeth (43.33%). In 22 samples (36.66%) the remnants were scored higher following microscopic evaluation, while in 8 samples (13.33%) the remnants were given 0 following radiographic and 2 following microscopic evaluation.

Conclusions Both retreatment systems left root filling material on the canal walls. ProTaper RetreatmentLeft significantly less
residual material than Reciproc following radiographic evaluation. Radiography underestimated the presence of remaining root filling material, which was detected using the microscope.

**R110**

D. Pesic*, I. Melih, V. Kolak, A. Nikitovic, M. Lalovic & A. Jakovljevic  
Department of Dental Pathology and Endodontics, School of Dental Medicine, Pančevo, Serbia

**Comparative SEM evaluation of the efficacy of several instrumentation techniques in removing Resilon during root canal retreatment**

**Aim** To compare the efficacy of three rotary and two hand instruments in removing RealSeal SE System 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 with BiocRaCe rotary instruments and enlarged to a size 40 before filling with laterally condensed RealSeal Se System. The samples were stored for three weeks in saline at 37°C in an incubator, before being randomly divided into 5 groups of 12 teeth each with regards to instrument used for retreatment: K files, Hedström files, ProFile, ProTaper Universal Retreatment System (PTUS) and D-RaCe. Canals were irrigated with 3% NaOCl and a final rinse using 17% EDTA. Following retreatment the roots were split longitudinally and three different areas (coronal, middle and apical thirds) of the root canal were evaluated and compared using SEM. The assessment and comparisons of present debris and remaining filling material were achieved using a predefined scale. Statistical analyses were performed with the Kruskal–Wallis test with Bonferroni post-hoc test.

**Results** D-RaCe system was the most effective in removing debris and filling material. The D-RaCe system was significantly more efficient than both K-files (p < 0.0112) and Hedström files in the coronal third (p < 0.032), while ProFile instruments were significantly more effective than K files in the middle third (p < 0.039). There were no significant difference between D-RaCe and PTUS systems. When comparing efficiency of each instrument in the three thirds of the root canal, a significantly greater amount of debris and residual filling material was found in the apical third (p < 0.05), except when Hedström files were used.

**Conclusions** All instrumentation techniques left RealSeal SE remnants inside the root canals. In the coronal third of root canals, D-RaCe instruments were more effective than hand instrumentation techniques, while in the middle third the ProFile system was more effective than K-files. Instrumentation technique had no influence on removal of debris and filling material in the apical third. The apical third was the area with the greatest amount of debris and residual filling material compared to coronal and middle thirds, except when Hedström files were used.

**R111**

N.Y. Akgül, F. Haznedaroglu & G. Kuthu  
Department of Endodontics, Faculty of Dentistry, Istanbul University, Istanbul, Turkey

**Evaluation of apical extrusion of debris during retreatment: R-Endo rotary instruments versus hand files**

**Aim** To compare *ex vivo* the amount of debris extruded during retreatment using stainless steel hand files (Hedström files, Dentsply Sirona, Switzerland) and R-Endo (Micro-Mega, France) nickel-titanium rotary instruments.

**Methodology** Eighty single-rooted freshly extracted human mandibular premolars were assigned into two groups consisting of 40 teeth each. Retreatment was performed with R-Endo and Hedström files. The experimental teeth were filled with standard conditions. 1.5 mL Eppendorf tubes were used for collecting material that was extruded during preparation. The amount of extruded debris was calculated by subtracting the postinstrumentation weight of the tubes from the preinstrumentation weight. The mean weight of extruded debris for each group was analysed statistically using SPSS in Windows (SPSS Inc., USA) and Student-t test was used to compare two groups (p < 0.05).

**Results** All instruments tested caused measurable apical extrusion of debris. Apical debris extrusion in the R-Endo group was significantly less than the hand instrumentation group (p < 0.001). The R-Endo required less time to reach working length and also less time for gutta-percha removal compared to hand instruments. There was a statistically significant difference between the groups.

**Conclusions** All instruments were associated with apical extrusion of debris. Hand files extruded significantly greater amounts of debris compared to R-Endo during root canal retreatment procedures.

**R112**

I. Ferreira1, S. Soares2, A.C. Braga3, M.A. Lopes2 & I. Pina-Vaz1,*  
1Department of Endodontics, Faculty of Dental Medicine of University of Porto, Porto, 2CEMMPRE, Center for Mechanical Engineering, Materials and Processes, Faculty of Engineering, University of Porto, Porto & 3Department of Production and Systems Engineering, ALGORITMI Centre, University of Minho, Braga, Portugal

**Chloroform – Is it avoidable in endodontic practice?**

**Aim** To evaluate the ability of several endodontic solvents for the dissolution of gutta-percha.

**Methodology** Stainless steel moulds were prepared and filled with gutta-percha. They were exposed to eucalyptol, xylene, chloroform, EndoSolv R, EndoSolv E and citrol, for 2 and 5 min. Distilled water was used as a negative control. Gutta-percha dissolution was quantified as a percentage, considering the difference between the initial and the final weight. The results were compared with factorial analysis of variance (ANOVA) using the IBM® SPSS® Statistics 23.0 software, considering a 0.05 significance level.

**Results** EndoSolv E was the best solvent. There were significant differences in the mean values of gutta-percha removal within the solvents studied, except when comparing chloroform with EndoSolv E. Similar to the control, EndoSolv R and citrol were not effective. The immersion time had an additive effect on the mean solubility of gutta-percha, as the longer time corresponded to an increased solubility.

**Conclusions** EndoSolv E, specially recommended for zinc oxide eugenol sealers, proved to be an effective alternative to chloroform in the solubility of gutta-percha. A solvent presenting less efficiency may be an important adjunctive step enabling the retreatment file to engage in the softened gutta-percha. The remaining apical gutta-percha can be better removed if it can be preserved as a solid mass. Greater dissolution ability may prevent the complete removal of the gutta-percha during retreatment. For further cleaning of the remaining gutta-percha on root canal walls a final irrigation with a more efficient solvent may be indicated in a short period of time.

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ROOT FRACTURE

R113
P. Linsuwanont* & S. Kulvitit
Department of Restorative Dentistry, Chulalongkorn University, Bangkok, Thailand

Fracture resistance of MTA apexified immature teeth after restoration with various materials

Aim To compare the fracture resistance of simulated immature teeth treated with MTA apexification, and restored with various materials; namely, fibre post, composite resin, MTA, and gutta-percha.

Methodology Fifty-six human maxillary incisors were divided into 6 groups, group 1: Intact teeth, group 2: Immature teeth, group 3: MTA apexified immature teeth restored with quartz fibre reinforced post, group 4: MTA apexified immature teeth restored with dual-cure luting/restorative resin, group 5: MTA apexified immature teeth where the entire root canal was filled with MTA, and group 6: MTA apexified immature teeth where the entire root canal was filled with gutta-percha. All teeth were subjected to thermocycling at 5°C and 55°C for 500 cycles and cyclic loading at 60 Newtons (N) for 160,000 cycles to simulate 6 months occlusal function. All teeth were tested for fracture resistance with a compression test at a speed of 1 mm/min using an Instron universal testing machine. Fracture patterns and load to fracture were recorded. One-way ANOVA and multiple comparisons Tukey HSD were used to analyze the difference of load to fracture among groups.

Results Most teeth fractured at the cervical level. The mean load to fracture of the simulated immature tooth group (962 N) was significantly lower than the intact tooth group (1988 N). The mean load to fracture of the fibre post (1691 N), composite resin (1623 N) and MTA (1921 N) groups were significantly higher than the simulated immature tooth group (962 N). The mean load to fracture of the gutta-percha group (1476 N) was significantly different from the simulated immature tooth group (962 N).

Conclusions Gutta-percha did not strengthen the root of simulated immature maxillary incisors. Fibre posts, composite resin and MTA increased the fracture resistance of simulated immature maxillary incisors. Therefore, after MTA apexification, fibre posts, composite resin or MTA should be used as a root filling material instead of gutta-percha in order to strengthen the immature tooth.

R114
P. Galeano1*, A. Isufi2, E. Pepla3, G. Schianchi2, F.V. Obino2, L. Testarelli2 & G. Gambarini2
1Department of Conservative and Endodontics, Private practitioner, Rome, Italy, 2Department of Endodontics, Sapienza, University of Rome, Rome, Italy & 3Department of Conservative Dentistry and Endodontics, Bristol University, Bristol, UK

Fracture resistance of endodontically treated teeth treated with different tapers of endodontic files

Aim To determine and compare the influence of the taper of endodontic files on the fracture resistance of endodontically treated teeth with conservative access cavities.

Methodology Thirty maxillary and 30 mandibular molars were selected based on similar dimensions. After cleaning, performing conservative access cavities and negotiating with size 10 K-files (Kerr Endo, USA) at the major apical foramen. Specimens were assigned to 3 subgroups for each tooth type (n = 10): Group A: control group, including intact teeth; Group B: canals were instrumented to length with .04 taper K3 NiTi rotary instruments (Kerr Endo) up to size 25; Group C: canals were instrumented to length with .06 taper K3 NiTi rotary instruments up to size 25. After shaping and filling of the root canals, adhesive procedures and performing restorations of access cavities with a traditional resin composite, the specimens were subjected to compressive force in a material static-testing machine until fracture occurred, the maximum fracture load of the specimens was measured (N) and the type of fracture was recorded as favorable or unfavorable.

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 with various canal tapers was similar in both maxillary and mandibular molars with no significant decrease in fracture resistance compared to intact specimens.

Conclusions No significant difference was observed in the mechanical fracture resistance of root filled molars when the canals were prepared with endodontic files with various tapers.

R115
N. Gencoglu* & C. Sivet
Department of Endodontics, Faculty of Dentistry, Marmara University, Istanbul, Turkey

Effect of filling technique on tooth resistance to vertical fracture after removal of fractured instruments

Aim To evaluate the effect of several root filling techniques on vertical root fracture resistance after the removal of fractured instruments using ultrasonics and the Masserrann kit.

Methodology Three-hundred forty-two extracted single and two control groups. 4 mm of F2 instruments were fractured in the coronal, middle or apical thirds of the canals. The fractured instruments were removed from the canals using ultrasonic tips or Masserrann kits. The groups were divided into 3 subgroups according to the root filling technique, which were lateral condensation, SystemB+Obtura and Resilon+Epiphany. The samples were subjected to a continuous vertical loading, using a universal testing machine for vertical fracture. Data were analyzed using one-way ANOVA and Tukey HSD test.

Results The force required to fracture the roots vertically was significantly higher in the positive control group than the experimental groups (p < 0.01). The roots from which the fractured instruments had been removed using ultrasonic tips required more force to fracture than roots in the Masserrann group in the middle and apical sections. The groups root filled with Resilon required significantly more force to fracture than the other techniques in all sections. Lateral condensation was associated with the least resistance in the middle section in the Masserrann group (p < 0.05).

Conclusions Removal of a fractured instrument from the middle and apical third of the canal decreased the force required to fracture the root vertically, regardless of the technique used for instrument removal. Resilon appears to compensate for root dentine loss that occurred as a consequence of attempts at retrieval of broken instrument.
Incidence of apical crack initiation and propagation during removal of root filling materials with various nickel–titanium rotary instruments

Aim To determine the incidence of crack initiation and propagation in apical root dentine after retreatment procedures performed using ProTaper Universal Retreatment, Mtwo-R, ProTaper Next, TF Adaptive systems and hand files with additional instrumentation.

Methodology One hundred and twenty extracted mandibular premolars with single canals were selected. One millimetre from the apex each tooth was ground perpendicular to the long axis of the tooth, and the apical surface polished. Twenty teeth served as the control group, and no preparation was performed. The remaining 100 teeth were prepared to a size 35 apical size with hand files and filled with gutta-percha and AH Plus sealer. Specimens were then divided into 5 groups (n: 20), and retreatment procedures were performed with the following devices and techniques: ProTaper Universal Retreatment, Mtwo-R, ProTaper Next, TF Adaptive and hand files. After retreatment, the additional instrumentation was performed using size 50 ProTaper Universal, Mtwo, ProTaper Next, TF Adaptive and hand files. Digital images of the apical root surface were recorded before preparation, after instrumentation, after filling, after retreatment, and after additional instrumentation. The images were then inspected for the presence of new apical cracks and propagation. Data were analyzed with the chi-square tests using SPSS 21.0 software.

Results All experimental groups were associated with crack initiation and propagation during retreatment processes. The Mtwo-R specimens were associated with more new cracks than the other groups after root filling removal (p < 0.05). Additional instrumentation with any instrument system did not cause significantly more cracks than the others (p > 0.05).

Conclusions Retreatment procedures and additional instrumentation after the use of retreatment files may cause crack initiation and propagation in apical dentine.

R118
H. Ari Aydinbelge, N.T. Azma* & M. Ozelik Yilmaz
Department of Endodontics, Selcuk University, Konya, Turkey

Dental crack formation after several obturation techniques

Aim To compare the incidence of dental crack after using various obturation techniques.

Methodology Seventy-five mandibular teeth with mature apices and straight root canals (<50) that had been extracted for peri-odontal reasons were selected and stored in distilled water. The teeth were randomly assigned to five groups of 15 teeth each. The groups were: 1) no canal preparation, 2) canal preparation, 3) canal preparation and root filling with cold lateral condensation, 4) canal preparation and root filling with warm lateral condensation, 5) canal preparation and root filling with a single cone. Afterwards, the teeth were horizontally sectioned at 3, 6, and 9 mm from the apex with a low-speed saw under water-cooling. All slices were then viewed through a stereomicroscope at 25× magnification and pictures were taken. The presence of dental crack formation was noted and analyzed using the Chi-square test.

Results Group 1 had no dental cracks. All the other treatments resulted in dental crack formation. In the 6 and 9 mm sections, treatments in groups 3 and 4 produced significantly more dental cracks than in the other groups (p < 0.05). The treatments in groups 3 and 4 produced significantly more dental crack in the 6 and 9 mm sections than 3 mm sections (p < 0.05).

Conclusions The single cone technique seems to be the safest root filling technique in respect to dental crack formation.
Abstracts

Methodology The SolidWorks 2014 software structural analysis program was used. Three-dimensional (3D) mathematical models simulating a mandibular premolar tooth with apical external resorption were created. Based on this model, eight experimental models simulating the root fillings were obtained. The experimental models are as follows: (1) MTA (Mineral Trioxide Aggregate) filling (MF); (2) MTA plug + root-filling (RMP); (3) MTA plug + fibre post (FMP); (4) MTA plug + stainless steel post (SMP); (5) Biodentine filling (BF); (6) Biodentine plug + root-filling (RBP); (7) Biodentine plug + fibre post (FBP); (8) Biodentine plug + stainless steel post (SBP). Coronal composite restorations were simulated and 300 N static load was applied to the buccal cusp of the simulated tooth vertically. Von Mises stresses values were calculated as MPa.

Results Maximum von Mises stress values were observed at the load application points, occlusal surfaces, buccal and lingual cervical regions. SMP and SBP models revealed maximum stress values in root dentine while stresses were reduced when the apically resorbed teeth were filled with MTA or Biodentine. The maximum von Mises stress values calculated were as follows (MPa): MF: 27.15; RMP: 27.29; FMP: 27.33; SMP: 28.60; BF: 27.17; RBP: 27.25; FBP: 27.30; SBP: 28.54.

Conclusions Maximum von Mises stress values were observed when stainless steel posts were used with MTA and Biodentine apical plug techniques. Filling the root canal entirely with MTA or Biodentine decreased the stress values in simulated mandibular premolar teeth with apical resorption.

R120
H.N. Li*, W.W. Chang & G.S. Cheung
Comprehensive Dental Care (Endodontics), Faculty of Dentistry, University of Hong Kong, Saiyingpun, Hong Kong

Incidence of dentinal defects after root filling ex vivo by two techniques

Aim To evaluate the incidence of dentinal defects, including partial thickness and full thickness cracking, after root canal instrumentation and root filling by ultrasonic compaction or cold lateral compaction.

Methodology A total of 118 extracted mandibular premolars were divided into 4 groups. The negative control group (n = 3) was left unprepared. The canals of all other teeth were instrumented with a reciprocating file (WaveOne®, large file; Dentsply Sirona, Switzerland) at working length. Group UF (n = 15) was left unfilled while the other canals were filled with gutta-percha and AH Plus (Dentsply DeTrey, Germany) either by cold lateral compaction (group CL, n = 50) or ultrasonic compaction (group UC, n = 50). Teeth were then sectioned horizontally 3, 6 and 9 mm from the root apex and inspected under a stereomicroscope. The presence of dentinal defects was recorded and differences were analysed using the Fisher’s Exact test for the categorical results and Shapiro–Wilk test for the continuous variables.

Results No crack was detected in the negative control group. Instrumentation using WaveOne large files alone gave rise to dentinal defects in 2/15 (13.3%) of the teeth. The incidence of dentinal defects after cold lateral compaction and ultrasonic compaction was 16% and 14% of teeth, respectively. There was no significant difference among the only-instrumented and two root filling groups (p > 0.05). No correlation was found between the appearance of cracking and level of the roots. The distribution of various types of dentinal defects was significantly different between the two root filling techniques (p = 0.009). Both techniques did not induce full thickness cracking on root dentine.

Conclusions The use of ultrasonic compaction for root filling did not show any significant difference with cold lateral compaction of gutta-percha regarding the amount of dentinal defect formation. Root canal treatment was not a direct cause of defects on root dentine under the ex vivo condition of this study.

R121
1Department of Endodontics, Fluminense Federal University, Niterói, Rio de Janeiro, 2Department of Endododontics, Estácio de Sá University, Rio de Janeiro, 3Department of Endodontics, Grande Rio University, Duque de Caxias, Rio de Janeiro, 4Medical Mycology, Federal University of Ceará, Fortaleza, Ceará, 5Nuclear Engineering Program, Federal University of Rio De Janeiro, Rio de Janeiro, 6Dentistry II, Federal University of Maranhão, São Luís, Maranhão & 7Department of Restorative Dentistry, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

Dentinal microcrack formation and canal preparation: a longitudinal in situ micro-CT study using a cadaver model

Aim To evaluate the development of dentinal microcracks after root canal preparation with Reciproc and ProTaper Universal systems, using an in situ cadaver model, by means of a micro-CT imaging system.

Methodology At autopsy, 8 maxillary bone-blocks having at least the premolar teeth (n = 16) were excised, scanned at a resolution of 13.18 μm, and randomly distributed into 2 groups (n = 8), according to the preparation protocol: Reciproc and ProTaper Universal groups. Root canals were prepared up to R25 and F2 instruments in the Reciproc and ProTaper groups, respectively. After the experimental procedures, the specimens were scanned again, and the registered pre and post-operative cross-section images of the roots (n = 19,060) were screened to identify the presence of dentinal defects.

Results In the Reciproc group, 9,176 cross-section images were analyzed and no cracks were observed. In the ProTaper group, 244 out of 9,884 cross-section slices (2.46%) had dentinal defects; however, these defects were already present in the corresponding pre-operative images, indicating that no new microcracks were observed after root canal preparation with the systems.

Conclusions Mechanical preparation of root canals with Reciproc and ProTaper Universal systems did not induce the formation of dentinal microcracks in a cadaver model.
SESSION 3: SATURDAY 16TH SEPTEMBER

BASIC SCIENCE PULP

R122
M.S. Pedano*, X. Li, S. Cokie, E. Putzeys, K.L. Van Landuyt & B. Van Meerbeek
KU Leuven (University of Leuven) Oral Health Sciences – BIOMAT & University Hospitals Leuven (UZ Leuven), Dentistry, Leuven, Belgium

Freshly-mixed-and-setting hydraulic calcium-silicate cements favour proliferation of human dental pulp cells

**Aim** To evaluate the bioactive effect of freshly mixed and setting hydraulic calcium-silicate cements (hCSCs) on human dental pulp cells (HDPCs) by means of an *in vitro* cell-proliferation assay.

**Methodology** Freshly mixed and setting cements were evaluated for their ability to stimulate the proliferation of HDPCs with a XTT colorimetric assay at different time points. To collect the eluates, disks occupying the whole surface of a 12-well plate were made using the hCSCs Pro-Root MTA or Biodentine. Immediately after preparing the disks (non-set), 3 mL of Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS) were added. The medium was left in contact with the disks for 24 h before being collected. To carry out the proliferation assay, cells from passage 3 to 6 obtained from at least 3 different patients were used. 1 × 10^4 HDPCs were seeded in 100-µL culture medium in 96-well plates. The cells were incubated at 37°C and 5% CO_2_ for 24 h. Once the cells were attached, the culture medium was replaced by 100 µL extracts dilutions (1:1; 1:2; 1:4; 1:10). A XTT assay was performed at day 1, 4 and 7 (refreshing the medium and eluates from the wells at day 3 and at day 6). The medium to collect the cements’ extracts was refreshed every 24 h (3 mL of DMEM +10% FBS) to simulate the washing effect of the blood/serum when the cements are expose to pulp tissue. Each extract was tested in triplicate per test and the experiment was repeated 3 times with cells from 3 different patients. Absorbance values of the positive and negative controls were adjusted to 100% and 0% and the relative formazan production was calculated.

**Results** For the 100% and 50% concentrations, freshly-mixed MTA stimulated the proliferation of HDPCs better than Biodentine (Mann-Whitney U test; p < 0.01). For the 25% and 10% concentrations, no significant difference was found between the materials.

**Conclusions** Using freshly-mixed-and-setting cements is a suitable test set-up for bioactivity testing of pulp-capping materials. Pro-Root MTA stimulated the migration of HDPCs better than Biodentine.

**Acknowledgements** Research Foundation – Flanders (FWO) – Belgium – Project number: G.0893.15

R123
A. Digka*, E. Zachou & K.L. Lyroudia
Department of Endodontology, Dental School, Aristotle University of Thessaloniki, Thessaloniki, Greece

Immunohistochemical identification of the blood vessels of a dental pulp polyp by CD34. Report of a rare case

**Aim** The immunohistochemical identification of the vessels of a dental pulp polyp by the use of a pan-endothelial marker for paraffin embedded tissues, CD34.

Methodology A 32 year old healthy female visited the Postgraduate Clinic of the Department of Endodontontology, Dental School, Aristotle University of Thessaloniki, Greece. Upon clinical examination a large pulp polyp was found in an extensive deep carious lesion of the mandibular right second molar, that filled the entire cavity and had a light pink colour. After the application of block anaesthesia, the pulp polyp was excised with a sharp sterile bone excavator and immediately placed in 10% formalin solution for 48 h. The specimen was embedded in a paraffin block and 5 µm sections were obtained using a microtome HM340 E (MICROM Laborgeräte, Germany). The Avidin: Biotinylated enzyme complex (ABC) technique was used for staining with CD34 (Novocarsta). Observation of the sections was conducted using a Zeiss LM (Axiostar, Zeiss, Germany). The regions of interest from each section were captured by a video camera, which was connected to the LM and to a computer video grabber.

**Results** The immunohistochemical staining of the polyp using CD34 antigen revealed extensive vascularization of the polyp in a mesenchymal background tissue. The endothelial walls were identified by the CD34 brown staining. A large number of vessels were dilated.

**Conclusions** The polyp associated with the hyperplastic pulpitis was fully vascularized showing at the same time an enormous number of undifferentiated mesenchymal cells, possibly stem cells derived from the dental pulp.

R124
E.E. Aslantas 1*, Ş. Öztürk 2, H. Aksel 1 & K. Ulubayram 2
1Department of Endodontics, Faculty of Dentistry & 2Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

Synergistic effect of demineralized dentine and dental pulp stem cells on VEGF and BMP-2 release and odontogenic differentiation of cells

**Aim** To investigate the release of VEGF and BMP-2 from demineralized dentine in the presence of DPSCs, and the effect of exogenous growth factor addition based on the release profile on odontogenic differentiation of cells.

**Methodology** Proliferation and morphology of human DPSCs on demineralized dentine discs (dd) were determined using MTT and SEM analysis, respectively (n = 3, assessed in triplicate). VEGF and BMP-2 release from dd in presence or absence of DPSCs were measured to determine the expression of these growth factors using ELISA analysis. In accordance with growth factor release profiles, the exogenous VEGF (VEGF-dd) and BMP-2 (BMP-2-dd) were added in osteogenic medium. DPSCs that were cultivated in dd and in monolayer culture plates (DPSC) that were used as the control groups. The odontogenic differentiation of DPSCs was then evaluated by mineralized nodule formation and the expressions of dentine matrix protein (DMP-1) and bone sialoprotein (BSP) using RT-qPCR analysis.

**Results** DPSCs were able to proliferate and attached onto the dentine surface. The release of BMP-2 from dentine discs was constant in a sustained manner up to 28 days regardless of the presence of DPSCs (p > 0.05) while the release of VEGF was markedly enhanced in the presence of DPSCs after 14 days (p < 0.05).
VEGF-dd and BMP-2-dd upregulated BSP and DMP-1 expressions as compared to dd and DPSC groups (p < 0.05).

Conclusions An increase in VEGF release from demineralized dentine occurred in the presence of DPSCs. The addition of VEGF and BMP-2 at specific time of cell differentiation enhanced the odontogenic differentiation of DPSCs on dentine matrix.

R125
K. Janjić1, M. Edelmayer3, U. Alhujazy1, B. Cvikl1, C. Kurzmann1, A. Moritz1 & H. Agis1,2,4
1Department of Conservative Dentistry and Periodontology & 2Department of Oral Surgery, School of Dentistry, Medical University of Vienna, Vienna, Austria

Do hypoxia and the hypoxia mimetic agent L-mimosine modify the production of Sclerostin and Dickkopf-1 and the pro-angiogenic capacity of dental pulp-derived cells?

Aim To reveal the impact of hypoxia and the hypoxia mimetic agent L-mimosine on the production of Sclerostin (Sost) and Dickkopf (Dkk)-1 in human dental pulp-derived cells (DPC) and their pro-angiogenic capacity.

Methodology DPC in monolayer, spheroid, and tooth slice cultures were exposed to hypoxia and L-mimosine. Resazurin-based toxicity and MTT assays were performed to determine cell viability. Sost, Dkk-1 mRNA and protein levels were evaluated using RT-qPCR and ELISA, respectively. The pro-angiogenic response to hypoxia and L-mimosine was validated based on the measurement of Vascular endothelial growth factor (Vegf), Interleukin (Il)-8, Stromal cell-derived factor (Sdf)-1, Angiogenin (Ang), and Angiopoietin-like 4 (Angptl4) were assessed at mRNA and protein levels.

Results DPC monolayer cultures, DPC spheroid cultures, and tooth slices remained vital upon treatment with hypoxia or L-mimosine. DPC monolayer cultures showed a downregulation of Sost and Dkk-1 mRNA levels in response to hypoxia and L-mimosine, respectively. Protein levels of SOST, but not DKK-1, were reduced by hypoxia. DPC spheroid cultures showed downregulation of Sost and Dkk-1 by L-mimosine at mRNA levels. At protein levels DKK-1 was downregulated by exposure to hypoxia. mRNA levels of Vegf, Il-8, Ang, Angptl4 were increased by treatment with L-MIM or hypoxia in DPC monolayer and spheroid cultures while Sdf-1 levels were downregulated. Sost, Dkk-1 mRNA and protein levels DKK-1 were downregulated by exposure to hypoxia. mRNA levels of Vegf, Il-8, Ang, Angptl4 were increased by treatment with L-MIM or hypoxia in DPC monolayer and spheroid cultures while Sdf-1 levels were downregulated. Sost, Dkk-1 mRNA and protein levels DKK-1 were downregulated by exposure to hypoxia. mRNA levels of Vegf, Il-8, Ang, Angptl4 were increased by treatment with L-MIM or hypoxia in DPC monolayer and spheroid cultures while Sdf-1 levels were downregulated.

Conclusions Hypoxia and the hypoxia mimetic agent L-mimosine in the presence of Sost and Dkk-1 while inducing a pro-angiogenic response in DPC. However, the specific response is dependent on the culture model. These results can serve as a primer for the development of hypoxia-based strategies for oral tissue regeneration.

Acknowledgements The authors thank M. Pensch for skillful technical assistance. The research on Dkk-1 and Sost production upon treatment with hypoxia and the hypoxia mimetic agent L-mimosine was supported by the European Society of Endodontology (research grant 2013). The research on optimization of the cell secretome by pre-conditioning with hypoxia and hypoxia mimetic agents was supported by the International Team for Implantology Research Grant 1085-2015.

BIOCOMPATIBILITY

R126
E.H. Ewesel1, *, J.M. El-Shafei1, D.H. El-Roubay2 & M.M. Bedier3
1Department of Endodontics & 2Department of Oral Pathology, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt

Assessment of the intraosseous tissue response to Biodentine compared with that of mineral trioxide aggregate (MTA)

Aim To assess and compare the intraosseous tissue response to Biodentine (Septodont, France) with that of MTA (ProRoot™, MTA, USA).

Methodology Twenty, white, adult, male, Wistar albino rats were selected. All the experimental procedures were carried out according to the protocol approved by the Ethical Committee of Research in the Faculty of Oral and Dental Medicine, Cairo University. The animals were submitted to general anaesthesia and a longitudinal groove parallel to the long axis of the shaft of the tibia was created. A sterile polyethylene tubes filled with one of the two experimental materials (n = 20) was then implanted in the right tibia of each animal while an empty tube was implanted in the left tibia of the same animal to serve as a control (n = 20). Thirty days after implantation, the animals were sacrificed and the implant sites were removed and prepared for histopathological examination and histomorphometric analysis. Statistical analysis was performed using the Kruskal-Wallis test followed by the Mann-Whitney U test for pair-wise comparisons; one-Way ANOVA was used for data with parametric distribution.

Results Histopathological examination revealed the deposition of newly formed bone trabeculae at the open ends of the implant sites with the presence of osteoblasts at the periphery of bone trabeculae and mild chronic inflammatory cell infiltration in Biodentine and MTA groups. The number of osteoblasts was significantly higher in Biodentine and MTA groups than the control (p < 0.05). The histomorphometric analysis revealed a significant increase in the area percentage of the newly formed bone with Biodentine and MTA compared to the control (p < 0.05) with no difference between the two experimental groups (p > 0.05).

Conclusions Biodentine and MTA had similar biocompatibility to intraosseous tissue. Biodentine seemed to be a bioactive material that could be used as a substitute for MTA.

R127
F.J. Rodríguez Lozano1, *, C.J. Tomás Catalá1, M. Collado-González2, L. Forner2, A. Lozano3 & C. Ilena4
1School of Dentistry, University of Murcia, Murcia & 2University of Valencia, Valencia, Spain

Effects of GuttaFlow Bioseal, GuttaFlow2, MTA Fillapex and AH Plus on cell migration and cell attachment of human periodontal ligament stem cells (hPDLCs)

Aim To investigate the effects of various endodontic sealers on mesenchymal cell migration and cell attachment of human periodontal ligament stem cells (PDLCs).

Methodology hPDLCs were cultured in contact with extracts of endodontic sealers up to 168 h in vitro. A scratch wound healing assay was used to determine their effects on cell migration. To assess cell attachment, hPDLCs were directly seeded onto the material surfaces and analysed by scanning electron microscopy.
To compare the effect of three commonly used bioceramic materials, GuttaFlow Bioseal and GuttaFlow2 but not in the case of AH Plus or MTA Fillapex. At 48 h, GuttaFlow Bioseal and GuttaFlow2 exhibited high and moderate cell migration respectively, whereas AH Plus and MTA Fillapex revealed low rates of cell migration (p < 0.001). Finally, SEM studies revealed a high degree of migration, cell spreading and attachment, especially when using GuttaFlow Bioseal discs at 168 h.

**Conclusions** GuttaFlow Bioseal exhibited better cell migration and attachment than GuttaFlow2, MTA Fillapex and AH Plus.

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**R128**
M.R.W. Ali1, A. Bletsu2,3, M. Mustafa2 & A. Brænden1
1Department of Clinical Dentistry, Faculty of Medicine and Dentistry, University of Bergen, Bergen  & 2Oral Health Centre of Expertise in Western Norway, Hordaland, Bergen, Norway

**Effect of MTA®, Biodentine®, and TotalFill® on proliferation, osteogenic and angiogenic differentiation of human bone marrow stem cells in vitro**

**Aim** To compare the effect of three commonly used bioceramic materials on cellular proliferation, and osteogenic/angiogenic differentiation of stem cells.

**Methodology** Commercially available human bone marrow stem cells (hBMSCs) were cultured (passages 2–4 were used). The cells were subjected to serial concentrations of MTA, Biodentine®, or TotalFill® eluates for 24 h, 3 and 7 days and cellular viability and proliferation was thereafter assessed by MTT assay. Gene expression of osteogenic (Alkaline Phosphatase (ALPL), Osteoprotegerin (OPG), Osteocalcin (OC), Collagen 1A1 (COL1)), or angiogenic (Vascular Endothelial Growth factor A (VEGFA), and Fibroblast Growth Factor-1 (FGF1)) factors was assessed by quantitative real-time PCR (qRT-PCR) after short stimulation of the cells (for 6 h and 24 h) with bioceramic eluates. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as reference gene. All experiments were performed in triplicate and cells not subjected to bioceramic eluates were used as controls. One-way analysis of variance (ANOVA) was used to compare the results (p ≤ 0.05).

**Results** The proliferation of hBMSCs was hampered by exposure to TotalFill® at 3 and 7 days and MTA at 7 days in a dose-dependent manner. Only the highest concentration of Biodentine eluate reduced cellular proliferation at 7 days. Biodentine increased the expression of ALPL whereas Biodentine® and TotalFill® decreased the expression of OC at 6 h stimulation. Biodentine® and TotalFill® increased the expression of OPG at 24 h. MTA decreased the expression of COL1 and FGF and increased the expression of VEGFA at the same time period. VEGFA expression was also increased by 24 h stimulation with TotalFill®

**Conclusions** Human BMSCs in vitro responded differently to the three bioceramic materials tested. Cell proliferation was mostly affected by TotalFill® stimulation. Certain osteogenic factors were increased by Biodentine® and TotalFill® stimulation. The gene expression of the angiogenic factor VEGFA was increased after MTA and TotalFill® stimulation.

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**R129**
H. Aksoy1, D. Deniz Sungur1, Ş. Öztürk2, Z. Yılmaz1 & K. Ulubayram2
1Department of Endodontics & 2Department of Pharmacy, Hacettepe University, Ankara, Turkey

**Biological impact of dentine conditioning by various chelating agents on the viability of dental pulp stem cells**

**Aim** To investigate the effect of dentine conditioning by phytic acid (inositol hexakisphosphate; IP6) and etidronic acid (HEDP) as alternative chelating agents to EDTA on the viability of DPSCs.

**Methodology** Dentine discs (2 mm thick and 6 mm diameter) were prepared from extracted human third molars and treated with 1.5% NaOCl for 5 min. Following that, the dentine discs were conditioned by using one of the following chelating agents for 5 min: 17% EDTA; 1% IP6 and 9% HEDP and distilled water (DW) as a control group (n = 4). DPSCs (2 x 10^5/disc) were seeded on dentine discs and incubated for 1 day, 3 days and 5 days. For each time points, the culture medium was removed and the viability of DPSCs was determined using MTT analysis (n = 4, assayed in triplicate). The obtained data were analyzed using two-way ANOVA and Bonferroni for post-doc comparisons (α = 0.05).

**Results** No significant difference was found in the viability of DPSCs amongst the groups at 1 day (p > 0.05) but there was a significant difference at 3 days and 5 days (p < 0.05). At 3 days, the largest number of viable cells were observed in the control group (DW) while significantly more viability were noted in EDTA group as compared to IP6 and HEDP groups (p < 0.05). Cytotoxicity of the agents were ranked as follows: DW<EDTA<IP6 = HEDP at 3 days while IP6 allowed more viability as compared to HEDP group at 5 days (p < 0.05).

**Conclusions** IP6 could be an alternative dentine conditioning agent for the proliferation of DPSCs. HEDP was associated with the lowest viability of DPSCs as compared to EDTA and IP6.

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**R130**
J.M. Santos1,*, S. Pereira2, D. Sequeira1, P.J. Palma1, A. Messias1 & A.C. Santos2
1Department of Dentistry & 2Department of Biophysics and Biomathematics Institute, IBIL, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

**Biocompatibility of silicon-based sealers in subcutaneous tissue**

**Aim** To evaluate the biocompatibility of a new silicon-based sealer (GuttaFlow Bioseal) in subcutaneous tissues of rats in comparison with GuttaFlow2 and AH Plus.

**Methodology** Twelve Wistar rats were selected. Each animal received 4 subcutaneous tissue implants: GuttaFlow Bioseal, GuttaFlow2, AH Plus and one empty Teflon tube (negative control group). Half the animals were euthanized at 8 days and the remaining 30 days after material implantation. Histological sections prepared from skin specimens were stained with haematoxylin & eosin and analysed using light microscopy. Scores were established for the inflammatory reaction, macrophage infiltrate, thickness of fibrous capsule and vascular changes. Differences between groups and periods of time were accessed using the Friedman test and all pairwise comparisons. The p value for significance was set at 0.05.

**Results** At 8 days, GuttaFlow Bioseal had the lowest inflammatory reaction compared to GuttaFlow2 and AH Plus. At day 30, AH Plus had higher inflammation scores than the negative control and GuttaFlow2 (p = 0.037). Overall, there was a reduction

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in inflammatory reaction from day 8 to day 30 ($p < 0.01$). Macrophage infiltrate increased over time in all groups except for the negative control. At day 30, the groups had different scores of macrophage infiltrate ($p = 0.004$), with GuttaFlow Bioseal and GuttaFlow2 having higher infiltrates than the negative control ($p = 0.002$ and $p = 0.007$, respectively). No significant differences were found in fibrous capsules between sealers. AH Plus was associated with a significant decrease of vascular alterations from day 8 to day 30 ($p = 0.026$), with most cases showing normalcy at the end of the follow-up.

**Conclusions** GuttaFlow Bioseal induced limited inflammatory reactions at both evaluation periods. The initial inflammatory reaction to GuttaFlow2 and AH Plus subsided at 30 days. All tested sealers were considered biocompatible 30 days after subcutaneous implantation.

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**CLINICAL TRIALS**

**R131**

N.A. Taha* & M. Khazali

Conservative Dentistry, Jordan University of Science and Technology, Irbid, Jordan

Partial pulpotomy in mature permanent teeth with clinical signs indicative of irreversible pulpitis: a randomized clinical trial

**Aim** To assess the outcome of partial pulpotomy using mineral trioxide aggregate (MTA) compared with Calcium Hydroxide (CH) in mature cariously exposed permanent molars.

**Methodology** Fifty permanent molar teeth with carious exposures in 50 patients aged above 20 years were included. Preoperative pulpal and periapical diagnosis was established based on history of presenting pain, results of cold testing and radiographic findings. After informed consent the tooth was anaesthetized, isolated via rubber dam and disinfected with 5% NaOCl before caries excavation. Partial pulpotomy was performed by amputating 2 mm of the exposed pulp, haemostasis was achieved and the tooth was randomly assigned for placement of either white MTA (White ProRoot, Dentsply Tulsa, USA) or CH (Dycal, L.D. Caulk, USA) as pulpotomy agents. Postoperative periapical radiographs were taken after placement of the permanent restoration. Clinical and radiographic evaluation was completed after 6 months, 1 and 2 years postoperatively. Statistical analysis was done using the Fisher’s exact test.

**Results** Clinical signs and symptoms suggestive of at least partial irreversible pulpitis were established in all teeth. Immediate failure occurred in 4 teeth. At 1 year MTA had a higher tendency toward success compared to the CH group, and the difference was significant after 2 years (83% versus 55%, $p = 0.052$ at 1 year, 85% versus 43%, $p = 0.006$ at 2 years). Gender did not have a significant effect on the outcome.

**Conclusions** MTA partial pulpotomy sustained a good success rate over 2 years in mature permanent teeth clinically diagnosed with at least partial irreversible pulpitis. More than half of CH cases failed within 2 years.

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**R132**

D. Angerame1, M. De Biasi1.*, V. Franco2 & L. Bevilacqua1

1Clinical Department of Medical Science, Surgery and Health, University of Trieste, Trieste & 2Private Practice, Rome, Italy

Three-year randomized clinical trial on the effectiveness of two integrated techniques for the treatment of maxillary central incisors with periapical pathosis

**Aim** To assess the effectiveness of two integrated shaping and filling techniques for the treatment of maxillary central incisors affected by chronic apical periodontitis.

**Methodology** The trial enrolled 60 patients with a maxillary central incisors needing root canal treatment affected by chronic apical periodontitis with periapical radiolucency smaller than 5 mm in diameter. Block-randomization was undertaken to allocate the patients to two treatment groups with different canal shaping and filling protocols: group 1 (G1, n = 30), Revo-S/One Step Obturator; group 2 (G2, n = 30) GTX/GTX Obturator. An independent examiner evaluated the patients at baseline, after 6, 12, 24 and 36 months by performing percussion and palpation tests. At the same time points, standardized periapical radiographs were taken to score the radiographic healing according to a previously published scale. For this analysis, the level of agreement with regard to the scores assigned by two blind evaluators was tested by weighted kappa statistics. Comparability of baseline clinical data, as well as the differences between the two groups and among time points concerning clinical and radiographic data were evaluated with non-parametric tests ($p < 0.05$).

**Results** All the patients were re-evaluated after 3 years. One patient per group complained of mild symptoms at the end of the observation period. Radiographic healing was progressive during the follow-up. The lesions were classified as totally healed, partially healed and not healed in 93.3%, 3.3% and 3.3% of cases in G1 and in 93.3%, 0% and 6.7% of cases in G2. The differences between groups were not significant ($p > 0.05$).

**Conclusions** The two integrated endodontic systems were equally effective in achieving high three-year success rates in the treatment of maxillary central incisors with chronic apical periodontitis.

**R133**

P.O. Sousa1, M.C. Ferreira1,2, A.D. Loguercio2 & C.N. Carvalho3,∗

1School of Dentistry, Ceuma University, São Luís & 2School of Dentistry, UEPG, Ponta Grossa, Brazil

Post-operative pain after instrumentation with different kinematics in root canal preparation: a randomized clinical trial

**Aim** To compare the absolute risk and post-operative pain intensity after root canal instrumentation in molars of patients, with ProTaper Next (Dentsply Sirona, Switzerland) or Reciproc (VDW, Germany) for a period of 7 days.

**Methodology** A randomized clinical trial was conducted with sixty-two patients with indication for root canal treatment in molars. The teeth were randomly allocated to one of the instrumentation techniques. Root canal instrumentation was performed in accordance with the manufacturers’ instructions. At the end of the first session the patient received a form with the NRS-10 and VAS scales for evaluation the post-operative pain levels (6 h, 12 h, 24 h, 2nd to 7th days), and devices for evaluating pain perceived on vertical percussion. In addition, the consumption of analgesics was evaluated. In the second session, seven days after the first, the root canals were filled. Intragroup (ProTaper and
Reciproc) analysis of the post-operative pain intensity in the different time intervals of evaluation was performed ($p < 0.05$), followed by evaluation between each time interval. The post-operative pain intensity was compared between the groups for the post-preparation time intervals ($p < 0.05$). In each time interval of evaluation, the absolute frequencies of the presence and absence of pain between the instrumentation techniques were also compared, as well as the absolute risk and intervals of confidence.

**Results** The post-operative pain results were analyzed in 58 participants (35 women and 27 men), 4 patients were lost to follow-up. The mean age of the patients evaluated was 30.7 years. No significant difference was observed between the systems for all the variables observed in all the time intervals analyzed ($p > 0.05$). The peak post-operative pain was observed in the first 24 h, with significant reduction from the 4th day after instrumentation for the two techniques evaluated. There was no significant difference in pain after vertical percussion and the consumption of analgesics ($p > 0.05$).

**Conclusions** The ProTaper Next and Reciproc systems caused the same level of post-operative pain, and presented the same absolute risk of generating pain.

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**R134**

C. Gur-Ilgen¹, D. Helvaciglu-Yigit²,³ & M. Ozcan²

¹Department of Endodontics, Faculty of Dentistry, Kocaeli University, Kocaeli, Turkey & ²Department of Dental Biomaterials Unit, University of Zurich, Zurich, Switzerland

**Clinical and radiographic evaluation of direct pulp capping using three different materials: a randomized clinical study**

**Aim** This prospective randomized clinical study aimed to assess the outcomes of one visit direct pulp capping with two calcium silicate-based materials (Biodentine™, ProRoot® MTA) and calcium hydroxide (Dycal).

**Methodology** One hundred twenty permanent molars with deep carious lesions in healthy adults aged 18–47 years were treated by direct pulp capping with three different materials. Teeth were randomly assigned to three groups (n = 40) according to material used for capping, as follows: Group 1: Dycal; Group 2: Biodentine and Group 3: ProRoot® MTA. During treatment; degree of bleeding, exposure size and location, cavity design were recorded. Clinical and radiographic evaluations were performed at 1, 3, 6 and 12 months. Teeth with no response to pulp vitality tests and those exhibiting clinical or radiographic signs and symptoms were considered to be failures. Intragroup comparisons of the observed values were analyzed using Chi-square and Fisher’s Exact test.

**Results** Ninety four teeth (78.3%) were available for clinical and radiographic follow-up evaluation ranging from 1 to 12 months. After 12 months, the overall success rate of direct pulp capping was 80.9%. The success rates of ProRoot MTA, Biodentine and calcium hydroxide groups were 82.9%, 82.4% and 76% respectively. Findings were non-significant ($p > 0.05$) between Biodentine and MTA groups. However calcium silicate-based materials were more successful than calcium hydroxide ($p < 0.05$). Age of the patients, gender, teeth location, degree of bleeding, the diameter of pulp exposure and location had no bearing on the success rate.

**Conclusions** Use of both calcium silicate-based materials for direct pulp capping were associated with better success rates than calcium hydroxide.

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difference ($p = 0.52$). With pain control during both access preparation and extirpation, percentage of success was lower, with $38.5\%$ ($n = 30/78$) in the articaine group versus $29.5\%$ ($n = 23/78$) in the lidocaine group, again with no significant difference ($p = 0.23$).

**Conclusions** Buccal infiltration with articaine can be an alternative to inferior alveolar nerve block in emergency access cavity preparation.

**EPIDEMIOLOGY**

**R137**

D. Donnermeyer$^{1, *}$, E. Schäfer$^2$ & S. Bürklein$^2$

$^1$Department of Periodontology and Operative Dentistry & $^2$Central Interdisciplinary Ambulance in the School of Dentistry, University of Münster, Münster, Germany

**Prevalence of apical periodontitis in a German population: a CBCT analysis**

**Aim** To determine the frequency and quality of root fillings (RCF) and the occurrence of apical periodontitis (AP).

**Methodology** Five hundred randomly selected full-size CBCT-images (Planmeca ProMax3D, FOV $= 8 \times 8$ cm, voxel size $\leq 200 \mu m$) were obtained from German patients ($40.6\% = \text{male}; 59.4\% = \text{female}; \text{age} = 50.21 \text{years}$). A total of 8254 teeth were examined for pathosis (AP, widened periodontal ligament) by analysing the multiplanar reconstruction of each tooth. The relationship between AP and existing RCF and the quality of RCF (homogeneity, length, extrusion of filling material) was documented. Frequency and correlations between the left and right side and gender distribution were analysed statistically using the Chi-square test.

**Results** The overall prevalence of AP was $3.8\%$, whereas for teeth with RCF it was $41.2\%$. Patients had approximately 1.4 root filled teeth ($8.2\%$ of all teeth). Root fillings terminated in $31\%$ more than 2 mm short of the radiological apex. $9.1\%$ of all teeth were associated with extruded root filling material. Additionally, $5.3\%$ of the teeth had untreated root canals. Regarding the right and left side as well as the gender of the patients no significant differences occurred ($p > 0.05$). In $37.1\%$ of teeth associated with AP the RCF terminated more than 2 mm short of the apex, but only $5.7\%$ of teeth were associated with extruded filling material. Further reasons were non-filled canals ($5\%$) followed by inhomogeneity of the RCF ($2.5\%$).

**Conclusions** About $41\%$ of root filled teeth were associated with AP and in more than one third of these teeth the RCF terminated more than 2 mm short of the apex.

**R138**

S. Devroey, R. De Moor, F. Calberson, D. Linden$^*$ & M. Meire

Department of Restorative Dentistry & Endodontology, Ghent University, Ghent, Belgium

**Cleaning of sealer-contaminated pulp chambers by dentists practitioners: results from a cross-sectional digital survey in Belgium**

**Aim** To assess the protocols for sealer removal from pulp chambers adopted by dental practitioners in Belgium.

**Methodology** A digital questionnaire was distributed by email to active dental practitioners in Belgium through the national dental societies. The questionnaire consisted of twelve multiple-choice questions on the type of sealer they used, sealer removal protocols, ‘orifice sealing’ and restoration of the pulp chamber after root canal treatment. Data were imported in a database and subjected to descriptive statistics.

**Results** A total of 901 questionnaires were completed. The majority of the respondents ($85.2\%$) were general dental practitioners (GDPs), $9.1\%$ were endodontists. The most frequently used sealers were epoxy resin ($58.0\%$), followed by calcium hydroxide ($17.9\%$) and medication-containing sealers ($9.2\%$). $62.2\%$ of the respondents used more than 1 sealer. $9.8\%$ of the respondents rarely or never removed the sealer from the pulp chamber, $70\%$ always did. A cotton pellet ($67.4\%$), air/water syringe ($30.2\%$) and microbrush ($28.2\%$) were the most popular methods to remove sealer remnants. Alcohol ($36.6\%$) was mainly used as a solvent, followed by no product ($32.5\%$) and water ($20.9\%$).

**Conclusions** Epoxy resin sealers are the most widely used sealers in Belgium. $10\%$ of the practitioners rarely or never removed the sealer from the pulp chamber. Among the ones who do remove the sealer, the use of a cotton pellet with alcohol was the most popular method.

**R139**

M. Neukermans$^{1, *}$, M. De Bruyne$^1$, M. Meire$^1$, J.P. Siquet$^2$ & R. De Moor$^3$

$^1$Department of Restorative Dentistry and Endodontology, University of Ghent, Ghent & $^2$Belgian Association for Endodontology and Traumatology, BAET, Brussels, Belgium

**Endodontic practice and referral behaviour by Flemish and Walloon dentists**

**Aim** This study sought to expose similarities and differences in aspects of endodontic practice and referral behaviour between Flemish and Walloon dentists. Another goal was to obtain an overview of contemporary endodontic practice in Belgium.

**Methodology** A questionnaire was attached to both the monthly magazine of the French-speaking Dental Society as well as the Flemish Society of Dentistry, reaching respectively 2400 and 4500 active dentists. The questionnaire consisted of an epidemiological part and multiple choice questions on endodontic practice and referral need. Postal return of the questionnaire was free of charge. Data were imported in a database, merged and subjected to descriptive and analytical statistics. Endodontic practice was compared to contemporary quality guidelines.

**Results** The combined response rate was $21.75\%$ ($n = 1423$). Fifty-three percent of the respondents were men, $73.7\%$ were general dental practitioners (GDPs). $32.5\%$ of the respondents worked in a group practice. There were no significant differences between Flanders and Wallonia regarding the use of rubber dam, microscope, K-files, Hedström files and reamers nor for the use of sodium hypochlorite. Overall, the use of EDTA ($58\%$ never), magnification ($58\%$ never) and activation of irrigants (almost $60\%$ never) was limited. Approximately $56\%$ of the respondents never or seldom used rubber dam. Walloon dentists used NiTi, single cone or hybrid condensation technique significantly more frequently compared to their Flemish colleagues. On the other hand, EDTA and a cold lateral condensation technique were more frequently used by Flemish dentists. Generally, younger respondents used rubber dam. NiTi and sodium hypochlorite significantly more frequently compared to their older colleagues. When asked to rate the importance of secondary care, endodontics scored an average of 7.17 out of 10. This number, however, differed significantly between Wallonia ($7.95$) and Flanders ($6.63$). Persistent pain or symptoms, difficult tooth anatomy, open apices, root canal obstruction, retreatment and perforation were identified as important factors for referral by well over half.

**Conclusions** Endodontic practice by Walloon and Flemish dentists does not differ considerably, but was not always in agreement with quality guidelines. Generally, more recently graduated dentists adopted more frequently used techniques in endodontics.
dentists tend to use present-day materials and techniques more frequently. The perceived need for secondary endodontic care in Belgium is substantial.

R140
K. Papaefthymiou*, K. Gulabivala & Y.L. Ng
Unit of Endodontontology, Department of Restorative Dentistry, UCL Eastman Dental Institute, University College London, London, UK

Prevalence and characteristics of cracks in teeth undergoing root canal treatment: a clinical observational study

Aim To investigate the prevalence, characteristics, and factors associated with cracks in teeth undergoing root canal treatment in the Department of Endodontics, Eastman Dental Hospital.

Methodology Patients scheduled for root canal treatment or retreatment of posterior teeth at the Endodontic Department of the Eastman Dental Hospital, over a period of three months, were included. Clinicians (n = 29) recorded information for each designated tooth, including demographic, pain, occlusal and restorative characteristics, as well as soft tissue findings and pulpal periapical diagnoses of the tooth. The presence, number, direction and extent of cracks, as well as canal orifice involvement were recorded at three stages: (1) after removal of coronal restoration; (2) after completion of access cavity preparation; and (3) after completion of chemo-mechanical preparation, using four methods of inspection (visual, transillumination, microscope, and combination of transillumination/microscope). Data were analysed descriptively.

Results Of 122 teeth included, cracks were present in 27%. All cracks were identified at the restorability assessment stage, with no further detection of cracks at subsequent stages. Most cracks were identified using the microscope. Cracks were more prevalent in: mandibular (35%) than maxillary (23%) molars; teeth whose opposing teeth had attritive wear facets (46%) than those that did not (26%); and teeth with intra-coronal restorations (31%) than those with cuspal coverage restorations (14%). Most frequently encountered patterns were single (70%) compared to multiple cracks (30%), and directed mesio-distally (55%).

Conclusions Cracks were present in 27% of the posterior teeth undergoing root canal (re)treement. All the cracks could be identified as early as the restorability assessment stage and presented most often as single cracks directed mesio-distally.

R141
E. Bołtacz-Rzepkowska & K. Sopińska*
Department of Restorative Dentistry, Medical University of Lodz, Lodz, Poland

Periapical status among adult patients in the Lodz region of Poland

Aim To evaluate the prevalence of apical periodontitis among adult patients in the Lodz region of Poland.

Methodology The survey enrolled 760 patients who were referred to the Central Clinical Hospital Medical University of Lodz for the first time. The study group was a systematic attempt of the population adopted in 2016. A panoramic radiograph was taken for each patient using Orthoralix 9200 (Italy). The periapical status and the RCT prevalence were evaluated. AP was recorded when more than double widening of the periodontal ligament space on the lateral aspect of a tooth or the presence of a radiolucency connected with the apical part of the root in the periapical region were seen (Genc et al. 2008). A tooth was classified as endodontically treated when there was a radiopaque material in the root canal and/or the pulp chamber.

Results Each of 493 adults (64.9%) had at least one tooth with AP. No difference between the prevalence of AP and gender was found (p = 0.561). The AP prevalence increased with age (p < 0.005). Lesions occurred most often (85.7%) in patients over 60 years old, the least frequently (29.9%) in patients below 30 years old. AP was observed in 6.7% of teeth, significantly more often (p < 0.0005) in molars (13.3%) and premolars (7.3%) than in incisors (3.1%) and canines (3.1%). The AP prevalence was associated with the prevalence of RCT. AP was detected in 37.9% of RCT teeth and in 3.1% of teeth without RCT (p < 0.0005).

Conclusions The AP prevalence among the population of the Lodz region was high and increased with age. AP was observed more often in the maxilla than the mandible and in posterior teeth compared with anterior. More than one third of endodontically treated teeth had AP, which indicates unsatisfactory quality of RCT in the study population.

R142
WITHDRAWN.

R143
M. Amato*, A. Savic & R. Weiger
Department of Periodontology, Endodontology and Cariology, UZB University School of Dental Medicine, University of Basel, Basel, Switzerland

Endodontic treatment in Switzerland: a survey of current treatment protocols

Aim To collect information about current treatment protocols of endodontic therapy by Swiss dentists and to compare them to the ESE quality guidelines and former Swiss and foreign data surveys.

Methodology The questionnaire collected information on the socio-demographic distribution of participants: age, gender, clinical experience and specialties, practice location and university of graduation. Specific questions referred to the number of root canal treatments (RCT) per month, tooth types treated, techniques and materials used and hours accumulated in continuing education (CE). Data of specialists, endodontic minded (EM) and general practitioners were analysed.

Results A total of 506 surveys were collected (response rate: 33.2%). The questionnaires were divided into three groups. The general practitioner group (GP) with 414 participants consisted of 81.8% of all respondents. The EM group were respondents from the Swiss Endodontic Congress (SSE), accounting for 14.4% of all respondents. The third group consisted of endodontic specialists (ES), making up 3.8% of the respondents. The majority of the participants were male (59.9%), the mean age was between 40 and 59 years and the average clinical experience was more than 20 years. 98.4% of the participants perform RCT regularly. 14.1% of all participants never used rubber dam during RCT, 13.7% reported using a microscope and 63.9% of all respondents used loupes during treatment routinely. Rotating instruments (43.2%) were mainly used, followed by conventional hand-instruments (37.8%) and reciprocating instruments (19%). GP rarely activated irrigants and used lower concentrations of NaOCl compared to ES. Most of the respondents (68.5%) performed multi-visit RCTs. 57% of GP preferred lateral compaction as the root filling technique whereas 89.5% of ES performed vertical compaction.

Conclusions The majority of Swiss dentists follow the ESE quality guidelines. Specialists were more likely to use new techniques, such as warm vertical compaction and to use microscopes.
Changes in periapical status, quality of root canal fillings and estimated endodontic treatment need in an urban German population after 20 years

Aim To assess the periapical status of root filled teeth, quality of root fillings and the endodontic treatment need of an urban German population after 20 years in a repeated cross sectional study.

Methodology Clinical and radiographic data as well as the performed operative procedures of 353 patients coming to a dental surgery in 2013 were evaluated. The periapical index score (PAI) was applied to assess apical pathosis. Descriptive and regression analyses were performed and the endodontic treatment need was calculated. Results were compared with data from the year 1993, obtained under comparable conditions.

Results A total of 9269 teeth were examined (26.2 teeth per patient; 1993: 24.8). In 207 individuals at least one tooth had a root filling, a necrotic pulp or an irreversible pulpitis. A total of 337 teeth had a root filling (3.6%; 1993: 2.7%). Prevalence of apical periodontitis in root filled teeth was 34% (1993: 61%). Quality of root fillings was good in 117 cases (15%; 1993: 14%). Minimum endodontic treatment need was 1.6% (1993: 2.3%), including teeth with clinical symptoms. Including symptomless teeth with apical periodontitis and poor quality of root filling, the treatment need was 2.9% (1993: 3.7%). Regression analysis showed that quality of root filling was a significant factor for periapical health (p = 0.01, odds ratio 3.4x), as well as type of instrumentation. Teeth treated with rotary files had a significantly better outcome (p = 0.02, odds ratio 2.0x).

Conclusions Quality of root fillings and the periapical status improved over the last 20 years in Germany. However, there is still a need for improvement of the quality of root canal treatment. Rotary instrumentation and a good technical quality root filling were a reliable predictor for periapical health.

Prevalence of conservative and endodontic treatments in patients affected by oncologic diseases. A retrospective clinical study

Aim To determine the prevalence of conservative and endodontic treatments administered to a cohort of patients with several types of cancer, and whether the condition was associated with other diseases, and to the use of medications, in a referral dental institution over a 4-year period.

Methodology A retrospective analysis was conducted using the medical records of 144 oncologic patients requesting dental treatment from January 2011 to January 2016. Dental therapies performed were divided in conservative and endodontic treatments. The variables recorded for each patient were demographic data, medical history, use of medications, administration of antibiotics before (prophylaxis) or after (therapy) dental treatment. The data were entered into a Microsoft Excel spreadsheet, and analyzed using IBM SPSS package version 21.0 for Mac.

Results The patients age ranged from 26 to 88 years and the male to female ratio was 0.58:1. A total of 431 dental treatments were performed on oncologic patients and out of them 325 were conservative treatments performed in 70.2% of individuals and 106 were endodontic therapies performed in 29.8% of patients. Antibiotic prophylaxis was given to 27.7% of individuals, and antibiotic therapy was prescribed in 25% of treated cases. Breast cancer was the most common type of cancer in the overall group (51.4%) and among women (80.2%). In addition to oncological pathology all the patients included in this study were affected by one or more medical disorders. The prevalence of associated medical disorders increased with age, but was concentrated between the 4th and 5th decades. The most commonly associated systemic conditions were cardiovascular (21%) and rheumatic (21%) diseases. One hundred twenty-six patients were under medical prescriptions, mostly in the 5th–7th decades; 69.9% of the individuals received from 1 to 4 medications/day and among patients receiving more than 8 drugs/day the number of females was significantly higher (p < 0.05).

Conclusions There is an increasing need for conservative treatment in patients who have cancer which is often associated with other medical conditions, and use of several medications. Dental practitioners should receive training to provide the best care possible and prevent complications.
this tooth. The pain was generally of low intensity and long lasting. In most cases, the patient experienced recurrent or occasional pain, but one in five with painful teeth had continuous pain. Although apical radiolucency, swelling and sinus tract were more common with painful teeth, pain in root filled teeth still remains partly unexplained.

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R147
N.M. Dydyk*  
Department of Prosthetic Dentistry, Danylo Halutsky Lviv National Medical University, Lviv, Ukraine

Periapical, endodontic, and prosthetic status of teeth in an adult Ukrainian population

Aim To determine the prevalence of apical periodontitis, the presence of root fillings and crowned teeth in an adult Ukrainian population using analysis of orthopantomograms (OPGs) and periapical radiographs.

Methodology A total of 705 individuals were examined: 326 males and 379 females. The status of 17 755 teeth was analyzed: 8 216 in males and 9 539 in females. The presence of an apical periodontal lesion was evaluated using the Periapical Index. The status of each tooth was recorded as ‘not root filled’, ‘root filled’, ‘crowned’, and ‘non-crowned’. The data on teeth were dichotomized: ‘0’ – the absence of certain characteristic and ‘1’ – its presence. Obtained data were recorded and processed by specially designed computer database.

Results Root filled teeth were detected in 586 (83.1%) of the 705 patients examined. A total of 2 235 (12.6%) teeth had been endodontically treated. Teeth in the maxilla were more frequently root filled (64.1%) than teeth in the mandible (35.9%) (p < 0.001). Frequency of endodontic treatment increased with age: from 4.4% in the age group of 15 – 19 years to 21.5% in the age group 55 – 64 years. The most frequently root filled tooth (33.6%) was the mandibular first molar, followed by the maxillary first molar and maxillary second premolar – 25.5% and 22.9% respectively. In 448 of 705 adults (63.5%) AP was diagnosed. 1 240 teeth (7.0%) had radiographic signs of periapical radiolucency. Apical periodontitis was evident in 2.0% of all non-root filled teeth. 41.6% of root filled teeth (930 of 2 235) had periapical lesions (scores 3, 4, 5 of PAI). 50% of the population had crowned teeth. The highest prevalence of AP was observed in the group of endo-treated crowned teeth – 44.3%.

Conclusions The prevalence of AP and frequency of endodontically treated teeth in the Ukrainian population is comparable with findings in other countries.

R148
K. Chaini1,*, D. Kavoura1, I. Oikonomou1, E. Arvaniti1, M.K. Georgopoulou1 & K. Tsiklakis2  
1Departments of Endodontics & 2Oral Diagnosis and Radiology, Dental School, National and Kapodistrian University of Athens, Athens, Greece

Prevalence of pulp stones in a Greek population

Aim To describe the prevalence of pulp stones in a Greek population by examining full-mouth periapical radiographs and to examine possible associations between pulp stones and gender, age, tooth type, side, arch, and dental status.

Methodology A total of 416 records were randomly selected from patients referred to a private maxillofacial radiology practice for full mouth periapical radiographs from 2012 to 2015. A total of 10 938 teeth were examined: only permanent teeth with complete roots were analyzed. Teeth with crowns or bridges that prevented adequate vision of the pulp chamber were excluded. Full-mouth radiographs that lacked half or more molars, those that pulp was not visible at half or more molars, those that lacked half or more teeth or the pulp was not visible at half or more teeth were excluded. For each patient name, gender, age and number of teeth was recorded. For each tooth with pulp stones, its status was recorded. The radiographs were examined by two examiners, using a monitor, with magnification and contrast changing as needed. Examiner reliability was calculated via replicate observations derived from double determination of 100 samples. The Cohen’s Kappa was calculated 92% and the strength of agreement was considered to be ‘very good’.

Results The parameters of gender, type of tooth, dental arch, side, age and tooth status (caries, restoration, periodontitis) were evaluated. Only type of tooth and tooth status were found to be significant (p < 0.05). The prevalence of pulp stones in this Greek population was 64.1%.

Conclusions Patient’s gender and age, dental arch and side did not affect the prevalence of pulp stones, in contrast with type of tooth and tooth status. Prevalence of pulp stones was high (64.1%) as observed on periapical radiographs.

R149
R. Higgins1,*, E.K. Zuk1, M. MacLuskey2, D.N.J. Ricketts1 & W.P. Saunders1  
1Department of Restorative Dentistry, Glasgow Dental Hospital and School, Glasgow & 2Department of Oral Surgery, Dundee Dental Hospital and School, Dundee, UK

Diabetes mellitus and the prevalence of apical periodontitis in a Scottish subpopulation

Aim To assess the prevalence of apical periodontitis in patients with diabetes mellitus and evaluate the relationship between apical periodontitis and glycosylated haemoglobin.

Methodology In a cross-sectional case-controlled study, the medical and dental records were examined for 503 patients with diabetes mellitus and 503 control patients. Dental panoramic radiographs were assessed for apical periodontitis using a modified periapical index score. Number of teeth, alveolar bone levels, and number of root fillings were also assessed from these radiographs. In the diabetic group, glycosylated haemoglobin (HbA1c) levels were reviewed and analysed with the data obtained from the radiographs. Statistical analyses were undertaken using Cohen’s k test, analysis of variance, independent t-tests (95% CI), and multiple regression.

Results In the diabetic group the mean number of teeth with apical periodontitis was 1.12 per patient, compared with 0.87 in the control group (p = 0.001). In diabetic patients with HbA1c levels ≥9%, the mean number of teeth with apical periodontitis was 1.8, compared with 1.0 in diabetic patients with HbA1c levels <9% (p = 0.002). The mean number of teeth per patient was 18.57 in the diabetic group and 20.51 in the control group (p = 0.003).

Conclusions On average, patients with diabetes mellitus have fewer teeth, but a greater proportion of teeth with apical periodontitis, when compared with non-diabetic patients. As a group, diabetic patients with high levels of glycosylated haemoglobin (HbA1c ≥9%) have a greater number of teeth with apical periodontitis than those with lower levels.
The complexity of non-surgical endodontic treatment in general dental practice in the UK: a prevalence study

Aim To assess the prevalence of non-surgical endodontic case complexity in general dental practice.

Methodology Thirty fully qualified dentists working within general dental practice across the UK were recruited. Each dentist assessed 10–15 consecutive potential endodontic cases as encountered in their day-to-day clinical practice. The data was collected using an online endodontic case assessment tool (E-CAT) recently developed at The University of Liverpool. The tool allowed the data to be recorded into a secure database. Information on tooth-related factors, systemic factors, oral diagnosis and patient-related factors was recorded. Three levels of complexity were defined for the analysis: class 1 (uncomplicated), class 2 (moderately complicated) and class 3 (highly complicated). The data was analysed to express period prevalence with a 95% confidence interval using SPSS 22 statistical software.

Results Overall, 385 endodontic cases were evaluated. The distribution of complexity over classes 1, 2 and 3 was 37.1%, 32.6% and 30.3% respectively. Root canal retreatments formed 23.1% of the cases encountered. The majority of the cases (66.7%) appeared to have <15 degree root curvature, 29.2% had 15–40 degree curvature and only 4.1% had >40° curvature. Teeth with existing extra-coronal restorations formed 19.8% of the cases encountered. Radiographically, visible and moderately reduced canal space was reported in 76.2% of the cases, while 20.5% had severely reduced canal space and only 3.3% were perceived to have invisible canal space. History of trauma was encountered in 9.2% of the evaluated cases.

Conclusions Relatively equal distribution across the three level of endodontic treatment complexity was observed. The prevalence of severe root curvature and severely reduced canal space was relatively low. Previously root filled teeth and teeth with pre-existing extra-coronal restorations formed a considerable proportion of the endodontic treatment encountered in general dental practice.

Acknowledgements The development of the E-CAT is partly funded by a research grant awarded by the European Society of Endodontology. Favourable ethical approval was granted by the North East research ethics committee. REC reference: 15/NE/0372.

HISTOPATHOLOGY OF PERIAPICAL LESIONS

Effect of chronic alcohol intake on induced periapical lesions

Aim To assess the effect of chronic alcohol consumption on induced periapical lesion in rats, through histologic and histomorphometric analysis, as well as radiographic density of the periapical region.

Methodology Thirty-two male rats were allocated into four groups (n = 8). G1 no alcohol intake (A.I.) no periapical lesion (P.L.); G2 with A.I. no P.L.; G3 no A.I. with P.L.; G4 with A.I. with P.L. A 20% alcohol solution (Groups 2, 4) or drinking water (Groups 1 and 3) was administered for sixty days. On the thirtieth day, periapical lesions were induced (Groups 3, 4) through the pulpal exposure of the mandibular left first molar with a diamond bur, under general anaesthesia. At the end, the animals were euthanized with an anaesthetic solution overdose, had their jaw removed and stored in a 10% formalin solution. Then, specimens were subjected to digital radiographic analysis conjointly with an aluminum step wedge to compare the density. Finally, specimens were decalcified with EDTA and proceeded the protocol for paraffin inclusion, 5 micrometers thick sections were cut and stained with haematoxylin and eosin for histological and histometric analysis. Multiple comparisons of results were performed by (ANOVA) followed by Tukey test. For non-parametric data, the Kruskal-Wallis test followed by Dunn’s test was used. The level of significance was 5%.

Results The histopathologic analysis revealed no inflammatory infiltrate and mild infiltrate on G1 and G2 respectively. G3 and G4 had an intense polymorphonuclear inflammatory infiltrate that reached the periapical alveolar bone. There were no significant differences associated with alcohol intake on the periapical lesion groups (G3, G4) p > 0.05. The histometric analysis revealed larger areas of bone resorption in groups with induced periapical lesion compared to the others with no lesion induced. However, alcohol consumption did not have a significant effect on lesion size (p > 0.05). Digital X-ray analysis also did not show a significant difference between groups regarding to alcohol intake (p > 0.05), but only regarding the presence or absence periapical lesions (p < 0.05).

Conclusions Although the groups taking alcohol had large numbers of inflammatory cells and resorption lacunae in almost the whole extent of the lesion, the magnitude of the inflammatory reaction and bone loss were similar to control groups.

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m-RNA expression of TNF-α levels In Type 2 diabetic and non-diabetic individuals with apical periodontitis

Aim To compare the gene expression levels of tumour necrosis factor-alpha (TNF-α) in teeth with apical periodontitis of diabetic and non-diabetic individuals.

Methodology The study was performed after taking ethical approval and a consent form from the patients. Fifteen type 2 diabetic patients with HgA1c level between 6 and 7, having chronic apical periodontitis were selected for the test group. Fifteen non-diabetic and systemically healthy patients were selected as the control group. Clinical samples were taken from teeth with periapical lesions with a radiographic diameter greater than 2 mm. After chemo-mechanical preparation and drying of canals, three paper points were introduced into the root canal, passing passively through the root apex 2 mm into the periapical tissues for 1 min and collected in TR101 reagent. The mRNA expression levels of TNF-α were determined by Real-Time PCR. Following the isolation of total RNA from each sample, cDNA was synthesized, and PCR reaction was performed. GAPDH was used as an
internal control. The Student’s t-test was used to compare the differences between the groups ($p < 0.05$).

**Results** Gene expression of TNF-α was significantly higher in diabetic patients than the control group ($p < 0.05$). The mean value of mRNA expression/GAPDH in diabetic patients was $1.606 \pm 0.048$, whereas it was $1.08 \pm 0.05$ in non-diabetics.

**Conclusions** The pro-inflammatory cytokine TNF-α was expressed significantly higher in periapical lesions of diabetic patients. It is one of the responsible factors for delayed bone healing. The treatment strategy can be changed according to these findings following supporting studies. Given the data of the present study novel endodontic treatment approaches could be developed for diabetic patients.

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**MODERN AND NEW TECHNOLOGY**

**R153**

N. Montis1,* C. Fattuoni2, L. Barberini1, A. Noto1, V. Fanos3 & E. Cotti1

1 Departments of Surgical Sciences, 2 Chemical and Geological Sciences, 3 Medical Sciences and Public Health & 4 Surgical Sciences, University of Cagliari, Cagliari, Italy

**Metabolomics fingerprint of chronic apical periodontitis with sinus tract: a pilot study**

**Aim** To verify the possibility of identifying the metabolic fingerprint of chronic apical periodontitis (CAP) through the metabolomics analysis of saliva.

**Methodology** Twenty-one patients were selected. Inclusion criteria were: age: 30–60 years, both genders, good systemic condition and periodontal health, presence of at least 20 teeth, absence of pharmacological therapy. Twelve patients affected by CAP with a sinus tract constituted the study group (Group 1) and 9 patients without clinical and radiographic signs of CAP were the controls (Group 2). Samples from saliva (2 mL) were collected from each subject and immediately frozen at −80°C. Metabolomic profiling was obtained using gas chromatography/mass spectrometry (GC/MS). The statistical approach was conducted by partial least square discriminant analysis (PLS-DA) to compare the two groups.

**Results** The PLS-DA model indicated a clear separation between the groups. Group 1 exhibited high concentrations of valero lactate, putrescine, proline, glycolic acid, lysine, aminopentane, glycine, phosphate and phenylalanine, while it was associated with low concentrations of glucose, sorbitol, maltose, propanediol, galactose and ethanolamine. The differences between the groups with regards to these metabolites were significant.

**Conclusions** The type of metabolites from saliva in the presence of CAP were closely related to bacterial catabolism and tissue necrosis, and this outcome may be associated to the presence of a sinus tract. These preliminary results indicate that metabolomics can identify the presence of CAP, based on saliva, and that there is the need for future studies with more samples.

**R154**

J. Grąęcka-Mańkowska1, A. Kowalczyk2, B. Zarzycka2 & H. Pawlicka1,*

1 Departments of Endodontics & 2 Microbiology and Laboratory Medical Immunology, Medical University of Lodz, Lodz, Poland

**Laser-assisted endodontics: bactericidal efficacy of various laser operation modes**

**Aim** To assess the antibacterial action of diode laser irradiation (Continuous Wave – 1250 mW, 1500 mW) and photo-activated disinfection (PAD) on Enterococcus faecalis in an infected tooth model.

**Methodology** Forty freshly extracted bovine teeth with single canals were prepared to apical size 45, autoclaved and contaminated with **E. faecalis**. After incubation for 48 h the specimens were randomly divided into three treatment groups and one control group ($n = 10$): Group 1 ($n = 10$) was treated with a high-power diode laser emitting at a wavelength of 980 nm and set at a power of 1250 mW, operated in CW mode; Group 2 ($n = 10$) was irradiated with the same laser device set at a power of 1500 mW (CW); Group 3 ($n = 10$) was subjected to photo-activated disinfection (PAD). Toluidine Blue Ortho (TBO) as a photosensitizing agent, was introduced into each canal for 60 s. After this pre-irradiation time, irradiation was performed with a diode laser emitting at a wavelength of 635 nm and set at a power of 100 mW. Laser light was applied through a single-use endodontic dif- fusor, which was gently moved up and down the canal during the irradiation time of 30 s. Group 4 ($n = 10$) was rinsed with saline solution and served as a positive control. After treatment, the dentine samples were collected and the CFUs/mg were counted. Values were subjected to the Kruskal-Wallis U-test, for group comparisons to the Mann-Whitney U-test.

**Results** The differences in the mean number of the colony forming units (CFU/mg) between all the groups analyzed were significant ($p < 0.001$). The greatest number of microorganisms was observed in the control group ($52.7 \times 10^8$ CFU/mg). Both diode laser applications are suitable for root canal disinfection, but do not eradicate all bacteria.

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**R155**

A. Arias1,* J.C. Macorra2 & O.A. Peter2

1 Conservative Dentistry, Complutense University, Madrid, Spain & 2 Department of Endodontics, University of the Pacific, Arbor A. Dugoni School of Dentistry, San Francisco, USA

**The effect of gamma irradiation and sterilization on body temperature fatigue behaviour of contemporary martensitic rotary instruments**

**Aim** To compare body temperature cyclic fatigue (CF) resistance of contemporary heat-treated NiTi rotary instruments after been submitted to γ-IR and/or sterilization.

**Methodology** Hylex EDM (HF) (size 25, .08 taper, manufactured by electrical discharge machining) and TRUShaper (TS) (size 25, .06 taper; manufactured by grinding and shape setting) instruments were selected. A total of 160 instruments (80HF and 80TS) were divided into eight groups ($n = 20$) each depending on the
sterilization process: TS-γIR and HF-γIR / TS-γIR-AC and HF-γIR-AC (γ-irradiated and autoclaved)/ TS- no-γIR and HF- no-γIR (non γ-irradiated)/ TS- no-γIR-AC and HF- no-γIR-AC (non γ-irradiated and autoclaved). CF resistance was tested in a water bath at body temperature (37°C ± 0.5°C). Instruments were rotated until fracture occurred in a simulated canal (angle 60°; radius 3 mm; centre of the curvature 5 mm from the tip) with the motor controlled by an electric circuit. Mean half-life, beta and eta parameters were determined and compared with Weibull analysis.

Results Both TS-no-γIR (mean-life = 101.5s, 95 CI% 91.7–112.3) and TS-no-γIR-AC (mean-life = 100.7s, 95 CI% 92.1–110.2) lasted significantly longer than TS- γIR (mean-life = 83.2s, 95 CI% 76–91.1) and TS-γIR-AC (mean-life = 78s, 95 CI% 69.9–86.9). Further autoclave sterilization of both TS-no-γIR and TS-γIR did not affect fatigue resistance significantly. A different pattern was observed with HF. γ-irradiation did not affect HF fatigue behaviour (HF- γ mean-life = 717.9s, 95 CI% 636.8–809.3; HF- no-γIR mean-life = 678.8s, 95 CI% 595.1–744.2); further sterilization significantly decreased fatigue resistance for both γ-irradiated (mean-life = 524.1s, 95 CI% 476.1–576.8) and non γ-irradiated instruments (mean-life = 570.6, 95 CI% 512.3–635.5). Overall, HF instruments lasted significantly longer than TS.

Conclusions γ-irradiation and sterilization affected differently the fatigue life span of contemporary martensitic rotary instrument manufactured with different methods.

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OUTCOME STUDIES

R156
A. Tifooni*, S. Patel & F. Mannocci
Department of Endodontics, King’s College London, London, UK

The Dental Practicality Index: reliability and validity in assessing the outcome of endodontically retreated teeth

Aim To assess the reliability and validity of the Dental Practicality Index in relation to the outcome of root canal re-treated teeth.

Methodology One hundred and thirty-seven posterior teeth with symptoms and/or signs of endodontic post treatment disease requiring root canal retreatment were included. Clinical and radiographic examinations including digital periapical and cone beam computed tomography were obtained pre-treatment and 1 year post-operatively. Root canal retreatment and cuspal coverage restorations were performed by Endodontic postgraduate students using a standardised technique. A calibrated and trained assessor assessed all cases using the DPI. Each of the restorative aspects; structural integrity, endodontic status, periodontal health as well as the context of treatment were assessed and giving an overall DPI score. A one year outcome of those teeth was assessed by clinical and radiographic examinations. The DPI score was then correlated to the outcome of treatment.

Results Teeth with a DPI score of 4 or above had 32% failures, whereas teeth with a DPI score of less than 4 had 10% failures.

Conclusions The Dental Practicality Index is a potentially promising tool to aid decision making on whether it is in the patient’s best interest to consider re-root canal treatment of a compromised tooth or not.

R157
M.C. She1, N.N. Chen1 & G.S.P. Cheung2
1Department of Restorative Dentistry, National Dental Centre Singapore, Singapore & 2Faculty of Dentistry, University of Hong Kong, Hong Kong

Clinical outcomes of teeth treated endodontically through pre-existing crowns

Aim To determine the incidence and treatment outcome using clinical and radiographic assessment as well as evaluate possible prognostic factors affecting treatment outcome when root canal treatment was provided through existing crowns.

Methodology The study population comprised of patients who had primary root canal treatment performed through pre-existing crowns from January 2011 to November 2014 at the National Dental Centre, Singapore. Teeth diagnosed with irreversible pulps or pulp necrosis, with or without apical periodontitis were included in the sample. After excluding teeth with incomplete data, or had extraction or re-treatment performed, as well as including only one tooth per patient, 197 patients were eligible for the study. One hundred and seventy-one patients (68.5%) recall rate) were examined clinically and radiographically for condition of the crown and periapical status. Periapical healing status was determined by two examiners who reviewed and compared immediate post-obturation and follow-up radiographs side by side. Treatment outcome was dichotomized into ‘favourable’ (healed/ healing in progress, absence of clinical signs and symptoms) or ‘unfavourable’ (uncertain/post-treatment disease, and/or clinical signs and symptoms). The Chi square test was performed to find significant associations (p < 0.05) between several covariates and the periapical healing status of the root filled teeth. Univariate and multivariate logistic regression analyses were performed on prognostic factors that were significant.

Results The incidence of such treatment modality was low, at 3.1% in 47 months. Favourable radiographic outcome for the sample of 171 teeth was 76.6% and 80.5% for per-tooth and per-root evaluation respectively. Including 16 teeth with treatment failure (re-treated or extracted) as having an unfavourable outcome, favourable outcome based on clinical and radiographic assessment was 61.5% for per-tooth evaluation based on a sample of 187 teeth. Multivariate logistic regression analysis revealed that maxillary teeth had a lower chance (OR = 0.46; 95% CI: 0.21–1.00; p < 0.05) of a favourable outcome as compared to mandibular teeth. Teeth with adequate length of root filling were most likely to exhibit favourable periapical healing outcome (OR = 3.2; 95% CI: 1.50–6.84; p < 0.001).

Conclusions Within the limitations of this study, favourable radiographic treatment outcome for this treatment modality was similar to other outcome studies. Root canal treatment through prosthetic treatment with intact margins and maintaining them as final restorations is a viable treatment option.

R158
L. Roskamp1, P.C. Trevilatto2, C.M. Souza2, E. Carneiro1, U.X. Silva Neto1, L.F. Fariniuk1 & V.P.D. Westphalen1
1School of Health and Bioscience & 2School of Life Sciences, Pontifícia Universidade Católica do Paraná, Curitiba, Brazil

Association of IL6 gene polymorphisms with the outcome of replanted teeth and analysis of the clinical aspects involved

Aim To investigate the association of clinical variables and polymorphisms in IL6 gene, with the outcome of avulsed and replanted teeth after 1-year follow-up.
Methodology Ninety-four patients who suffered avulsion and had their teeth replanted and treated endodontically were selected. Periapical radiographs were taken after replantation and after 1 year. To determine the IL6 gene polymorphisms, the DNA of the oral mucosa cells were collected, and the analysis performed by Real Time-PCR. Univariate and multivariate statistical evaluation were used to verify the association of clinical and genetic variables and the absence or presence of external root resorption in the replanted teeth (p < 0.05).

Results An association of age, extra-alveolar time and storage medium with root resorption was observed in a univariate analysis. An extra-alveolar time longer than one hour and the rs2069843 polymorphism of the IL6 gene were significantly associated with external root resorption in multivariate analysis.

Conclusions An extra-oral time longer than one hour, and the rs2069843 in IL6 gene were associated with susceptibility for the development of external root resorption in avulsed and replanted teeth one year after trauma.

R159
E. Wigsten*, P. Jonasson & T. Kvist
Department of Endodontology, Institute of Odontology, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

Indications and one year follow-up of root canal treatment in a Swedish public dental health setting. Results from a prospective cohort-study

Aim To study the indications and the one-year outcome of root canal treatment performed in the Swedish public dental service.

Methodology The indications for initiating root canal treatment were recorded consecutively by general dental practitioners during a period of eight weeks at eight different clinics in the public dental service, county of Västra Götaland, Sweden. The following variables were registered: gender, age, tooth group and any symptoms present. The follow-up was made by access to the patient records. The 1-year outcome was evaluated as either not completed or completed treatment with a permanent root filling or extracted tooth. The Chi-square test was used for statistical analyses.

Results One hundred and nine teeth in 107 patients (49 (46%) men and 58 (54%) women) were included. The mean age was 49 years. Molars were the most commonly treated tooth (48%) followed by 38 (35%) premolars. The root canal treatment was initiated because of symptoms in 64 teeth (62%) and in 100 teeth (92%) it was a primary root canal treatment. At the 1-year control 72 teeth (66%) were completed with a permanent root filling. Of the remaining teeth 19 (17%) were not completed and 18 (17%) had been extracted. Molars were completed to a significantly less degree (48%) compared to premolars (82%) and incisors and canines (84%) (p = 0.001). There was no significant difference in respect to gender, age or symptoms.

Conclusions The findings in this prospective study suggest that in Swedish public dental health root canal treatment is frequently initiated because of symptoms from previously non-root filled teeth. In addition, root canal treatment in molars was associated with particular treatment difficulties.

R160
S. Huang1*, N.N. Chen1, V.S. Yu2 & J.N. Lui1
1Restorative Dentistry, National Dental Centre of Singapore & 2Faculty of Dentistry, National University of Singapore, Singapore

Long-term outcome and survival of teeth after endodontic microsurgery: a 5–9 year follow-up study

Aim To evaluate retrospectively the 5–9-year success and survival rates of teeth that underwent endodontic microsurgery (EMS), the various predictors for healing, and patient satisfaction levels at long-term recall. Short and long-term outcomes were also compared.

Methodology Patients who had EMS from 2007 to 2010 were invited to participate. Eligible patients were recalled for a clinical and radiographic examination. A satisfaction survey was administered at recall, and patients returned the same survey 2 weeks later by post. A blinded radiographic evaluation was carried out by two independent, calibrated examiners. Outcome was determined based on clinical and radiographic findings and associated with pre-, intra- and post-operative variables. Survival status and reasons for extraction of all teeth were determined. Multivariate Cox regression, Kaplan-Meier and Pearson’s correlation analyses were used to identify prognostic factors, survival rate and test-retest reliability respectively.

Results Of 151 patients invited to participate, there were 25 discontinuers. 40 dropouts, and 76 participants. The recall rate was 66.1% after excluding discontinuers. Thirty teeth were extracted: 6 due to surgical failure, 20 for unrelated reasons, 4 for unknown reasons. The 6 teeth that were extracted due to surgical failure were included in the computation of healed rates. Outcomes were categorized as healed (77.6%) and not healed (22.4%). Multivariate analysis revealed that adjusted hazard ratio for failure was 4.27 × (95% CI: 1.07,17.01; p = 0.039) higher for teeth treated with IRM than with MTA. The 9-year survival rate was 91.8%. Patients reported high levels of satisfaction with Pearson’s correlation scores ranging from 0.666 to 0.810 (p < 0.001). Teeth classified as ‘healed’ in the short-term, mostly remained healed at the long-term, whereas those with ‘uncertain healing’ at short-term had varied outcomes in the long-term.

Conclusions The healed rate was 77.6%, functional retention rate was 87.1%, and 9-year survival rate was 91.8%. This study suggests that MTA was associated with a higher healed rate than IRM. However, this should be interpreted with caution as root-end filling material was not randomly allocated at the time of treatment. Patients reported high levels of satisfaction with EMS at the long-term recall. The satisfaction survey used showed high test-retest reliability, but should be validated in future studies.

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R161
C. Prati, C. Pirani, F. Zamparini*, MR Gatto & M.G. Gandolfi
School of Dentistry, Endodontic Clinical Section, Master in Clinical Endodontology, Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italy

A 20-year clinical study: functionality and success of root canal treatments

Aim This prospective cohort study aimed to evaluate endodontically-treated teeth checked periodically for at least 20 years.

Methodology A large number of patients were managed between January 1989 and January 1996 for various endodontic reasons (deep caries/pulpitis, periapical lesion, prosthetic reason, retreatment for re-exacerbate lesion) and followed-up for at
least 20 years. Teeth were endodontically treated by a single specialist and restored definitively by four different operators. During the routine hygiene recall-program (approx. every 2 years for 20 years), teeth were clinically and radiographically blindly assessed to detect clinical symptoms, presence of apical radiolucency (PAI), quality of root filling, presence of apical extrusion and quality of coronal restoration (coronal status). A Chi-square-test was performed to detect statistically significant pre-, intra- and post-operative factors associated with root canal treatment disease (p < 0.05). Multilevel analysis was performed to explore factors associated to endodontic failure/disease development. A Kaplan Meier analysis was used to examine tooth survival.

Results At 20-year recall, 196 teeth in 70 patients (39M, 31F, mean age 37.7 ± 8 years) were analyzed. 153 (78.1%) were functional and 41 (20.9%) had been extracted. Out of these, 39 were lost for non-endodontic reasons (tooth fracture, periodontal diseases, deep carious/not-restorable tooth) and 2 as a result of the sequelae of endodontic pathology. 128 teeth (65.3%) did not have endodontic disease, were not retreated (PAI ≤ 2) and were defined as healthy. Twenty-seven teeth (11.7%) developed an endodontic lesion, or showed a stable/unaltered periapical radiolucency (PAI ≥ 3) during the 20 year follow-up; these teeth were defined as endodontically diseased. Pre-operative disease, initial PAI and coronal status significantly influenced (p < 0.05) the final outcome/success rate.

Conclusions In the long-term, approximately 80% of treated teeth remained functional. Teeth were more frequently extracted for non-endodontic reasons rather than for endodontic failure/sequelae. Endodontically treated teeth that received and maintained an adequate hygiene therapy and coronal restoration were associated with long-term success.

R162
C. Lindström* & T. Kvist
Department of Endodontology, Institute of Odontology at The Sahlgrenska Academy, Gothenburg, Sweden

Incidence of apical periodontitis in root filled teeth following intra-radicular post placement

Aim To explore the incidence of apical periodontitis, following placement of intra-radicular posts, and its dependence on the quality of previous root filling.

Methodology Two observers evaluated the patient records and intra-oral radiographs from the public dental health organisation of Kalmar county, Sweden. The records consisted of all teeth receiving an intra-radicular post in 2009. Included in this study were teeth that had an existing root filling, older than one year, and where no sign of apical periodontitis was present. Root filling quality was evaluated as good or poor. Each patient was allowed to contribute with one tooth only. Available information in patient records and from follow-up radiographs were analysed for signs of occurrence of apical periodontitis over a 6-year period. Statistical analyses were completed using Fisher’s exact test.

Results Intra-radicular posts had been placed in 534 teeth; 175 teeth in 94 women and 81 men fulfilled the inclusion criteria. The mean age at baseline (2009) was 58 years. Twenty-nine teeth (40%) had a good quality root filling and 44 teeth (60%) had a poor quality root filling. During the 6-year period, 73 teeth (42%) had at least one follow-up radiograph. Twenty-one teeth (29%) developed signs of apical periodontitis. For teeth with a good quality root filling, the incidence was 6 teeth (21%). Whilst for teeth with poor root filling quality the corresponding incidence was 15 teeth (34%). The difference in root filling quality was not significant (p = 0.21).

Conclusions Findings from this study suggest that placement of intra-radicular posts in previously root filled and apically healthy teeth may be a risk factor for the development of apical periodontitis. Further studies, including more patients and a control group, are needed to provide supporting evidence.

R163
S. Mezzina1,*, E. Schirru1, O. Ottonello1, M. Mura1, P. Usai2, V. Piras1, M. Susnik1, F. Ideo1 & E. Cotti1
1Department of Conservative Dentistry and Endodontics & 2Gastroenterology Unit, Università Degli Studi di Cagliari, Cagliari, Italy

Healing of apical periodontitis in patients with autoimmune diseases under anti-TNFalpha medications

Aim To evaluate the healing response following root canal treatment (RCT) of apical periodontitis (AP) in patients with inflammatory bowel diseases (IBDs) treated with biologic medications (BMs).

Methodology Nineteen patients (22 teeth with AP) affected by IBDs and under treatment with BMs were included (IBD study group). Fourteen patients (22 teeth with AP) without systemic disease and not under any medication represented the control group. Following primary and secondary RCTs, healing of AP was followed-up clinically and radiographically every three months until the end of the study (24 months). Two calibrated endodontists evaluated the extension of AP using the periapical index score (PAI). Data were recorded and statistical analysis using student t-test, Chi-square, Wilcoxon signed-rank test and ANOVA was conducted (p-value < 0.05 was considered statistically significant).

Results At time zero, both groups had similar PAI values (2.8 ± 0.8 IBD: 3.1 ± 0.8 Control). Twenty-two (IBD group) and 18 teeth (control group) were diagnosed as healed at the 24-month follow-up, with an average PAI through the follow-up period lower for the IBD group (IBD 1.56 ± 0.48; Controls 2.01 ± 0.79) (p < 0.05). IBD group had a 18% higher healing risk than Controls (p < 0.05). The beginning of healing was noticeable earlier in the IBD group compared to Controls (3 and 6 months, respectively). Similarly, complete healing occurred earlier in the IBD group (6 months; Controls 10.5). Analysis of variance revealed PAI values of the IBD group 0.47 less than the Control (β = 0.47, p < 0.05, 95% CI [−0.62, −0.32], and 0.07 smaller (β = −0.07, p-value < 0.0001, 95% CI [−0.078, −0.059]) during the follow-up period.

Conclusions Healing of AP was faster in IBD patients under biologic medication. This study suggests a possible positive association between BMs and healing of AP after adequate root canal treatment. Further studies with a larger cohort are needed to confirm these findings.
TRAUMA/REGENERATION

R164
D. Tsiafas1,*, K. Kodonas2, C. Gogos1, C. Tziafas1 & S. Papadimitriou1
1 IHBM College of Dental Medicine, MBR University of Medicine and Health Sciences, Dubai, UAE, United Arab Emirates, 2 School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

EDTA-conditioning of circumpulpal dentine induces regenerative events following removal of coronal pulp in miniature swine teeth

Aim To evaluate the pulp response after complete removal of the coronal pulp and EDTA-conditioning of circumpulpal dentine of the coronal pulp chamber without any therapeutic dressing.

Methodology On the occlusal surfaces of 4.2 fully developed permanent teeth of 3 healthy miniature swine. Class 1 cavities and pulp exposures (2.5 mm X 2.5 mm) were prepared. After the complete removal of the coronal portion of the pulp the haemorrhage was controlled and the amputated pulp was covered with thin polyurethane film. Teeth were divided into 3 groups: Cotton pellets soaked in normal saline (control group), or 17% EDTA solution, experimental groups A and B were placed in the pulp chamber for 3 min. After cleaning of the pulp chamber with saline the film was removed and the pulp cavity was left empty in teeth of Group A, or was filled with sponge of swine collagen in teeth of Group B. Teeth were restored with Teflon discs and glass ionomer. Pulp response was evaluated histo-morphologically after 10 weeks.

Results Traces of mineralized tissue were found along the root dentinal walls of control teeth. The pulp responses of experimental groups were characterized by the following changes: Group A: deposition of large amounts of mineralized matrix along the root dentinal walls and at the site of pulp amputation. Group B: pulp growth and deposition of osteotypic mineralized matrix along the conditioned dentinal walls without any evidence of odontoblast-like cells differentiation, or complete root canal obliteration was seen.

Conclusions The conditioning of circumpulpal dentine with EDTA without use of therapeutic dressing, exerted an in vivo morphogenetic activity on the cells in amputated pulps.

R165
Sir John Walsh Research Institute, Faculty of Dentistry, University of Otago, Dunedin, New Zealand

Angiopoietins and Tie2 receptor expression in immature permanent teeth

Aim To investigate the distribution of angiogenic factors ANG1, ANG2, their receptor Tie2, and the endothelial cell-surface marker CD34 in the apical papilla (AP) of immature permanent teeth using immunohistochemistry; and their mRNA levels in the AP compared with the coronal pulp using q-PCR.

Methodology Ethical approval was obtained from the University of Otago Human Ethics Committee (H15/002). Ten unerupted immature third molar teeth were extracted, decalcified in 10% EDTA, and embedded in paraffin. Sections were stained by immunohistochemistry using anti-ANG1, anti-ANG2, anti-Tie2, and anti-CD34, and visualized using the chromogen DAB. A pyogenic granuloma served as a positive control. Non-specific anti-IgG was the isotype negative control. Slides were examined using light microscopy and the distribution of markers described. Eight extracted immature third molars were used for mRNA analyses. Teeth were sectioned transversely below the CEJ, and the coronal pulp and AP were removed for RNA extraction. mRNA levels for ANG1, ANG2, Tie2 and CD34 were determined using TaqMan assays. Data analyses were performed with GraphPad Prism®, using Wilcoxon tests at $p < 0.05$.

Results Endothelial cells were the main cell type expressing these markers centrally in the AP, ANG1 (perivascular), Tie2 (cell surface), and CD34 (cell surface) had more immunopositive staining than ANG2 (intracellular). ANG1, ANG2 and Tie2 (TEK) genes were more expressed in the AP compared with the coronal pulp but these did not reach significance ($p > 0.05$).

Conclusions The presence of angiopoietins and their receptor mRNA and protein in the AP of immature teeth suggests the potential for these growth factors to contribute to physiological development and pulp healing following injury.

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R166
Y.I. Kwon*, H.J. Ko & MR Kim
Department of Conservative Dentistry, Asan Medical Center, University of Ulsan, Seoul, Republic of Korea

The effect of enamel matrix derivatives on root resorption after delayed replantation: Micro-computed tomographic and histologic analyses

Aim To determine the inhibitory effect of enamel matrix derivative gel on root resorption after delayed replantation of teeth in vivo.

Methodology Thirty-three premolar roots of four beagle dogs were used. Extracted teeth received conventional root canal treatment and were kept air-dried for an hour. Extracted teeth were divided according to surface treatment; control group ($n = 13$) and EMD group ($n = 20$). Roots of EMD group had topical Emdogain® (Straumann, Switzerland) applied. Surface-treated roots were then replanted in their original sockets and resin-wire splinted for a week. All animals were sacrificed twelve weeks after surgery. Micro-computed tomography were taken to score the amount of root resorption; 0 (no root resorption) to 4 (complete root resorption). The samples were further processed as undecalcified tissue preparation and stained with haematoxylin-eosin for histological evaluation including morphometric assessments, which calculated proportions of inflammatory resorption and replacement resorption. Data were analyzed by Mann-Whitney U test in each evaluation ($\alpha = 0.05$).

Results Micro-CT revealed root resorption involving dentine in all specimens. The control and EMD groups were associated with mean score of 2.31 ± 0.95 and 2.10 ± 1.02, respectively. The EMD group had lower mean scores, but these were not significantly different ($p > 0.05$). In the histomorphometric evaluation, the mean proportions of inflammatory resorption and replacement resorption in the control group were 62.11 ± 19.56% and 36.50 ± 18.16%. These proportions were similar in the EMD group, resulting in 53.46 ± 29.18% and 44.41 ± 29.89%, respectively. The EMD group had less proportion of inflammatory resorption and greater proportion of replacement resorption than the control, but these values were not significantly different ($p > 0.05$).

Conclusions Enamel matrix derivatives did not provide inhibitory effects on root resorption after delayed replantation in vivo.
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R167
B.N. Lee*, V.A. Ngô, H.S. Chang, I.N. Hwang, W.M. Oh & Y.C. Hwang
Department of Conservative Dentistry, School of Dentistry, Chonnam National University, Gwangju, Republic of Korea

The effect of leptin on angiogenesis and odontoblastic differentiation in human dental pulp cells

Aim To investigate whether leptin promotes odontogenic differentiation and angiogenesis in human dental pulp cells (hDPCs).

Methodology Leptin’s cytotoxicity on hDPCs was examined using an WST-1 assay at 24 h after leptin treatment. Gene expression was analyzed by qRT-PCR. The Western blot assay was used in three different conditions to measure the odontogenic, angiogenic protein expression and MARK pathway involvement. ALP staining and ARS staining were performed. One-way ANOVA and student’s t-test was used with SPSS 23.0 software.

Results Cell viability was not significantly different (p > 0.05) among four groups after 24 h of culturing. Leptin at 10 ng/mL concentration significantly increased the mRNA level of DSPP after 1 and 3 days in hDPCs (p < 0.05). Leptin treatment significantly increased the mRNA level of DMP-1 (at 0.1 ng/mL), FGF (at 10 ng/mL after 3 days) and VEGF (at 1 and 10 ng/mL after 1 day) in hDPCs. Leptin at 0.1, 1 and 10 ng/mL concentration significantly enhanced the ALP expression and calcified nodule formation (p < 0.05). Leptin enhanced ERK, p38 and JNK phosphorylation within 5 min of treatment. Leptin-induced DSPP and VEGF protein expression and mineralization was appreciably blocked with the presence of MAPK inhibitors in hDPCs.

Conclusions Leptin stimulated odontogenic differentiation and angiogenesis in hDPCs through activating MAPKs (ERK, p38, and JNK) activity in vitro.

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R168
N. Scott1,2*, W.M. Thomson2 & P. Cathro1
1Postgraduate Endodontic Programme, 2Department of Oral Sciences & 3Department of Oral Rehabilitation, University of Otago, Dunedin, New Zealand

Traumatic dental injuries (TDIs) in the most recent NZ National Oral Health Survey (NZOHS)

Aim To determine the prevalence and associations of TDIs in New Zealand adults by analysing data from the 2009 National Oral Health Survey (NZOHS).

Methodology Information about TDIs in a representative sample of 2,209 New Zealand adults was collected as part of the NZOHS. This included self-reported information and a clinical examination of the maxillary 6 anterior teeth. Data were weighted and analysed using Stata.

Results Of the approximately 40% who reported previous orofacial trauma, 70% (that is, 28% of the overall population) reported that this included a TDI. More males than females had experienced orofacial trauma (51% and 31% respectively; p < 0.05) but there was no significant difference with TDIs. The most common injury reported was a chipped or broken tooth (67%). Examination of the 6 maxillary anterior teeth revealed a 23% overall TDI prevalence, with more males than females affected (27% and 20% respectively). TDIs were more prevalent in the 35–44 age group (33%). Teeth 11 (15%) and 21 (13%) were the most frequently affected. The most common clinical TDI observation was evidence of treatment or an untreated enamel fracture. These were more common among males and those in the 35–44 age group.

Conclusions TDIs in the NZ adult population constitute an important public health issue, given that many will need life-long follow-up and treatment.

R169
Department of Conservative Dentistry, Seoul National University Dental Hospital, Seoul, Republic of Korea

Location of mineral trioxide aggregates (MTA) plug and radiographic root change in regenerative endodontic procedure (REP): a preliminary study

Aim To evaluate the effect of the location of MTA plugs on radiographic root changes during regenerative endodontic procedures in immature permanent teeth with non vital pulps.

Methodology Seventeen cases of REP conducted in the Dept. of Conservative Dentistry (Seoul National University Dental Hospital, Seoul, Republic of Korea) were collected according to inclusion criteria: 1) pulp necrosis with or without periapical lesion, 2) no root fracture, internal resorption, or ankylosis, 3) healthy periodontium, and 4) restorability. Each patient’s demographic, pre-operative clinical and radiographic data were investigated. Cases were divided into 2 groups by the location of apical border of the MTA plug: Group C (coronal 45%, n = 10) and Group A (apical 55%, n = 7). A series of periapical radiographic images (pre-operative, post-operative, and 1-year follow-up) were standardized using ImageJ software (ver. 1.51)). Calibrated radiographic images were digitally assessed by a single observer, 3 times, to determine the degree of root lengthening, root thickening, apex closure, and healing of PA lesions after 1-year. Each radiographic outcome was quantified using a pre-determined grading criteria. Statistical analysis was conducted by adopting Mann-Whitney test to verify the difference between two groups. p value < 0.05 was considered to be statistically significant.

Results Patients screened by inclusion criteria had an average age of 12.3 years, with no significant prevalence of gender (M:F = 9 (52.8%); 8 (47.1%)), Types of tooth were various including premolars (64.7%, n = 11), incisors (29.4%, n = 5), and a molar (5.9%, n = 1). The patients had diverse pre-operative clinical symptoms (perfusion, mobility, palpation, fistulation, bite, etc.), which were eliminated after 1-year. 2 cases among 17 had a positive response to vitality test (e.g. ice test, EPT). Radiographic success scores were compared between the two groups. Average values of success scores were higher in Group A (10.0) than in Group C (8.0); however, the difference was not significant (p > 0.05).

Conclusions The average value of radiographic success score was higher in the group with more apically positioned MTA plugs, but the difference was not significant. Further prospective studies are proposed to identify the ideal position of MTA plugs for REP based therapies.
Effect of various irrigation protocols on the survival of human stem cells in the apical papilla in organotype root canal models

Aim To evaluate the effect of different irrigation protocols on survival of human stem cells of the apical papilla (SCAP) with WST-1 assay and SEM analysis.

Methodology SCAPs were isolated from immature human third molars and CD73, CD90 and CD105 coexpressing cells were used. Forty-three standardized organotype root canal models were prepared for WST-1 assay and randomly divided into 1 control (n = 3) and 8 experimental groups (n = 5). Standardized organotype root canal models were irrigated with 1 of 9 protocols: (1) no irrigation, (2) 5% EDTA, (3) 17% EDTA, (4) 1% NaOCl/5% EDTA, (5) 2.5% NaOCl/5% EDTA, (6) 5% NaOCl/5% EDTA, (7) 1% NaOCl/17% EDTA, (8) 2.5% NaOCl/17% EDTA, (9) 5% NaOCl/17% EDTA. Following these procedures, isolated SCAPs were mixed with platelet-rich plasma (PRP) and seeded into the organotype models and cultured for 3 and 7 days. The mandibular premolar teeth were used for SEM analysis. The prepared root canals were split longitudinally. After irrigation protocols as in the WST-1 assay, isolated SCAP mixed with PRP were seeded into the root canals. After 7 day incubation, the samples were prepared for SEM analysis. Statistical analysis was performed using Kruskal-Wallis H test with the Bonferroni post-hoc test.

Results Irrigation with only 17% EDTA (Group-3) and 2.5% NaOCl followed by 17% EDTA (Group-8) had the highest SCAP viability on day 7 and in these groups, SCAPs in small communities attached on dentine surface were more consistent than the other groups. Final irrigation with 17% EDTA increased SCAP viability more than 5% EDTA, following NaOCl irrigation at both times. In the groups where 5% NaOCl was used (Group-6 and 9), absorbance values decreased from the third day to the seventh day.

Conclusions Dentine conditioning with irrigation protocols that included 5% NaOCl had irreversible negative effects on SCAP survival. However, irrigation protocols that included 2.5% NaOCl followed by 17% EDTA might be beneficial for regenerative endodontic therapies.

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Effects of three covering materials on human pulp derived stem cells used clinically for capping blood clots in regenerative endodontics

Aim To analyze human pulp derived stem cells (HPDSCS) after exposure to several light-cured (LC) materials and MTA used clinically for covering blood clots in regenerative endodontics.

Methodology Cells were derived from pulp tissue of freshly extracted human third molars. Teeth were caries free with incomplete root development. The ages of the donors were 15–22 years. Pulp tissues from four different donors were cultured after enzymatic digestion based on the Grontros’ protocol for obtaining HPDSCS. After reaching confluence, the cells were trypsinized and seeded on 24-well-plates with 10 000 cells per well. After 24 h, the cells were exposed to the following materials (n = 192 in all groups): 1. MTA (Dentsply/Germany); 2. Calcimol LC (CM; Voco/Germany); 3. Ultrablend plus (UB; Ultradent/USA); and controls. The materials were prepared according to manufacturer’s instructions, placed in inserts (pore size 0.4 μm), and then into wells after 24 h. After 1, 4, 7 and 11 days cells were analyzed by Casy cell counter (Roche Innovatis/Germany) focusing on viability/%, viability/mL, and on mean diameter of the cells. Additionally, HE staining was performed. Statistical analysis was achieved by one-way ANOVA followed by a post hoc analysis (Tukey’s HSD test). Significance level was p ≤ 0.05.

Results Viability/% showed no differences between the groups on day 1. On day 4 and 7 the viability/% of the cells exposed to UB was significantly lower compared to the MTA or control group. On day 11 the UB and CM groups had significantly lower viability/% than cells of the control group. Viability/mL was significantly lower than in the MTA and control groups on days 1, 4, 7 and 11 in the UB and CM groups. Mean diameter of HPDSCS was significantly larger in the UB group than in the other groups on day 1, while on day 4 this was true for the UB and the CM groups; this was confirmed by HE staining.

Conclusions At the beginning of the observation period the HPDSCS exposed to LC materials had significantly larger diameters than those incubated with MTA or in the control group. At the middle and end of the trial, the viability of HPDSCS exposed to LC materials decreased significantly.

Evaluation of a fluorescence-aided identification technique (FIT) for removal of composite bonded trauma splints: an ex vivo comparative study

Aim To evaluate a fluorescence-aided identification technique (FIT) in comparison with a conventional light source for removal of a composite resin bonded Titan-Trauma-Splint (TTS), regarding time needed, loss of tooth structure and remaining composite resin.

Methodology Twenty-two maxillary models with six bovine teeth (PDL: 13–23) were fabricated. Two models served for calibration of the experimental set-up. A pre-operative digital surface-scan was performed and a TTS was applied under standardized conditions using composite resin. Two dentists removed splints from five models using FIT and five with a conventional light source. For FIT, a 405 nm light-emitting headlamp (prototype) was used. Time was recorded. A post-operative scan was superimposed on the pre-operative scan using OraCheck software. A qualitative and quantitative analysis of tooth defects and composite resin remnants was performed. Data were analyzed statistically.

Results Compared with the conventional technique (CT), FIT was significantly faster (mean: 162.6 s versus 268 s) (p < 0.0001), led to significantly fewer and smaller tooth defects (mean volume:
versus –0.04 mm³ versus –0.33 mm³ (p < 0.0001), and significantly less composite resin remained on the tooth surface (mean volume: 0.02 mm³ versus 0.28 mm³) (p < 0.0001).

Conclusions FIT allowed the reliable removal of composite with fewer resin remnants and tooth defects in a shorter period of time compared to the conventional method. Therefore it is highly recommended for splint and composite resin removal.

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R173

M.T. Albuquerque¹,², A.E. Munchow³, Á. Lopez³, R.L. Gregory³ & M.C. Bottino⁴

¹Department of Clinical Dentistry, Federal University of Bahia, Salvador, Brazil, ²Department of Restorative Dentistry, Federal University of Juiz de Fora, Juiz de Fora, Brazil & ³Department of Biomedical and Applied Sciences, Indiana University School of Dentistry, Indianapolis, USA

Augmentin nanofibres as a novel drug delivery strategy for root canal disinfection

Aim To develop and characterize Augmentin (amoxicillin and clavulanic acid) nanofibres as a novel intracanal drug delivery strategy for root canal disinfection prior to regenerative endodontic procedures.

Methodology Amoxicillin (AMX) and clavulanic acid (CLV) were added to polydioxanone (PDS) polymer solutions (PDS), at two distinct concentrations, namely 5 wt.% and 15 wt.% and electrospun into nanofibres. Scanning electron microscopy (SEM), tensile testing, and Fourier-transform infrared spectroscopy (FTIR) were performed to characterize the morphology, mechanical, and chemical characteristics of the Augmentin-incorporated electrospun fibres. Antimicrobial properties were assessed using the agar diffusion test against Porphyromonas gingivalis, Prevotella intermedia, Enterococcus faecalis, Actinomyces naeslundii, Lactobacillus casei and Staphylococcus aureus. The Inhibition halos were measured after 48 h. Data were analyzed using ANOVA and Tukey tests (p < 0.05).

Results FTIR and SEM analyses demonstrated effective incorporation of AMX and CLV into the fibres, and fairly smooth fibres at the nanoscale, respectively. In detail, AMX and CLV incorporation led to thinner fibres when compared to pure PDS fibres. Overall, the mechanical properties of the fibres were reduced upon AMX and CLV incorporation. Furthermore, the presence of AMX and CLV did not affect the stretching ability of the fibres. Augmentin nanofibres (5 and 15 wt.%) exhibited antibacterial action against the microbial species tested in a dose-related fashion.

Conclusions Collectively, the synthesized Augmentin nanofibres were associated with significant antimicrobial action against several root canal bacteria holding promise as an alternative disinfection strategy for use in regenerative endodontics.

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R174

A.C. Pereira¹,², J.M. Lazzari¹, A.C.C.L. Cerqueira-Neto³, V.G.A. Pecorari⁴, A.A. Zaia¹, B.P.F. A. Gomes⁴, J.F.A. Almeida³ & A.J. Soares¹

¹Department of Restorative Dentistry, Piracicaba Dental School, State University of Campinas, Piracicaba & ²Department of Biostatistics, Paulista University, São Paulo, Brazil

Evaluation of internal bleaching in traumatized teeth submitted to pulp revascularization

Aim To evaluate internal bleaching of discoloured traumatized teeth submitted to pulp revascularization and the satisfaction of patients with the results of treatment.

Methodology Fourteen teeth of twelve patients were divided into three groups: Group TAP (n = 4): discoloured teeth treated with triple antibiotic paste (ciprofloxacin, metronidazole and minocycline) as intracanal dressing, and cervical sealing with white MTA; Group CHP (n = 4): discolour teeth treated with calcium hydroxide and 2% chlorhexidine gel as intracanal medication, and cervical sealing with white MTA, and a Control Group (DT) (n = 6): discoloured teeth after dental trauma, treated endodontically. The bleaching agent was sodium perborate mixed with distilled water changed every 7 days, for a maximum of five applications. Colour evaluation was measured by a spectrophotometer at the beginning (T0) and at the end of internal bleaching (T1). A validated questionnaire was used to assess satisfaction of patients. Data were collected based on the CIELAB-CIE1976 (L*a*b*) system and analyzed using analysis of variance of repeated measures and Tukey’s test. The Pearson correlation test was used for analysis between discoloration time and number of sessions. To verify the difference in the scores of the visual analogue scale, the Wilcoxon test was applied.

Results Independent of the group, the T1 values were significantly higher than T0 values (p = 0.0006). In the analysis of L* values, independent of the group, final L* was significantly higher than values of L* initial (p = 0.0006). There was no significant difference between the means of ΔE (p = 0.48) and ΔL (p = 0.42).

All groups resulted in a similar tooth bleaching. Only in group TD, in which teeth had been discoloured for several years needed more bleach applications (p = 0.0005). In the analysis of patient satisfaction, there was a significant difference between the initial and final values (p = 0.003). At the end of the treatment, 72.7% of the patients were satisfied, while 27.3% where very satisfied.

Conclusions Internal bleaching was effective for discoloured traumatized teeth after pulp revascularization, with satisfactory aesthetic results.

OTHER

R175

E. Cotti¹, K. Abramovitch², J. Jensen³, E. Schirru¹*, D.D. Rice², U. Oyoyo³ & M. Torabinejad¹

¹Department of Conservative Dentistry and Endodontics, Università degli Studi di Cagliari, Cagliari, Italy, ²Department of Radiology and Imaging Sciences, ³Department of Endodontics & ⁴Department of Dental Education Services, Loma Linda University, Loma Linda, USA

The influence of adalimumab on the healing of apical periodontitis in ferrets

Aim To evaluate the effect of adalimumab (anti-TNFα), on healing and healing time of experimentally-induced apical periodontitis (AP) in ferrets.
**Methodology** AP was induced in the canines of twelve male ferrets. Three ferrets (12 teeth) provided the positive controls for the histological evaluation; the canines of the remaining nine ferrets were randomly divided into 3 treatment groups with 12 teeth each in the following manner: Group 1: conventional root canal treatment (RCT) and systemic subcutaneous administration of anti-TNFα every two weeks for 90 days; Group 2: RCT and a single periapical administration of anti-TNFα prior to canal obturation, via root canal; Group 3: RCT only (control). Cone beam computed tomography (CBCT) of the jaws were taken at baseline health (T0); AP confirmation (T1); 30 (T2), 60 (T3) and 90 (T4) days post RCT to monitor healing. Two calibrated radiologists assessed the CBCTs independently and blindly for AP identification and quantification. Interclass correlation agreement was measured and rank-based ANCOVA was used for statistical analysis of lesion size. Level of significance was set at $p < 0.05$.

**Results** AP was induced in all teeth. Following RCT, all AP lesions in the 3 groups were associated with a significant reduction in size. Specific pairwise comparisons of the related samples demonstrated a decreasing trend in lesion size with healing time in all three groups, most pronounced for Group 2 (local adalimumab). No significant difference was noticed between groups ($p > 0.05$). The examiners’ interclass correlation coefficient was 0.977.

**Conclusions** Both systemic and local anti-TNF did not hinder AP healing in this animal model, and a faster healing response when these medications are used may also be anticipated. These findings encourage follow-up studies with larger sample sizes.

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**R176**

S. Ali$^{1,4}$, S.M. Faisal$^2$, A. Zoya$^1$ & A. Ahmed$^3$

$^1$Conservative Dentistry & Endodontics, $^2$Molecular Immunology Laboratory, Interdisciplinary Biotechnology Unit, Aligarh Muslim University, $^3$Ali Garh & $^4$Hybridoma Laboratory, National Institute of Immunology, New Delhi, India

**The role of inflammatory cytokines in the pathogenesis of periapical granuloma and radicular cyst in the population of Aligarh region of north India**

**Aim** To determine the level of inflammatory cytokines IL-4, IL-17, IFN-γ, IL-6 in the pathogenesis of human radicular cysts and periapical granulomas.

**Methodology** The study was carried out on 40 apical periodontitis and 20 healthy human subjects. The ethical clearance for this study was taken from the institutional ethical committee. The participants and patients were informed and their written consent was obtained. Serum levels of cytokines were measured using commercially available cytokine detection kits employing ELISA. The data was analyzed by the Microsoft excel software. The statistical analysis was achieved with the unpaired student’s t-test and differences with a $p$-value $\leq 0.05$ were considered to be statistically significant.

**Results** The plasma level of inflammatory cytokines was elevated in patients with periapical pathosis as compared to that of healthy participants. Increased expression of the IFN-γ was observed in the cases having radicular cysts as compared to those with apical granulomas. On the contrary, the level of IL-4 and IL-6 cytokine (Th2 type cytokine) expression was upregulated in cases with apical granuloma. Nevertheless, the levels of IL-17 cytokines were significantly augmented ($p \leq 0.001$) and maintained in both periapical pathoses. Periapical granulomas displayed a regulatory environment characterized by high Th2 type cytokines, while radicular cysts had both Th1 and Th2 inflammatory reactions with the presence of high levels of IFN-γ ($p \leq 0.01$).

**Conclusions** The level of inflammatory cytokines and specific biochemical markers in serum can be exploited as potential markers for the pathogenesis of apical periodontitis. The data of the present study further suggests the differential expression of inflammatory cytokines in radicular cysts when compared to periapical granulomas. Finally, granulomas may evolve into a radicular cyst due to the continuous exposure of Th2 inflammatory cytokines.

**R177**

C. Inostroza$^1$, C. Brizuela$^1$, P. Luz-Crawford$^2$, A.M. Vega$^3$, F. Carrion$^3$ & M. Atari$^4$

$^1$Dental Faculty, $^2$Medicine Faculty, Universidad de los Andes, Santiago Chile, Chile, $^3$Medicine Faculty, Universidad del Desarrollo, Santiago Chile, Chile & $^4$Regenerative Medicine Research Institute, Dental Faculty, Universidad Internacional de Catalunya, Barcelona, Spain

**Inflamed pulp: a promising new source of functional mesenchymal stem cells**

**Aim** To evaluate the functional characteristics of mesenchymal stem cells derived from the human dental pulp of 5 patients diagnosed with irreversible pulpitis (inflamed pulp).

**Methodology** Five dental pulp explants diagnosed with irreversible pulpitis were cultured in Alpha-MEM culture medium, 10% FBS, Pen Strep (1%) at 37°C and 5% CO2. Cultures were monitored weekly with a light inverted microscope. The cell population in step 4 was characterized with specific immunotype markers of mesenchymal stem cells and hematopoietic cells features by flow cytometry. In addition, their ability to differentiate into osteogenic, chondrogenic and adipogenic lineages was evaluated. Dental pulp stem cells derived from healthy donors were used as controls for the functional analysis.

**Results** Cells isolated from the dental pulp with irreversible pulpitis grew on a culture plate with a spindle morphology and adherence to plastic. These cells expressed ≥95% of surface markers; CD105, CD73 and CD90 and expressed ≤3% of the markers; CD34 (endothelial cell markers), CD14 or CD11b (macrophage and monocyte markers), CD79α or CD19 (B cell markers) and HLA class II. Differentiation assays showed that cells derived from the inflamed pulp were able to differentiate into osteogenic and chondrogenic lineage with a lower potential for adipogenic differentiation compared to dental pulp stem cells derived from healthy donors.

**Conclusions** The inflamed tissue of the dental pulp could be a promising new source for obtaining functional mesenchymal stem cells.

**R178**

S. Staffoli$^1,2$, N.M. Grande$^2$, G. Plotino$^1$, G. Gambarini$^3$, L. Testarelli$^1$, L. Fortunato$^1$ & A. Polimeni$^1$

$^1$Department of Oral and Maxillofacial Science, Sapienza University of Rome, Rome & $^2$Department of Health Sciences, Magna Gracia University, Catanzaro, Italy

**Influence of heat treatment, environmental temperature and cross-section design on cyclic fatigue resistance of NiTi rotary instruments**

**Aim** To determine how cross-section design, heat treatment and environmental temperature affect the fatigue life of NiTi files of the same size and taper under cyclic fatigue stress in simulated root canals.
Methodology Three groups of One Shape instruments (OS) produced by the same manufacturer (Micro-Mega, Besançon, France) size 25, .06 taper with two different cross sections (A and B) were tested: Group1, OS section A; Group2, OS section B; Group3, OS section B Heat Treated. Cyclic fatigue testing was performed in artificial canals with a 5 mm radius and 60° angle of curvature 5 mm from the tip. The test was performed in a liquid environment at two different temperatures: 0° and 20° C. A total of 120 instruments were rotated at 300 rpm until fracture occurred and the number of cycles to failure (NCF) was recorded. Data were analyzed by one-way Anova and the Bonferroni test ($p < 0.05$).

Results Mean values of NCF recorded for the different groups ($\pm$SD) were: 462 (60) Group 1 at 20° C; 473 (83) Group 2 at 20° C; 1513 (154) Group 3 at 20° C; 2461 (115) Group 1 at 0°C; 2628 (131) Group 2 at 0°C; 3879 (676) Group 3 at 0°C. There were significant differences between Groups 1 and 2 versus Group 3 at both the temperatures tested ($p < 0.05$), while there was no difference between Group 1 and 2. There were significant differences between the two temperatures in all the different groups of instruments tested ($p < 0.05$).

Conclusions Cross section was not a significant variable influencing fatigue resistance. Heat treatment of the alloy was a significant variable influencing the fatigue resistance of the instruments tested; Group 3 obtained the best performances for both temperatures tested. Temperature was also a significant variable influencing the fatigue resistance for both traditional NiTi (Groups 1 and 2) and heat-treated instruments (Group 3).

Cyclic fatigue testing of Blue NiTi alloy reciprocating files at different environmental temperatures

Aim To investigate how environmental temperature can affect the fatigue life of nickel–titanium (NiTi) rotary files manufactured from Blue alloy.

Methodology Cyclic fatigue testing of NiTi instruments was performed in stainless steel artificial canals with a 5 mm radius of curvature and a 60° angle of curvature located 5 mm from the tip of the instrument. Eighty R25 Reciproc Blue instruments (20 for each Group) of the same size and taper, 0.25 mm tip size and variable taper reducing from .08 at the tip to .04 at the shaft, were rotated in the artificial root canal until fracture occurred and the number of complete rotations to failure (NRF) were recorded. The test was conducted using a thermostatic bath with electronic and infrared controls in a water based liquid media at temperatures of: 0°, 20°, 35° and 39° C. Data were analysed by one-way Anova and the Bonferroni test; the significance was determined at the 95% confidence level ($p < 0.05$).

Results Mean values of NRF ($\pm$SD) were respectively: 2724 (189) at 0° C, 1974 (401) at 20° C, 705 (290) at 39° C. There were significant differences among all of the groups tested ($p < 0.05$).

Conclusions Temperature was a significant variable influencing the fatigue resistance and mechanical properties of NiTi instruments. As the environmental temperature increased the resistance of the instruments to flexural fatigue decreased, with an increase of 180% in fatigue life when 0° was compared to the clinical temperature present in root canals (35°C). A decrease of 26% was observed between 35°C and 39°C, demonstrating great sensibility of this type of alloy even to minimal changes of the environmental temperature. Further studies are needed to determine how to apply these observations clinically.
Author Index

Abdul Razek, A.A. R103
Abed Galil, K. R018, R053
Abiad, R.S. R018, R053
Abouelsaad, N.S. R053
Abramovitch, K. R175
Abu Bakr, A.Y. R136
Abu-Haimed, T.S. R064
Adl, A. R013
Adorno, C.G. R044
Ahmed, A. R176
Ahmed, G. R136
Ahmed, H. R087
Ahn, K.B. R011
Akgül, N.Y. R111
Aksel, H. R085, R124, R129
Al Masan, A.A. R058
Alada, L. R101
Albaaj, F. R093
Albuquerque, M.T. R057, R173
Alhujazy, U. R125
Ali, I.A. R103
Ali, M.R.W. R128
Ali, S. R176
Alim, B.A. R052
Almarche, A. R012
Almeida, J.F. R032
Almeida, J.F.A. R019, R022, R174
Almeida, M.M. R151
Almenar, A. R012
Almeida, M.M. R151
Almeida, M.M. R151
Andrade, F.B. R021
Andrei, M. R077, R109
Andrade, D. R097, R132
Ani, I. R107
Anil, C. R030
Ardebil, D.M. R048, R049
Ari Aydinbelge, H. R118
Ari, R. R015
Ar, R. R041
Arruda-Vasconcelos, R. R020, R023
Arslan, D. R060, R061
Arslan, H. R043
Arvaniti, E. R148
Arslan, H. R043
Arslan, H. R043
Arica Çelik, D. R152
Askervelyi Örs, S. R085
Aslanta, E.E. R124
Astara, G. R145
Atari, M. R177
Atav Ates, A. R062
Axmann, D. R171
Aytore Kosar, M. R084
Azmaz, N.T. R118
Baaij, A. EP1
Back, S.H. R011, R169
Bago, I. R088, R107
Balbi, S.B. R089
Barabanti, N. R075
Barberini, L. R153
Barbosa-Ribeiro, M. R020, R023
Barcellos, A.S.P. R099
Bärdsen, A. R128
Bartha, V. R008
Barut, G. R041
Basturk, F.B. R028, R084
Bazargani, A. R013
Bedier, M.M. R126
Bedini, R. R102
Belhari, K. R090
Belladonna, F.G. R003, R121
Belli, S. R095, R119
Benedetti, M. R032
Bernardinelli, N. R108
Beverlaqua, L. R132
Bhakta, S. R071
Bhambr, G. R013
Bicego-Pereira, E.C. R020, R023
Bleier, M. R029
Blnzarus, D.C. R109
Bleza, A. R128
Blundell, K.B. R079
Bołtacz-Rzepkowska, E. R141
Bortoluzzi, E. R003
Bottenberg, P. R066
Bottino, M.C. R173
Boyaci, H. R027
Boyle, L. R150
Bozbult, P. R041
Braga, A.C. R112
Brizuela, C. R177
Badimur, A. R088
Bukiet, F. R047
Bürklein, S. R069, R137
Calberson, L. R086, R138
Camilleri, J. R080
Canali, L.C.F. R108
Canpolat, F. R104
Carneiro, E. R038, R059, R158
Carrion, F. R177
Carvalhal, J.C.A. R121
Carvalho, C.N. R133
Carvalho, R.F. R099
Casagrande, R. R121
Castagnola, R. R179
Castaldo, A. R097
Cathro, P. R168
Cathro, P.R. R087, R094
Cehreli, Z. R081
Cerqueira, A.R. R067
Cerqueira-Neto, A.C.C.L. R174
Cerutti, A. R075
Çetin Canbaz, U. Ç. R071
Chaini, K. R148
Chalupa, M. R010
Chandler, N.P. R087, R117
Chang, H.S. R167
Chang, S.W. R011
Chen, N.N. R157, R160
Cheung, G.S. R120
Cheung, G.S.P. R157
Cíntia, T.A. R151
Cohane, A. R095
Cobankara Funda, K. R029
Cohen, T. R014
Coker, S. R102
Collado-González, M. R127
Connert, T. R144, R172
Cotes, C. R099
Coti, E. R145, R153, R163, R175
Croft, K. R024
Cruz, A.T. R059
Cruz, A.T.G. R038
Cuéllar, M.R.C. R021
Curtis, D.A. R048, R049
Cvičk, B. R125
Dal Fabbro, R. R151
Dunlap, O.K. R070
De Biasi, M. R097, R132
De Bruyne, M. R025, R055, R139
De Deus, G. R121
De Moor, R. R025, R086, R138, R139
De Moor, R.J. R055
De Moor, R.J. G. R014
De-Jesus-Soares, A. R032
Del Fabbro, M. R083
Delboni, M.G. R022
Deniz Sungur, D. R071, R129
Dervens, K. R033
Dettori, C. R145
Dettwiler, C.A. R172
Devroy, E. R086, R138
Di Nardo, D. R100
Diedemasa, G. R029
Didilescu, A.C. R077, R109
Diegriz, C.D. EP2
Digka, A. R123
Dimitriu, B.A. R077, R109
Dinci, C. R152
Dincer, A. R061, R083
Dionfiso, T.J. R021
Dodd, M.N. R099
Doganay, E. R043
Doganazhiyska, V.D. R050
Domingues, P.B.A. R099
Donnermeyer, D. R137
Author Index

Lee, J.K. R068
Lee, W. R011
Leelapornpisid, W. R031
Lenhardt, M. R097
Li, H.N. R120
Li, X. R122
Lim, S.M. R011
Lima, A.R. R019, R022
Lind, S.L. R049
Linden, D. R138
Lindström, C. R162
Linsuanont, P. R113
Lipski, M. R002, R046, R074
Llena, C. R012, R127
Logani, A. R017
Loguercio, A.D. R133
Lopes, M.A. R112
Lopes, R.T. R121
Lopez, A. R173
Lopez-Lopez, A. R012
Löst, C. R008, R144
Löst, C.W. R171
Lozano, A. R127
Lucateli, R.L. R080
Lui, J.N. R160
Luz-Crawford, P. R177
Lyons, K.M. R123
Macario, T.R.L. R099
MacLuskey, M. R149
Macorra, J.C. R155
Maddalone, M. R083
Maier-Schell, S. R171
Malec, M. R051
Mannheim, J.G. R008
Mannocci, F. R156
Marciano, M.A. R032, R080
Marnich, A.C. R032
Martín Peral, J. R036
Masi, O.V. R094
Matsumoto, M.A. R080
Mattos, B. R038
Mayordomo Sánchez, C. R036
Medioni, F. R090
Medioni, E.M. R089
Meena, N. R072
Meinel, M. R035
Meire, M. R025, R086, R138, R139
Meire, M.A. R014, R055
Mele, L. R145
Melih, I. R039, R092, R110
Meller, C.A. R172
Meltem, Ç. R070
Messias, A. R130
Messias, A.L. R067
Mezzena, S. R163
Miccoli, G. R100
Michelini, F. R106
Midena, R.Z. R021
Milne, T.J. R165
Mira, A. R012
Moadad, E.M. R079
Mozzami, F. R013
Moldoveanu, G.F. R077, R109
Montis, N. R153
Morandini, A.C. R021
Moritz, A. R125
Mota de Almeida, F.J. R007
Munchow, E.A. R057, R173
Mura, M. R163
Mustafa, M. R128
Nagas, E. R081
Nagata, J.Y. R057
Navani, H.A. R165
Neukermans, M. R139
Ng, Y.L. R140
Ngö, V.A. R167
Nikitovic, A. R039, R092, R110
Noto, A. R153
Novak-Frazer, L. R031
Nowicka, A. R002, R046, R074
Obino, F.V. R114
Oh, W.M. R167
Oikonomou, I. R148
Olczak, K. R001
Olsson, S.R. R096
Onay, E.O. R091
Osman, E. R018
Ottolengo, O. R163
Ozoy, U. R175
Ozbay, Y. R040
Ozcan, M. R134, R135
Ozcilek Yilmaz, M. R118
Ozguven Akbulut, M. R170
Özk, A.R. R153
Özturk, G.C. R052
Öztürk, . R124
Öztürk, . R129
Özyürek, T. R065, R116
Palatynska-Ulatowska, A. R051
Palma, P.J. R130
Panopoulos, P. R075
Papadimitriou, S. R164
Papaefthymiou, K. R140
Passini, M.R. R032
Paster, B.J. R032
Patel, B. R093
Patel, S. R156
Pawlicka, H. R001, R051, R154
Pe iulienč, V. R076
Pecorari, V.G. R032
Pecorari, V.G.A. R174
Pedano, M.S. R122
Pepl, E. R100, R102, R114
Pereira, A.C. R174
Pereira, S. R130
Pérez-Higuera Sánchez-Escalonilla, J.J. R036
Perleu, P. R077, R109
Pesci, D. R039, R092, R110
Peter, O.A. R155
Piasecki, L. R038, R059, R063
Pichler, J.B. R008
Pigg, M. R096, R146
Pina-Vaz, I. R112
Pirani, C. R106, R161
Piras, V. R163
Plotino, G. R054, R102, R178, R179
Polimeni, A. R178
Prati, C. R106, R161
Preston, A.J.P. R079
Provenzano, J.C. R121
Putzeys, E. R122
Qualtrough, A. R031
Rakesh, Y. R030
Ranjit, S. R017
Rautemaa-Richardson, R. R031
Rebollosa de Burrio, E. R036
Reichenmiller, K.M. R171
Részka, K. R046
Reus, A. R034
Reymus, M.R. EP2
Ricci, C. R090
Ricci, C.R. R089
Rice, D.D. R175
Rich, A.M. R165
Ricketts, D.N.J. R149
Rijckaert, E. R066
Rodrigues, C.T. R108
Rodríguez Lozano, F.J. R127
Rosaria Gatto, M. R106
Roseiro, L. R067
Roskamp, L. R158
Russeri, A. R179
Rover, G. R003
Roy, T.S. R017
Russell, A.A. R117
Sakalauskienė, A. R076
Sakhaei Manesh, V. R015
Salas, M.S. R087
Saédidas, L.M.P. R151
Santos, A.C. R130
Santos, C.F. R021
Santos, J.M. R130
Santos, M.P. R063
Sapmaz, C. R062
Saunders, W.P. R149
Savic, A. R143
Sazak Ovecoglu, H. R028
Scarlatescu, S.A. R077, R109
Scartozzi, M. R145
Schäfer, E. R069, R137
Schell, S. R008
Schianchi, G. R114
Schirru, E. R163, R175
Schumacher, I. R171
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