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ABSTRACTS

RP1

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Potential systematic error in laboratory experiments on microbial leakage through filled root canals: an experimental study

Aim To assess the routes of bacterial leakage in a commonly used two-chamber model designed to evaluate root fillings.
Methodology Fifty-one intact human mandibular premolars with fully developed roots were used. They were left completely intact ($n = 23$), or were accessed, instrumented and either left open ($n = 5$) or root filled with gutta-percha and AH Plus ($n = 23$). All teeth were sealed between two chambers using sticky wax. The apical root aspects were left uncovered. The upper chamber was seeded with *Enterococcus faecalis*. An enterococci-selective broth was used in the lower chamber. Leakage was assessed for 120 days and compared using survival statistics ($\alpha < 0.05$). Subsequently, roots were trans-sected, stained using a 'live' DNA stain (Syto59) and inspected using confocal laser scanning microscopy. An *E. faecalis*-specific RNA probe was used for fluorescence *in situ* hybridisation (FISH).
Results Leakage started to occur from day 56, with further occurrence essentially identical between root filled teeth and intact counterparts ($P = 0.71$). All the trans-sections showed fluorescence related to Syto59 between the cementum layer and the sticky wax. Fluorescence was also observed between the root filling and the tubular dentine, whilst it was absent at the interface between root filling and sclerotic dentine. Secondary dentinal tubules, i.e. lateral branches connecting the main counterparts, contained fluorescent material. FISH revealed that Syto59 exclusively stained *E. faecalis*.
Conclusions The current experimental method proved to be unsuitable to compare root fillings. Histology revealed interesting observations regarding the relationship of dentine structure and bacterial leakage, which warrant further investigation.

RP2

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The effectiveness of enzymatic irrigation on the removal of an endodontic stressed multi-species model biofilm

Aim To establish a nutritionally stressed multi-species model biofilm and to investigate the dynamics of biofilm killing and disruption by trypsin and proteinase K with or without ultrasonic activation.
Methodology Stressed biofilms comprising *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Actinomyces radicidentis*, *Streptococcus mitis* and *Enterococcus faecalis* OMGS 3202 were grown on hydroxyapatite discs or on endodontically prepared single rooted teeth in modified fluid universal medium containing serum proteins. The ability of 1% trypsin and 1% proteinase K with or without ultrasonic activation to kill and disrupt the biofilm was determined. Positive control irrigants, 1% NaOCl and 0.2% w/v chlorhexidine gluconate and an untreated control were also included. Each treatment was replicated five times. The biofilms were investigated using confocal laser scanning microscopy (CLSM) with LIVE/DEAD staining and quantitative microbial culture. Data were analysed using *bio*Image_L and SPSSPC (Ver 16.0).
Results Nutritional stress in the multi-species biofilm was apparent as the medium pH became more alkaline (from 5.58 ± 0.1 to 5.94 ± 0.13), glucose was not present in the supernatant and serum proteins were degraded. The CLSM analysis showed a decrease in the percentage of viable bacteria at the surface level of biofilm in response to nutritional starvation. Trypsin and proteinase K were effective in biofilm killing since their aerobic viable counts (6.69 ± 0.13 and 6.60 ± 0.02 respectively) were significantly lower ($P < 0.01$) than the control (7.86 ± 0.03) and chlorhexidine (7.72 ± 0.05) groups. Sodium hypochlorite was the most effective agent significantly ($P < 0.001$) reducing the recovery on all medium with mean values of <1. Moreover the percentages of mean biovolumes for dead populations with trypsin and proteinase K were 55% and 37%, significantly less than the control group ($P < 0.05$). Ultrasonic activation significantly improved the action of all irrigants ($P < 0.001$), except for NaOCl.
Conclusions Proteinase K and trypsin may have a future in clinical practice as endodontic irrigants for the treatment of both primary and refractory endodontic infections.

RP3

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Characterizing the sonochemical efficacy of passive ultrasonic activation

Aim To evaluate the effect of multiple ultrasonic refreshment/activation cycles and temperature on the reaction rate of NaOCl with bovine dentine and to characterize the sonochemical efficiency of passive ultrasonic activation under experimental conditions.
Methodology The root canal walls of 24 standardized root canals in bovine incisors were exposed to a standardized volume of sodium hypochlorite (NaOCl) at different temperatures (25 and 35°C) and exposure times (20, 60 and 180 min). An irrigation protocol was tested, where the irrigant was refreshed and ultrasonically activated four times for 20 s followed by a 40 s rest interval, with no refreshment and no activation as the controls. The reaction rate

was determined by measuring the iodine concentration using an iodine/thiosulfate titration method. A standard calorimetry and chemical dosimetry of tert-Butil Nitrite were performed to characterize the sonochemical efficiency of the PUI.

Results Refreshment, activation and exposure time increased the reaction rate of NaOCl ($P < 0.05$). During activation, the temperature of the irrigant increased up to 10°C. This temperature rise was insufficient to enhance the reaction rate of NaOCl ($P > 0.125$). Sonochemical activity happened at a constant rate during one minute of passive ultrasonic irrigation (PUI) and its occurrence was not altered by dentine ($P > 0.05$).

Conclusions The reaction rate of NaOCl when activated by ultrasound was enhanced due to convection and cavitation. Temperature rise of the irrigant during ultrasonic irrigation was not sufficient to alter the reaction rate.

RP4

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Attachment and growth of dental pulp stem cells on dentine in presence of extra calcium

Aim To differentiate dental pulp stem cells (DPSC) to odontoblast-like cells (ODPSC) and to investigate their attachment and growth on dentine in the presence of extra calcium by colorimetric assay and scanning electron microscopy (SEM).

Methodology After isolation of DPSC, they were differentiated to ODPSC. Morphology and characterization of both cells were examined by immunocytochemistry, histochemistry, inverted phase contrast microscopy and SEM. Standard dentine discs from human molar teeth were prepared and divided into three groups. While the dentine discs in Group 1 did not receive any extra treatment, the discs in Group 2 were treated with acidic calcium phosphate precipitation (CPP) solution. In Group 3, the discs were suspended in phosphate buffered saline containing calcium. DPSC or ODPSC (3×10^4 cells mL^{-1}) were seeded on all discs and incubated for 7, 14 or 21 days. Proliferation rate of cells was assessed with MTT assay. Attachment and growth of 7-day cell cultures on extra dentine samples were examined by SEM. Balanced-ANOVA with factorial design and Tukey's test were used for statistical analysis ($P = 0.05$).

Results Histochemical staining and immunohistochemistry demonstrated that isolation of DPSC and their differentiation to ODPSC were successful. MTT assay showed that number of cells on dentine surfaces was increased by time periods regardless of type of treatment and cells ($P < 0.05$). While DPSC and ODPSC showed similar proliferation rates at 7 and 14 days ($P > 0.05$), the number of ODPSC was higher than DPSC in 21-day samples ($P = 0.039$). Calcium-treated dentine surfaces always had lower number of cells; being significant for only CPP-treated surfaces ($P < 0.01$). According to SEM observations, both types of cells demonstrated good attachment and proliferation on dentine surfaces regardless of type of dentine treatment.

Conclusion Because the nature of dentine surface itself showed good adhesive characteristics with ODPSC and DPSC, additional calcium treatment of dentine surfaces may not be necessary.

SESSION 1: THURSDAY 15 SEPTEMBER 2011

ANATOMY

R1

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Root canal morphology of mandibular premolars

Aim To assess the root canal morphology of mandibular premolars under clinical conditions.
Methodology Clinical evaluation was conducted on 66 mandibular premolars from both sides of the jaw in patients of different age and gender. Three groups of patients were formed according to their age: (i) 21–40, (ii) 41–60, and (iii) over 61 years old. The number of root canals was established by assessing pre-operative radiographs, when negotiating canal orifice(s) and finally during instrumentation. Teeth were divided into two groups with respect to the number of canals: (i) single, and (ii) multiple canals. Root canal morphology was characterised based on the Vertucci classification. Chi-square was used to evaluated the differences between groups ($\alpha = 0.05$).
Results Overall 74.2% of teeth had only one root canal (Vertucci I) with 25.8% having multiple canals. There was no significant difference in the number of canals between 1st and 2nd mandibular premolars ($P > 0.05$). Multiple canals were detected significantly more frequently in males (82.3%) than females (17.7%) ($P < 0.05$). Eight of 17 mandibular premolars with multiple canals had Vertucci type V morphology, six had Vertucci IV, two had Vertucci II, and one had three root canals (Vertucci type VIII). In 63.6% of teeth, the number of canals was visible on the radiographs and in 36.4% during instrumentation of the root canal(s). Single canals were detected in 65.3% and 34.7% of teeth radiographically and during instrumentation, respectively. Multiple canals were detected in 58.8% and 41.2% of teeth radiographically and during instrumentation, respectively.
Conclusions Mandibular premolars had one root canal in the majority of cases. Multiple root canals were more often detected during instrumentation than single canals and tended to be gender-specific but not tooth-specific.

R2

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Analysis of mandibular first molars by micro-computed tomography (μCT)

Aim To evaluate the morphology of mandibular first molars by using μCT.
Methodology Twenty-five extracted human permanent mandibular first molars were evaluated. Each tooth was scanned using μCT (SkyScan 1072; SkyScan b.v.b.a., Aartselaar, Belgium). The following details were recorded for each tooth: number and type of root canals (Weine 2004) and apical foramina; presence of accessory canals, loops and isthmuses, and the distance between the anatomical apex and major apical foramen.
Results The sample of mandibular first molars presented most frequently with a distal and a mesial root (71.9%), and with three root canals (75%). MB and ML canals were completely independent in 12.5% of samples. Isthmuses were found mainly in the coronal and middle thirds of the root (25%; 75%), whereas accessory canals and loops were mainly found in apical third of the root (59.4%). Two apical foramina were identified in the mesial roots of 62.5% of the specimens, while three separate apical foramina were present in 31.2% of the molars studied. The mean distance between major apical foramen and anatomic apex was 0.53 ± 0.37 mm in mesial roots and 0.87 ± 0.48 mm in distal roots.
Conclusions Mandibular first molar teeth display significant variations of root canal anatomy, with a high incidence of isthmuses, accessory canals and loops. The use of μCT offers a non-invasive reproducible technique for the 3-D assessment of root canal systems, providing detailed and informative images of canal anatomy.

R3

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CBCT and microCT study of maxillary molars with fused roots

Aim To investigate the prevalence of maxillary molars with fused roots and to describe their internal anatomy using microcomputed tomography (microCT).
Methodology One hundred clinical cone beam CT examinations (iCAT, voxel size 0.3) were explored with emphasis on the first and second maxillary molars. The presence of the following types of anatomical variation were identified: palatal root fused with mesial root; palatal root fused with distobuccal root; mesiobuccal and distobuccal roots fused and palatal root isolated; C-shaped morphology. For the laboratory-based microCT study, 18 extracted first and second maxillary molars with fused roots were scanned at 18-μm resolution using a SkyScan 1076 microCT. Similar to the CBCT examination the anatomical variations were classified into four categories.
Results From 100 clinical cases evaluated, 33 fused molars were found in 22 patients. In eight patients, the anatomical variability was present bilaterally. The highest incidence was found in second molars (28 cases), the most common fusion occurring between the palatal and mesiobuccal roots. Three one- rooted maxillary molars were found (C-shaped morphology). The laboratory study showed 11 two-rooted maxillary molars and seven one-rooted maxillary molars. From the one-rooted teeth, three showed C-shaped roots canals and four showed confluence of the two buccal canals. The two-rooted maxillary molars showed fusion between the palatal and mesiobuccal roots in seven cases, the palatal root fused with the distobuccal in two cases and the mesiobuccal and distobuccal roots fused in two cases. In these cases a MB2 canal or accessory anatomy, presenting as isthmuses connecting the buccal and palatal canals were usually present.
Conclusions Fused roots were encountered in 22% of the maxillary molars examined in this study. Among these, there was great anatomical variability.

R4

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Effect of age on the density of dentinal tubules at three levels of tooth roots

Aim To assess the influence of patient age and position along the root on the density of dentinal tubules in an *ex vivo* model.
Methodology Twelve single-rooted teeth were allocated into three groups according to patient age, Group 1: 15–24 years, Group 2: 25–44 years, Group 3: 45 years and over. The teeth were decoronated and the roots embedded in acrylic resin and cut into 1 mm thick cross-sections. Representative slices from the coronal, middle and apical portions were cut mesio-distally yielding one useable half. SEM images (850×) were taken of the central portion of the root canal lumen. The number of dentinal tubules in an area of 9200 μm^2 was counted.
Results The number of dentinal tubules was highest in the most coronal section of roots (416 ± 122) and lowest in the apical region (271 ± 136). This trend was seen across all age groups. Teeth from older patients (group 3) had the lowest number of dentinal tubules (259 ± 99) and those of younger patients had the highest (455 ± 74).
Conclusions The density of dentinal tubules decreased in a corono-apical direction and with increase in patient age.
Acknowledgement We wish to thank Miss Liz Girvan of the Otago Centre for Electron Microscopy for her guidance.

R5

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The 'butterfly' optical effect: a SEM investigation

Aim To examine the distribution of dentinal tubules in teeth with the 'butterfly' effect – a difference between mesio-distal and bucco-lingual shades of dentine in root sections.
Methodology Thirty single-rooted teeth were allocated according to patient age; Group 1: 15–24 years, Group 2: 25–44 years, Group 3: 45 years and over. The teeth were decoronated and the roots embedded in acrylic resin and cut into 1 mm-thick cross-sections. Sections were viewed under a light microscope and coded (1 or 2) according to presence or absence of the effect. Each tooth was given an overall score (max 20) when all levels of the root had a butterfly appearance. Two teeth with the highest score were selected from each group, and a control group of two teeth (minimum score 10) selected. From each of the eight teeth two adjacent, consecutive cross sections were chosen. The most coronal was cut mesio-distally and the other bucco-lingually. SEM images (850×) were taken of the central portion of the canal lumen and the number of tubules in an area of 9200 μm^2 counted.
Results The mean number of dentinal tubules was highest in the root sections cut mesio-distally (418) and lowest in those cut bucco-lingually (116). This trend was consistent across all age groups, and the control teeth did not follow this pattern.
Conclusions Root sections with the butterfly effect have a lower density of tubules mesio-distally, corresponding to the 'wings' of the butterfly. This pattern was observed in teeth from all age groups and it was absent in controls
Acknowledgement We wish to thank Miss Liz Girvan of the Otago Centre for Electron Microscopy for her guidance.

R6

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Occurence of hypercementosis in a German population

Aim To assess the occurrence of hypercementosis in a selected German dental school patient population.
Methodology A total of 800 full-mouth periapical radiographs from patients recorded from August 1983 to February 2008 were screened (convenient sample). The radiographs were evaluated under optimal conditions using double magnifying glasses. A total of 14 589 teeth including third molars were evaluated (maxillary premolars and molars: 4740; maxillary incisors: 4155; mandibular premolars and molars: 5289; mandibular incisors: 4405). The relative incidence of hypercementosis and the correlation regarding the location (left- versus right-side occurrence, maxillary versus mandibular teeth, and incisors versus premolars and molars) and between males and females were recorded and analyzed using the chi-square test.
Results Eleven patients were found to have hypercementosis, 10 females and one male ($P < 0.05$). Of these, five had only one hypercementosed tooth (45.46%) and six had more than one (54.54%). In total, 22 teeth associated with a hypercementosis were found. Seven maxillary teeth were associated with a hypercementosis (31.82%) and 15 mandibular teeth (68.18%) ($P = 0.198$). No significant differences were found regarding left- and right-side occurrence of the affected teeth ($P > 0.05$). Hypercementosis was significantly more often associated with premolars and molars than with incisors ($P < 0.01$).
On the patient level, the occurrence of hypercementosis was 1.33%, while for the tooth level the occurrence was 0.12%.
Conclusions The occurrence of hypercementosis among this selected German population was rare. Female patients were approximately 10 times more often affected than male patients. Mandibular teeth were approximately twice as often associated with hypercementosis as compared to maxillary teeth.

R7

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Size and location of the apical constriction in molars: a micro computed tomography study

Aim To study the size and location of the apical constriction in molars using micro computed tomography (μCT).
Methodology Twenty molars with 67 root canals from patients aged between 24 and 43 years (mean 35 years) were selected. The reason for extraction was either unrestorable deep caries or periodontal disease. The teeth were checked for absence of apical resorption and fixed on stubs for the μCT scans. CT data with a

voxel size of 27 μm were obtained using the Inveon MultiModality SPECT/-CT scanner (Siemens, Germany) and reconstructed using the manufacturer's software (V1.4.3.6). A custom made software (Canal measure) was used to perform multi-threshold segmentation and analyze the apical third of the root canals. The smallest cross sectional area of the canal was defined to be the apical constriction. The size of the canal at the apical constriction was recorded as well as the distance to the apical foramen (AC-F) and radiographic apex (AC-A). For each canal type, the means of the recorded parameters were calculated and compared using the Tukey–Kramer test.
Results The type of canal had no influence on AC-F and AC-A. The mean distance of AC-F was 0.7 mm (95% CI: 0.5–0.8 mm; range: 0.4–1.4 mm) and of AC-A was 1.4 mm (95% CI: 1.2–1.5 mm; range 0.7–2.7 mm). The mean size of apical constriction in the distal canals of mandibular molars (ISO 40) was significantly different from all other canals (ISO 20–25).
Conclusions The location of the apical constriction in relation to the apical foramen and radiographic apex was highly variable. In the distal canals of mandibular molars, the size of the apical constriction was larger than in other molar canals.

IMAGING

R8

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A preliminary evaluation of metal artefacts in Cone Beam Computed Tomography images

Aim To determine the extent of artefacts created by metal restorations in CBCT images *ex vivo*.
Methodology Maxillary and mandibular jaw models were fabricated by inserting sound extracted human teeth in Frasaco models (Frasaco, Germany). Teeth were fixed in the model at their anatomical position using silicone putty. Multiple CBCT scans were performed using Kavo 3D eXam (Kavo, Germany) at voxel size of 0.2 and 0.4 mm and 180° rotation. Sound teeth were used for the first CBCT scans. For the following scans, one sound tooth was replaced with one restored with amalgam. The amalgam-restorations were either Class I or II placed in first molar or second premolar positions. Two experienced operators evaluated the image quality for the presence of artefacts using Kavo eXamVision software. The evaluated teeth were: restored, neighbouring, antagonist and contralateral teeth. Each tooth was evaluated at the axial, facial and transverse aspects. Logistic regression analysis was performed to determine the effect of restoration type and position on the extent of artefacts. Inter-operator agreement was also evaluated.
Results Size and location of amalgam-restorations had no effect on artefacts ($P = 0.89$). Regardless of the voxel size, metal streak artefacts were present in restored and both neighbouring teeth. Artefacts were limited to the crowns of the teeth and did not extend to the roots. Less accentuated beam hardening artefacts in the form of radiation beam shadow were observed in the 0.4 mm voxel images. Operators disagreed on the presence of metal artefacts in only one tooth.
Conclusions In CBCT images, metal artefacts resulting from amalgam restorations were limited to the crowns of restored and proximal teeth. Antagonist and contra lateral teeth as well as the roots of restored teeth were not affected by the presence of Class I or II amalgam-restorations.

R9

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CBCT to evaluate three-dimensional filling of the root canal system *ex vivo*

Aim To evaluate three-dimensional root canal filling using CBCT.
Methodology The sample comprised 40 maxillary and 40 mandibular premolars extracted for orthodontic reasons. After preoperative radiographs were taken, pulp tissue was removed and chemomechanical canal preparation was performed using the ProTaper system, accompanied by 5% sodium hypochlorite, and 17% EDTA irrigation. Root canal filling was accomplished using lateral compaction of gutta-percha and AD Seal sealer (META, Biomed). Postoperative radiographs and CBCT were employed to evaluate three-dimensional filling of the root canal system.
Results After analysing postoperative radiographs, 88% of the cases were judged to have complete canal filling. CBCT data indicated that only 68% of the cases had complete three-dimensional canal filling because of lack of instumentation of the second root canal in mandibular premolars, the third root canal in maxillary first premolars and type V Vertucci canals.
Conclusions The results of this *ex vivo* investigation suggest that CBCT may be an important aid in evaluating root canal treatment, especially in cases of complex endodontic anatomy.

LOCAL ANAESTHESIA

R10

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Efficiency and duration of anaesthesia with Ubistesin (Articaine 1 : 400 000) during outpatient dental treatment in a dental school

Aim To test the efficiency and duration of anaesthesia with Ubistesin (Articaine 1 : 400 000 adrenalin; 3M ESPE, Neus, Germany) during dental treatments such as restorations, endodontic treatments or root planing.
Methodology A total of 380 patients (52.1% female, 47.9 % male) with an age range from 18 to 78 years were included. Overall 515 maxillary teeth and 311 mandibular teeth underwent treatment and had to be anaesthetized. In total 287 patients were treated with a block (conduction) anaesthetic (1.7 mL) and in 97 cases an infiltration anaesthetic (1–1.7 mL) was used. The dental treatment included tooth preparation, restorations as well as periodontal therapies such as scaling and root planing and other minor dental procedures. All patients received a questionnaire to indicate the duration of anaesthesia.
Results In 327 (86%) patients it was not necessary to give a supplementary injection during the dental treatment; for 369 patients it was possible to complete the dental treatment and most of the patients (377) had no after-pain. In most cases the treatment involved removal of caries (42%), root canal access (8%), scaling and root planing (17%), restoration (11%) and others (22%). The average time course of the treatments was: commencing 5 min after the anaesthesia, repeat injection after 54 min, to finish the dental treatment within 90 min. The mean time for normal sensation to return was 164 min. All patients had satisfactory

anesthesia following Articaine (1 : 400 000), although the individual sensation of pain constituted a factor that was not easy to estimate.
Conclusions In this study population; satisfactory local anaesthesia was achieved using Articaine with only 1 : 400 000 epinephrine. Dentists should justify the use of agents with higher vasoconstrictor concentration on a case by case basis.

APEX LOCATORS/WORKING LENGTH

R11

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An investigation into the relationship between apical root impedance and canal anatomy

Aim To investigate *ex vivo* the effects of canal anatomy parameters on the impedance characteristics of the apical 5 mm of root dentine.
Methodology Fifty-nine roots from 42 extracted teeth were selected. Impedance measurements were taken using a frequency response analyser at eleven levels in the root (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 mm short of the apical terminus) at 14 frequencies ranging from 1120 to 100 000 Hz. The impedance characteristics were used to select the best fitting equivalent circuit from a pool of 37 for each level within the root. Apical anatomy was evaluated by staining and clearing the dentine and the roots were divided into two groups; 11 had simple (Vertucci type I) and 48 had complex anatomy. The effects of anatomical parameters (broad canals, number of foramina and canals, isthmuses, lateral canals, ramifications) were analysed using multinomial logistic regression and cluster analysis. Equivalent circuits were used as the single outcome to describe the impedance characteristics.
Results Equivalent circuit model 16 was found to be the most prevalent in both groups whilst circuit 10 appeared exclusively in the 'simple' group. Circuits 13, 18, 26, 27 and 30 occurred mainly in the complex group. Multinomial logistic regression using 'model 16' as the base outcome revealed all the anatomical parameters had significant ($P < 0.05$) influence on the impedance characteristics. The anatomical parameters and distance from the apical terminus were used to generate 12 cluster groups of 'root dentine' which were found to have significant ($P < 0.0001$) associations with the groups of dentine represented by the 12 equivalent circuit models. Both analyses revealed that the distance from the apical terminus had no significant ($P > 0.05$) influence on equivalent circuits.
Conclusion Complexity of canal anatomy had a significant effect on the impedance characteristics of the corresponding dentine.

R12

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Working length determination with the impedance ratio method for deciduous and immature permanent teeth

Aim To determine optimal measuring parameters of the impedance ratio method for working length determination (WLD) for primary and immature permanent teeth *ex vivo*, and to evaluate its accuracy.
Methodology Electrical impedance was measured on 11 extracted primary teeth (group A) and 10 extracted permanent teeth with incomplete root formation (group B) using a QuadTech 1920 precision impedance analyzer. A file electrode was inserted into the root canal; the second electrode was placed in saline solution surrounding the tooth. Measurements were performed in a frequency range from 20 Hz to 1 MHz, and repeated with different distances of the file tip from the major apical foramen. The measured impedances were analysed as a function of distance of the file tip to the apical foramen. Parameters that would result in optimal WLD (highest number of samples with the distance from the major apical foramen within ±0.5 mm) were evaluated.
Results For group A, optimal WLD was obtained at frequencies of 50 and 0.5 kHz, and 50 and 1 kHz for the impedance ratios 0.51 (95% CI: −0.47, 0.04 mm) and 0.56 (95% CI: −0.49, 0.03 mm), respectively; whilst for group B at frequencies of 10 and 0.5 kHz, 5 and 1 kHz, and 10 and 1 kHz for the impedance ratios 0.65 (95% CI: −0.68, 0.12 mm), 0.76 (95% CI: −0.69, 0.16 mm), and 0.66 (95% CI: −0.62, 0.06 mm), respectively. The limit of ±0.5 mm was attained in 82% and 60% of all measurements for group A and B, respectively.
Conclusions The accuracy of the ratio method depended on the set of frequencies used for evaluation and on the selected impedance ratio. The optimal measuring parameters for primary and immature permanent teeth were different. The results could drive further advancement of these measuring devices.

R13

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Accuracy of the apex locating function of different endodontic motors

Aim To compare the accuracy of the apex locating function of different endodontic motors; VDW Gold, X-Smart™ Dual and SIROEndo Pocket during root canal treatment using an *ex vivo* model.
Methodology Sixty freshly extracted human anterior teeth were selected. Following access cavity preparation, the length of root canals was measured visually with a K-file 1 mm short of apical foramen (true working length – TWL). All samples were mounted in alginate and randomly divided into three groups. Group A: before root canal preparation, apex locator function of VDW Gold was used according to the manufacturer's recommendations and working lengths measured (WL1-A). During root canal preparation, constant length monitoring was performed with VDW Gold (WL2-A). Group B: before root canal preparation, apex locator function of X-Smart™ Dual was used according to the manufacturer's recommendations and working lengths measured (WL1-B). During root canal preparation, constant length monitoring was performed with X-Smart™ Dual (WL2-B). Group C: before root canal preparation, apex locator function of SIROEndo Pocket was used according to the manufacturer's recommendations and working lengths measured (WL1-C). During root canal preparation, constant length monitoring was performed with SIROEndo Pocket (WL2-C). Differences between working lengths were calculated.
Results There were no statistically significant differences between both working lengths and the three endodontic motors.
Conclusions Within the limitations of this *ex vivo* study, the combination of electronic apex locators and constant length monitoring functions in endodontic motors were able to achieve accurate length control during root canal preparation.

R14

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Accuracy of Dentaport ZX for determining the working length in retreatment cases *ex vivo*

Aim To evaluate the accuracy of the Dentaport ZX apex locator for working length determination during root canal retreatment on mesiobuccal and distal canals of mandibular molars *ex vivo.*
Methodology Fifteen extracted human mandibular molars with two separate mesial canals and apical foramina and one distal were used. After access cavity preparation, the crowns were removed to provide a horizontal reference surface for length measurement. The length between the coronal surface and file tip positioned at the major diameter was determined as major foramen length (MFL). Root canals were prepared with ProTaper files (Dentsply Maillefer, Switzerland) to 1 mm short of the MFL and filled with gutta-percha and AH Plus (Dentsply De Trey GmbH, Germany). One week later, root fillings were removed using ProTaper retreatment files (Dentsply Maillefer). After removing the root filling, the length between the coronal surface and file tip positioned at the major diameter was determined as retreatment major foramen length (RMFL). The electronic measurements were taken at the Mod1 and Mod2 which were seen on the Dentaport ZX (J. Morita Corp., Japan) display. The length between the coronal surface and file tip at Mod1 and Mod2 were measured and determined as Mod1 length (Mod1L) and Mod2 length (Mod2L). The Mod1L and Mod2L were compared with RMFL, RMFL-0.5 and RMFL-1.
Results For both of the mesiobuccal and distal canals, no significant difference was found between the RMFL/Mod1L and RMFL-0.5/Mod2L ($P > 0.05$) while significant differences were detected between the RMFL-0.5/Mod1L, RMFL-1/Mod1L and RMFL-1/Mod2L ($P < 0.05$).
Conclusion The Dentaport ZX accurately detect the major foramen in root filled teeth undergoing retreatment *ex vivo.*

R15

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In vivo accuracy of five electronic apex locators in determining the apical foramen in molar teeth

Aim To compare *in vivo* accuracy of five electronic apex locators (EALs) in determining the apical foramen in molar teeth. Null hypothesis: there is no difference in accuracy amongst five EALs.
Methodology In 150 root canals of 82 M indicated for extraction, electronic working lengths were determined to the major apical foramen using one of the five EALs (randomised, 30 canals per EAL). When stable readings were achieved, files were fixed with composite resin. Following extraction, the apical 3–4 mm of roots with files inside the canals were exposed and photographed under a stereo microscope (SZ-TP; Olympus Corp., Japan) at 26× magnification with a built-in scale of 0.01 mm precision. Distances from the file tip to the tangent of the major foramen and root apex were measured and statistically analysed.
Results The null hypothesis was upheld since no statistically significant differences were found amongst the five EALs in determining the position of the apical foramen ($P > 0.05$; ANOVA). Average tip-to-foramen distances were: 0.148, 0.165, 0.169, 0.187 and 0.189 mm for Apex NRG XFR, Dentaport ZX, Propex I, Raypex 5, and Apex Pointer$^+$, respectively. No measurements beyond the foramen were recorded with the ApexNRG XFR, one with the Apex Pointer$^+$ (0.129 mm), two with the Dentaport ZX (0.103 mm), three with the Raypex 5 (0.147 mm), and six with the Propex I (0.226 mm) with an overall average of 0.176 ± 0.072 mm. In only one of 150 cases did the file tip extend to the root apex, whilst the remaining 149 were on average 0.677 ± 0.292 mm short of the apex with a significant difference only between Apex NRG XFR and Propex I (Kruskal–Wallis test, $P = 0.0141$).
Conclusions The tested EALs were accurate in locating the apical foramen, within a clinical tolerance below ±0.5 mm. In 12 of 150 cases (8%) the file tip was beyond the major foramen.

R16

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Clinical evaluation of the accuracy of two electronic apex locators

Aim To examine the clinical reliability of two latest-generation electronic apex locating devices, Propex (Dentsply Maillefer) and Apex Finder mod. 7005 (EIE/Analytic Technology).
Methodology The study sample comprised 81 consenting patients, with 100 teeth and 180 root canals undergoing treatment. Propex was used in 86 root canals and Apex Finder in the remaining 94. All measurements were made after ProFile instrumentation of the coronal and middle third of the root canals and irrigation with 5% NaOCl. Thereafter, a file was introduced into the canal and forwarded until the apex locator showed that it reached the apex. A radiograph was then taken and the distance from the end of the file to the radiographic root apex was measured. Apex locator measurements were considered accurate when the distance between the tip of the file and the radiographic root apex ranged from 0 to 2 mm short of the apex.
Results Propex was accurate in all 86 root canals (success rate: 100%). The Apex Finder had an overall success rate of 80%. In detail, the Apex Finder was accurate in 40 of 46 root canals with vital pulps (success rate: 87%), in 17 of 21 root canals with non vital pulps (success rate: 81%) and in 15 of 23 root canals in retreatment cases (success rate: 65%).
Conclusions The clinical accuracy of both electronic devices was high. Propex was found more accurate clinically than the Apex Finder.

R17

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Influence of clinical experience on the accuracy and consistency of estimating radiographic working length adjustment

Aim To determine the influence of clinical experience on the accuracy and consistency of estimating the optimum radiographic working length for single-rooted teeth.
Methodology Ethical approval was obtained and 40 radiographs of single-rooted teeth showing files at different lengths from the radiographic apex were selected from patient records. Radiographs were digitally scanned, arranged as PowerPoint presentations on CDs and distributed to: Group 1: 35 fourth year undergraduate students (class of 2010), Group 2: 35 fourth year undergraduate students (class of 2009), Group 3: 10 endodontic postgraduates. Evaluators were asked to indicate the adjustment needed to achieve correct working length for each tooth. After 2 weeks, the same

evaluators reassessed half of the images. A gold standard group of three endodontists reached a consensus for each image. Data were analyzed using the Kruskil–Wallis test to compare inter-group scores and Kappa scores were calculated to determine intra-examiner consistency.

Results The estimations of Group 3 were the most accurate. Significant differences ($P < 0.05$) were found between groups 3 and 1 but not between groups 3 and 2. The accuracy of group 2 was not significantly better than group 1. Individuals' Kappa scores varied between the two assessments without a consistent pattern being identified. Intra-group consistency was maintained for all groups despite the 2-week elapse between the assessments.

Conclusions The clinical experience of the assessors influenced their accuracy of estimating adjustments required to achieve appropriate radiographic working length in the single rooted teeth studied. Intra-examiner and intra-group consistencies were not influenced by clinical experience or a two-week elapse between assessments.

R18

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Root canal length determination using Cone Beam Computed Tomography

Aim To assess the accuracy of Cone Beam Computed Tomography (CBCT) in determining root canal length *ex vivo*.

Methodology The real lengths of 25 root canals were determined under a stereomicroscope, Stemi 2000 c (Carl Zeiss GmbH, Germany) with 4× magnification. A file was introduced into the root canal until the tip was flush to the apical foramen. Two operators performed the measurements and repeated each measurement twice. Teeth were then integrated in an A-RS mandibular model (Frasaco, Germany) and CBCT scanned using KaVo 3D eXam (KaVo Dental GmbH, Germany) at a voxel size of 0.2 mm and 180° rotation. Canal length to the apical foramen was measured using the manufacturer's software (eXam Vision). The measuring tool provided was used to draw a line from the corresponding cusp tip to the canal orifice. Another straight line was drawn from canal orifice to the apical foramen regardless of the canal curvature. The total length of both lines was recorded to be the CBCT canal length. For each canal the difference between real and CBCT length was determined. Paired *t*-test with alpha set to 0.5 was performed. Inter-operator agreement was determined for real and CBCT lengths.

Results The mean difference between real and CBCT lengths was 0.3 mm. Although *t*-tests indicated a significant difference ($P = 0.0001$) the clinical relevance of 0.3 mm difference is minor. Inter-operator agreement for real length (mean 0.2 mm) was not statistically significantly different ($P = 0.67$) from that of CBCT length (mean 0.3 mm).

Conclusions CBCT images can be used for accurate determination of root canal length *ex vivo*.

R19

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Working length variation during mechanical preparation of curved root canals with rotary instruments *ex vivo*

Aim To compare the endodontic working length (WL) measured at different stages of mechanical preparation of severely curved root canals in extracted teeth using sequences of new type S5 nickel-titanium rotary instruments.

Methodology The material consisted of 17 selected root canals of extracted molars and premolars with curvature more than 20° defined by the radiological Schneider's method. Horizontally sectioned roots were cemented in acrylic in order to obtain mechanical resistance. Root canals were prepared using a crown-down technique with a sequence of five rotary instruments S5 (Poldent) driven by a cordless endodontic S5 Endo Motor with a speed range 150–300 rpm. Sodium hypochlorite 2% and Filecare EDTA compound (VDW, Germany) were used to remove the organic remnants and smear layer. Initial and subsequent working length measurements were determined by means of size 08 or 10 C-Files (VDW) and an electronic caliper with an accuracy 0.01 mm. A consistent reference point was set for the anatomical opening of the root canal (mechanical resistance due to acrylic); the second point was on the bottom edge of the silicone stop.

Results The average working length of initially negotiated canals was 15.34 mm (±1.38 SD), while the final WL after full chemo-mechanical preparation was 15.05 mm (±1.4 SD). The average reduction of WL after preparation was 0.28 mm with respect to the initial value. The biggest average variation in WL (0.07 mm) was observed after the third S5 instrument. The differences between instruments were not statistically significant.

Conclusions Mechanical root canal preparation using S5 Ni-Ti rotary instruments with increased taper and when using a crown-down technique reduced the final working length of severely curved root canals in extracted teeth.

IRRIGANTS/DISINFECTION: IRRIGANT AGENTS

R20

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In vitro evaluation of endodontic irrigant cytotoxicity

Aim To compare the biocompatibility of currently used intracanal irrigants with Tetraclean, an antibiotic-based solution.

Methodology Mouse embryonic fibroblasts BALB 3T3 were growth in Dulbecco's minimal essential medium and exposed to increasing dilutions of 5% and 2.5% sodium hypochlorite (NaOCL), 0.2% chlorhexidine and 0–5 mg mL^{-1} Tetraclean (Ogna Laboratori Farmaceutici, Italy). After 24 h, cell mitochondrial activity was evaluated by the methyltetrazolium test (MTT). The Results were expressed as the percentage of unaffected cells and statistical analysis was performed by Mann–Whitney *U*-test ($P < 0.05$).

Results All the irrigants decreased cell viability in a dose related manner. Cytotoxicity based on concentrations which caused 50% inhibition of cell proliferation (ID50) were ranked as follows: 5% NaOCl (1.1 mg mL^{-1}) > 0.2% Clorhexidine (1.4 mg mL^{-1}) > 2.5% NaOCl (2.0 mg mL^{-1}) > Tetraclean (2.3 mg mL^{-1}).

Conclusions Since root-canal irrigants and medicaments can reach periradicular tissues, they should combine their antimicrobial properties with acceptable biocompatibility. This *in vitro* study demonstrated that among the irrigants tested the new antibiotic-based irrigant Tetraclean was less cytotoxic than NaOCl and Chlorhexidine solutions.

R21

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Cytotoxicity of different root canal medicaments on cultured human gingival fibroblasts

Aim To compare the cellular toxicity and apoptotic effect of different root canal medicaments; calcium hydroxide, 1% chlorhexidine gel, bioactive glass (S53P5) and two medicated guttapercha points (Roeko Active Points, Roeko Calcium Hydroxide PLUS Points) on cultured human fibroblasts.
Methodology The effect of the extract of each root canal medicaments on the viability of human gingival fibroblasts was determined after 24 h and 7 days incubation using a WST-1 cell viability assay. Patterns of cell death were analyzed using the Cell Death Detection ELISA plus kit. The Bonferroni Adjusted Kruskal–Wallis and Conover's multiple comparison tests were applied to evaluated the results ($P < 0.05$).
Results Bioactive glass and calcium hydroxide plus resulted in lower *in vitro* toxicity than other agents tested at 24 h and 7 days. Chlorhexidine gel had the highest toxicity at 24 h and Active point at 7 days. The difference was statistically significant ($P < 0.017$). Considering the patterns of cell death, Active Point induced more apoptosis than other agents at 7 days but the difference was not statistically significant among the groups ($P > 0.05$).
Conclusions Taking into consideration the limitations of this *in vitro* experiment, points containing calcium hydroxide and bioactive glass were the least cytotoxic medicaments.

R22

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Ability of $Ca(OH)_2$ based root canal medicaments to maintain their alkalinity *in vitro*

Aim The aim of the study was to assess different $Ca(OH)_2$ products for their ability to maintain alkalinity against acid and in the presence of dentine powder.
Methodology A constant volume of 0.5 mL of six commercial $Ca(OH)_2$ products, Biokalkki®, Calasept®, Ultracal®XS, DentalSystems CaOH-gel®, Calxyl®blue, $Ca(OH)_2$-gutta-percha Plus points® and pure $Ca(OH)_2$ powder in distilled water, were compared *in vitro* for their ability to maintain alkalinity in 100 mL distilled water by titrating them with 1 mol hydrochloric acid. Saturated solutions of each product were also tested in the presence of standardised dentine powder for their ability to resist dentine buffering effect. Measurements were repeated three times for each specimen and the mean values and standard deviations were calculated.
Results All $Ca(OH)_2$ products formed saturated solutions with pH ranging between 12.96 and 13.55. To maintain a pH value of 11, Calasept and pure $Ca(OH)_2$ powder in distilled water tolerated most hydrochloride acid (9.1 ± 0.05 mL and 8.3 ± 0.0 mL, repectively) followed by DentalSystems CaOH-gel® (7.1 ± 0.2), Ultracal®XS (6.5 ± 0.26), Biokalkki® (6.2 ± 0.3), Calxyl®blue (4.9 ± 0.3) and $Ca(OH)_2$-gutta percha Plus points® (1.5 ± 0.09). Dentine powder was able to reduce the pH down to 8.5 in saturated solutions of all the products within 24 h.
Conclusions Additives in $Ca(OH)_2$ products reduced the amount of soluble $Ca(OH)_2$ in the constant volume simulated main canal. Different types of $Ca(OH)_2$ products all formed saturated solutions with high pH but the effect was greatly reduced by dentine.

R23

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The effects of Er:YAG laser on intra-canal irrigation *in vitro*

Aim To clarify the working mechanism of intra-canal irrigation by pulsed lasers.
Methodology Using a high-speed camera, vapour and cavitation bubbles induced by Er:YAG laser were visualized in an aqueous environment within a glass root canal model. In addition, the motion of glass-bead tracers was captured in the root canal model.
Results The vapour bubbles produced by cone-shaped and flat tipped laser tips were different in shape and longevity. In the root canal model, vapour bubbles grew up and down along the canal wall, followed by the repeated formation and collapse of many smaller cavitation bubbles. The analysis using tracers showed rapid agitation caused by laser irradiation.
Conclusions Within the limitations of this laboratory model, these results suggest that the mechanism of Er:YAG laser irradiation in root canal irrigation might be attributed to high-speed fluid motion due to bubble formation and collapse. An Er:YAG laser with a cone-shaped tip may be promising for intra-canal irrigation.

R24

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Apical extrusion of irrigant with Nd:YAP laser at different depths and power settings *ex vivo*

Aim To evaluate the apical extrusion of irrigant from root canals of extracted teeth following activation with Nd: YAP laser at different depths and power settings.
Methodology Fifteen straight single-rooted teeth were decoronated and working lengths determined. The root canals were then instrumented with Mtwo rotary (VDW, Munich, Germany) NiTi instruments to size 35, 0.04 taper and irrigated with 3 mL NaOCl (3%). Apical patency was tested with a size 10 hand file. After a final rinse with 17% EDTA (Vista Dental, USA) the roots were dried. 20 mL irrigant (H_2O) was mixed with 10 mL fuchsine (Certa, Belgium), and the coloured irrigant placed in the root canals. The positive control was a combination of the liquid and fiberoptic without activation; the negative control was the liquid in the root canal without fibreoptic. A 200 μm fibreoptic activated by a Nd: YAP laser (Claremont Lokki, France) with a wavelength of 1340 nm was placed at 5, 7 and 10 mm from the apical working length. The liquid was activated at three power settings (180 mJ, 0.9 w, 5 Hz; 280 mJ, 1.4 w, 5 Hz; 360 mJ, 1.8 w, 5 Hz). Apically extruded irrigant was visualized by placing a white napkin 10 mm from the apex. The score was given, extrusion = 1, no extrusion = 0. Data were analysed by the non-parametric Kruskal–Wallis test.
Results The positive and negative control groups showed no extrusion. In the experimental groups, the occurrence of extrusion was higher with shorter distance of the fibre from the apex (KW, $P < 0.0001$) and higher power setting (KW, $P = 0.001$).
Conclusions The Nd: YAP laser could be used safely for activating root canal irrigant at 10 mm from the apical working length in extracted teeth. Extrusion increased with higher power settings.

R25

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Interactions between sodium hypochlorite and bioactive glass 45S5

Aim To test the compatibility of bioactive glass 45S5 (BG) particles with a sodium hypochlorite solution. Whether NaOCl maintained its soft tissue dissolving effect, whilst BG should continued to form hydroxyapatite on its surface in suspension was also evaluated.
Methodology Soft tissue dissolution was assessed by immersing a total of 144 bovine pulp tissue specimens (n = 12 per group per time) in standardized suspensions (200 mg in 360 μL) of BG in either physiological saline (0.9% NaCl) or 1.0% NaOCl. NaOCl and saline solutions served as controls. Tissue pieces of similar weight (37 ± 23 mg) were immersed for 10, 100, and 1000 min, and tissue weight loss was recorded using a precision balance. Data at each time point were compared between groups using one-way ANOVA followed by Bonferroni correction. The alpha-type error was set at 0.05. To assess precipitate formation, BG particles were either immersed in 0.9% NaCl or 1.0% NaOCl for up to 4 weeks at 37°C and then subjected to Raman spectroscopy.
Results Necrotic pulp tissue specimens gained weight when they were immersed in saline solution. Counterparts immersed in BG/saline suspensions first gained weight ($P > 0.05$ compared to saline after 10 min), but lost weight after 1000 min ($P < 0.05$ compared to saline). The effected weight loss was immediate and significantly stronger when NaOCl was in the system ($P < 0.05$ compared to other groups). It was similar between 1% NaOCl and the BG/1% NaOCl suspension at 100 and 1000 min ($P < 0.05$, each). Immersion of BG in either solution showed the corresponding spectroscopic peaks for carbonated hydroxyapatite at 960 and 1080 cm^{-1}.
Conclusions Both compounds maintained their desired effect. BG was compatible with sodium hypochlorite regarding the outcomes investigated in this study.

R26

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Evaluation of different irrigation protocols on chemical smear layer formation

Aim To evaluate the formation of chemical smear layers following different irrigation protocols by Scanning Electron Microscopy (SEM) and Electron Dispersive Spectroscopy (EDS).
Methodology Fifty-five teeth were instrumented using distilled water (DW) as irrigant. They were then subjected to a protocol that consisted of ultrasonication for 10 min in an ultrasonic bath in 17% EDTA, followed by 10 min in a 5.25% sodium hypochlorite (NaOCl) bath, and a phosphate buffer solution bath (to eliminate EDTA and hypochlorite residues) followed by a DW bath (10 min each), in order to eliminate the smear layer produced during the initial preparation. The teeth were then divided into eleven groups, according to the irrigation regimen. The irrigants used were: 5.25% NaOCl, 2% chlorhexidine gel (CHXG) and solution (CHXS), 17% EDTA, 10% citric acid (CA) and 37% phosphoric acid (PA). The teeth then were prepared and analyzed by SEM and EDS. The groups evaluated were: Group 1-NaOCl/DW/CHXS; Group 2-NaOCl/DW/EDTA/DW/CHXS; Group 3-NaOCl/DW/CA/DW/CHXS; Group 4-NaOCl/DW/PA/DW/CHXS; Group 5-CHXG/DW/EDTA/DW; Group 6-CHXG/DW/CA/DW; Group 7-CHXG/DW/PA/DW; Group 8-CHXG/DW/EDTA/DW/CHXS; Group 9-CHXG/DW/CA/DW/CHXS; Group 10-CHXG/DW/PA/DW/CHXS, and Group 11-only DW.
Results In Groups 1, 2 and 3, chemical smear layer (CSL) formation was observed. Group 4, using phosphoric acid between NaOCl and CHXS, did not induce CSL formation. In Groups 5–10, where CHXG was used alone or in sequence with EDTA, CA, PA and CHXS (without being used with NaOCl), a chemical smear layer was not observed.
Conclusion The sequential use of NaOCl and CHX induced chemical smear layer formation despite intermediate flushes with distilled water, EDTA and citric acid. (Supported by FAPESP 2009/53976-0, 2010/50817-5; CNPq 302575/2009-0 and CAPES).

IRRIGANTS/DISINFECTION: DENTINE INFECTIONS

R27

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Influence of patient age on bacterial invasion, distribution, and viability in root dentine

Aim To assess the influence of patient age on the distribution of viable bacteria in different levels of root dentine in an *ex vivo* model.
Methodology Thirty single-rooted teeth were assigned according to patient age into Group 1: 15–24 years, Group 2: 25–44 years, Group 3: 45 years and over. Cementum was removed and the teeth decoronated. The root canals were prepared using a crown-down rotary technique. Smear layer was removed in an ultrasonic bath with 18% EDTA followed by 5.25% NaOCl. Samples were washed ultrasonically and stored in saline for one week. Roots were then inoculated with *Enterococcus faecalis* and incubated for ten days with replenishment of medium every second day. They were stained with LIVE/DEAD® stain (Baclight Bacterial Viability Test; Invitrogen, USA), and cross-sectioned along their entire length at 1 mm intervals. Bacterial concentrations from the cervical, middle, and apical thirds of each root were quantified using confocal laser scanning microscopy (Zeiss LSM 510, Axioplan 200; Carl Zeiss Ltd., Germany).
Results Concentrations of viable bacteria were higher in the inner third of the dentine compared to peripherally ($P < 0.001$). Density of bacterial penetration was significantly higher in the cervical parts of the root than in both middle and apical locations ($P < 0.001$). Teeth from older patients (Group 3) had significantly fewer bacteria present in their dentine than either Group 1 ($P = 0.004$) or Group 2 ($P = 0.02$).
Conclusions The pattern of viable bacterial invasion decreased in a corono-apical direction. A decrease in the bacterial density in the tubules was observed with increase in patient age.
Acknowledgement We thank Mr Andrew McNaughton for his guidance with confocal microscopy and Professor Peter Herbison for statistical advice.

IRRIGANTS/DISINFECTION: ANTIMICROBIAL ACTIVITY

R28

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Antibacterial effect of root canal lubricants

Aim To assess the antibacterial activity of 10 widely used root canal lubricants.

Methodology The following lubricants were selected- RC Prep (Premier Dental Products, USA), Endo Prep (Professional Dental Supplies, Australia), Endosure Prep Crème (Dentalife, Australia), Prep-Rite (Pulpdent Corp., USA), Glyde (Dentsply Maillefer, Switzerland), SlickGel ES (SybronEndo, USA), KY Jelly (Johnson & Johnson, Australia), File-Eze (Ultradent, USA), File-Rite (Pulpdent Corp., USA) and Alpha Glide (Komet, Germany). Five serial dilutions (10-fold) of each lubricant were tested by addition to bacteriological growth media inoculated with either *Enterococcus faecalis* or *Pseudomonas aeruginosa*. Preparations were incubated at 37°C and samples spot plated onto tryptic soy agar after 6, 12 and 24 h. After 24 h incubation, agar plates were examined for growth and minimum inhibitory concentrations (MIC) determined. The experiment was repeated three times.

Results RC Prep, Endo Prep, Endosure Prep Crème, Prep-Rite, Glyde and SlickGel ES demonstrated antibacterial activity against both species, with detectable antimicrobial activity at dilutions of one part in a hundred. Endo Prep, which contains 0.85% cetrimide, performed considerably better at all time intervals than the other lubricants, with antimicrobial activity at one part in one thousand. KY Jelly (which contains 0.25% chlorhexidine) displayed antibacterial effects at a one in ten concentration. File-Eze, File-Rite and Alpha Glide had little or no antibacterial effect. All lubricants that displayed an antibacterial effect against both bacterial species had increased efficacy over time.

Conclusions Within the limitations of this *in vitro* investigation, most root canal lubricants had antibacterial properties, and the endodontist's choice might be influenced by these findings.

R29

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Root canal disinfection with 6% NaOCl at different exposure times using two irrigation techniques *ex vivo*

Aim To evaluate the disinfection of root dentine using 6% sodium hypochlorite at different exposure times and using two irrigation techniques: Conventional (syringe) and passive ultrasonic irrigation (PUI).

Methodology Seventy freshly extracted teeth were used. After cleaning and shaping of the root canals using the crown down technique with ProTaper (Dentsply Maillefer, Switzerland) rotary NiTi files, the smear layer was removed and teeth were sterilized. The teeth were divided into four groups of 15 teeth each (A, B, C and D) and were contaminated with *Enterococcus faecalis*. The remaining 10 served as positive and negative control groups. Groups A and B were irrigated using a conventional technique and 6% NaOCl, which was left for 10 (group A) and 15 min (group B). After irrigation, a size 25 Hedström file (SybronEndo) was introduced to file the dentine walls to produce dentine shavings and paper points were use for debris collection. The other two groups were irrigated as previously shown for 9 (group C) and 14 min (group D), with an additional last minute of passive ultrasonic irrigation. Then the teeth and the paper points were placed in a brain heart infusion (BHI) broth and were incubated for 24 h. Disinfection of the samples was determined by the presence or absence of turbidity in the broth. Data were collected and statistically analyzed (chi square test).

Results Presence of infection was observed in groups A and B. In groups C and D, there was absence of turbidity. A statistically significant difference was found between all groups A, B, C and D ($P < 0.05$).

Conclusions The results of this *ex vivo* study revealed that 6% NaOCl aalong with passive ultrasonic irrigation was more effective than conventional irrigation in eliminating *E. faecalis*, regardless of the exposure times.

R30

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The effect of three irrigation techniques on the reduction of intracanal *Enterococcus faecalis ex vivo*

Aim To compare intracanal bacterial reduction promoted *ex vivo* by three irrigation techniques with regard to the standardized volume of NaOCl and standardized irrigation time.

Methodology Forty-eight single-rooted teeth with straight canals of approximately the same volume were instrumented with ProTaper istruments and sterilized in plasma. Forty-two root canals were innoculated with an *Enterococcus faecalis* suspension, and the remaining six canals were filled with sterile broth and served as negative controls. After 24 h of incubation, 36 root canals were randomly distributed into three groups of 12 roots each according to the irrigation technique used; group 1, conventional syringe irrigation using needle; group 2, automated-dynamic irrigation using RinsEndo; group 3, passive ultrasonic irrigation (PUI) using Piezon Master 400. The remaining six root canals served as positive controls. Antimicrobial efficacy of the irrigation techniques was analysed in regard of standardized volumes of 3% NaOCl (20 mL) in the first protocol, and in regard of standardized irrigation time (45 s) in the second protocol. Samples taken immediately after irrigation were cultured and the colony-forming units (CFUs) were counted.

Results When the volume of the irrigant was standardized, RinsEndo was more efficacious than PUI ($P < 0.01$), however, there were no statistically significant differences between other techniques. When the irrigation time was standardized, there were no significant differences between any irrigation technique ($P > 0.05$). The highest percentage of minimal counts of *E. faecalis* CFUs was noted in RinsEndo group in both protocols.

Conclusions On the basis of this *ex vivo* investigation, RinsEndo was superior to PUI only when the volume of the irrigant was standardized. However, RinsEndo seemed to be more beneficial because it caused higher bacterial reduction in both protocols when using the least amount of irrigant, and provided longer contact time.

R31

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Antimicrobial activity of varying concentrations of two different chemical solutions against *Candida albicans* biofilms

Aim To compare the antimicrobial activity of different concentrations of tetraacetylethylenediamine + sodium perborate (TAED + P) with that of sodium hypochlorite (NaOCl) against *Candida albicans* biofilms.

Methodology Biofilms were generated using cellulose nitrate membrane filters. Membranes were inoculated with a suspension of *C. albicans* in BHI agar. Plates were then incubated for 48 h at 37°C. After incubation, the membranes were transferred into tubes containing 5 mL of the selected antimicrobial solution or sterile

saline solution. Samples were taken after 2, 5, 20 and 30 min, transferred into tubes containing neutralizing broth for 5 min, diluted and then plated. After incubation, CFU/membrane were determined. Three replicates were performed for each sample. Statistical analysis was performed by using ANOVA and Tukey–Kramer tests at a P level of 0.05 to determine the interaction between time and concentration of the chemicals tested on biofilm viability.

Results Significant differences ($P < 0.05$) were found in the comparisons of all the concentrations of the two antimicrobial agents tested, TAED + P, NaOCl against the control group after each time point. No statistically significant differences were found between NaOCl and TAED + P solutions when used undiluted or at high concentrations at various time points. While all concentrations of NaOCl proved to be effective against *C. albicans* biofilms after 30 min of exposure, *C. albicans* biofilms treated with TAED+P were totally eradicated at 20 and 30 min at all dilutions tested.

Conclusions The antimicrobial agents investigated in this experimental biofilm model showed a different efficacy correlated with the concentration and the duration of the exposure. Both TAED + P and NaOCl were able to completely eliminate *C. albicans* biofilms at different exposure times when used at high concentrations, indicating that TAED + P has at least similar antimicrobial activity to NaOCl under the conditions tested.

R32

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The efficiency of poly(hexamethylene biguanide) hydrochloride (PHMD) as a root canal irrigant

Aim To test the efficacy of poly(hexamethylene biguanide) hydrochloride (PHMD) as a root canal irrigant against *Enterococcus faecalis* and *Pseudomonas aeruginosa* in an experimental model *ex vivo*.

Methodology Crowns and apical portions of 22 extracted single-rooted human teeth were removed with diamond burs and the root canals instrumented with Hedström files to size 40. Smear layer was removed with sequential usage of 17% EDTA and 2.5% NaOCl and then sterilized in autoclave at 121°C for 15 min. Specimens were randomly divided into even groups and mounted with sticky – wax at the bottom of cell culture wells. Eleven specimens were inoculated with an overnight broth culture of *E. faecalis* (ATCC 51299) and eleven with *P. aeruginosa* (ATCC27853). After 48 h incubation and irrigation with saline, dentine samples were collected from inner root canal walls with Hedström files size 50 before and after irrigation with 10 mL of 1% PHMD (2 mL every 2 min) followed by irrigation with 2 mL saline. Samples were put into vials containing 2 mL of sterile saline and 10-fold dilutions, were plated on blood agar (Oxoid, UK) after 30 s vortexing. Colony forming units (CFU) were counted after 48 h incubation at 37°C. Wilcoxon Signed Ranks test was used for statistical analysis.

Results After treatment with PHMD *P. aeruginosa* was eradicated from all specimens and *E. faecalis* from eight of 10 specimens. In two samples that grew *E. faecalis* after treatment reduction was from 10^4 to 10^1 CFU mL^{-1}. These results were statistically significant for both groups of microorganisms tested ($P < 0.01$).

Conclusions PHMD in 1% concentration effectively reduced the number of *E. faecalis* and *P. aeruginosa* in an *ex vivo* experimental tooth model and therefore its role as an endodontic irrigant should be studied further.

R33

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Antibacterial effects of neutral electrolyzed functional water on *Enterococcus faecalis*-derived biofilm

Aim The purpose of this study was to investigate the antimicrobial effects of Neutral electrolyzed functional water (NEW) containing high levels of hypochlorous acid at concentrations of 600–700 ppm (pH 7.5) on *E. faecalis*-derived biofilm.

Methodology The effects of NEW were compared with those of 5% NaOCl, 2% Chlorhexidine and sterilized water (DW). *E. faecalis* biofilms were induced on tissue-culture plates. An overnight culture of *E. faecalis* grown in brain heart infusion broth was seeded (initial concentration, 10^{7-8} cells mL^{-1}) with trypticase soy broth (containing 0.25% glucose), and was incubated under aerobic conditions for 48 h to allow biofilm formation. After incubation, biofilms were irrigated with PBS and treated with test solutions. Remaining biofilms were stained with crystal violet and were quantified using a microplate reader. Morphological studies using a confocal laser microscope (SLM) and a scanning electron microscope (SEM) were also performed.

Results NaOCl was able to disaggregate and remove biofilms, while treatment with NEW caused a high degree of biofilm disaggregation. SEM analysis showed that 5% NaOCl eliminated the bacteria completely. NEW was capable of disrupting and removing the biofilm, but not completely eliminating bacteria. However, SLM analysis showed no viable bacteria after exposure to NEW treatment.

Conclusions On the basis of this *in vitro* investigation, PPW is able to effectively kill *E. faecalis* in biofilm, and had a moderate disrupting effect on the biofilm.

R34

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The effect of photodynamic antimicrobial chemotherapy (PACT) on *Enterococcus faecalis* in human root canals *ex vivo*

Aim To determine the antibacterial effect of photodynamic antimicrobial chemotherapy (PACT) on *Enterococcus faecalis* biofilms in experimentally infected root canals.

Methodology The canals of one hundred single-rooted extracted teeth were prepared using ProTaper instruments up to F2. Teeth were embedded in methacrylate, autoclaved and infected with a clinical isolate of *E. faecalis* for 3 days. Samples were taken using paper points to determine the presence of *E. faecalis* in the root canals. For the antimicrobial treatment, the teeth were divided into two groups. In the first group, teeth were treated using the PACT system (Cumdente, Germany), consisting of the PACT light source at 635 nm in combination with the photosensitizer PACT-Fluid Endo, a tolonium chloride solution and sampled with paper points. In the second group, root canals were rinsed with 10 mL of 3% sodium hypochlorite. After sampling with paper points, root canals were treated with PACT-Fluid Endo and the PACT light source, and another sample was taken. Survival fractions of the samples were calculated by culturing on blood agar and counting colony-forming units.

Results Treatment of root canals with PACT alone caused a reduction of bacterial load, resulting in a 92.7% kill of *E. faecalis*. Rinsing root canals with 3% sodium hypochlorite achieved a reduction of 99.9% and the combination of sodium hypochlorite disinfection and PACT reduced bacterial viability by 99.9%.
Conclusions On the basis of this *ex vivo* investigation, photodynamic antimicrobial chemotherapy killed *E. faecalis* at significant levels. PACT is not an alternative but a possible supplement to existing protocols of root canal disinfection. Disruption of the biofilm prior to PACT and the adjunct use of a sodium hypochlorite based conventional approach remain mandatory.

R35

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Antibacterial efficacy of nonthermal atmospheric pressure plasma against *Enterococcus faecalis*

Aim To test the antimicrobial efficacy of nonthermal atmospheric pressure plasma (NAPP) against *Enterococcus faecalis*.
Methodology Freshly prepared Brain-Heart-Infusion (BHI) agar plates (90 mm) were inoculated with 0.2 mL BHI broth culture of *E. faecalis* ATCC 29212 at different McFarland concentrations. NAPP was applied for 30, 60 s, 3 or 5 min on petri dishes with a plasma pencil. The inner electrode was powered by 7 kHz frequency, 20 kV high voltages. The gas used was He/O_2 (ratio 99/1) with a flow rate of 10 L min^{-1}. Petri dish-pencil distance was 10 mm. After treatment, all of the plates were incubated at 37°C for 24 h. The diameters of the inhibition zones were measured for all application times. For quantitative evaluation, 1 mL broth cultures of 0.5 McFarland *E. faecalis* were prepared for five eppendorf tubes. NAPP was applied for 0 (growth control), 30, 60 s, 3 or 5 min. Serial dilutions were prepared to reduce the number of bacteria to a countable point. Ten microlitre from the diluted bacteria was then spread onto agar plates. After 24 h incubation at 37°C, the number of colonies was enumerated.
Results The mean diameters of the inhibition zones were 2 × 2 mm for 30 s, 3 × 3 mm for 60 s, 3 × 3 mm for 3 min, 4 × 4 mm for 5 min applications for the samples prepared in two McFarland. In the control group, the number of bacterial colonies was 5.5×10^8 cfu mL^{-1}. It was 5×10^8, 4.4×10^8, 3.2×10^8 and 1.8×10^8 cfu ml^{-1} for 30, 60 s, 3 and 5 min applications, respectively.
Conclusions Although there was less than 1-log difference in the bacterial counts, NAPP application reduce the number of *E. faecalis* colonies *in vitro* especially at 5 min applications. Further studies are needed for total inhibition of this species.

R36

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The antimicrobial efficacy of irrigants and calcium hydroxide on persistent endodontic microorganisms

Aim To detect the antimicrobial efficacy of sodium hypochlorite, chlorhexidine gluconate and calcium hydroxide on microorganisms isolated from endodontic retreatment cases.
Methodology Thirty-three patients in need of root canal retreatment were selected for collection of microbiological samples. The root filling material was removed and canals were sampled using sterile physiological saline solution and sterile paper points. Determination of microbial species was based on series of biochemical tests using identification kits. The agar diffusion test was used to evaluate the susceptibility of microorganisms to sodium hypochlorite (2.0%), chlorhexidine gluconate (0.2%) and calcium hydroxide.
Results Sodium hypochlorite created the largest zones of bacterial inhibition (0–42 mm; mean 17.9 ± 14.4 mm), compared with chlorhexidine gluconate – 0–26 mm (mean 8.5 ± 8.0 mm) and calcium hydroxide – 0–6 mm (mean 1.8 ± 1.4 mm). There was a statistically significant difference in antimicrobial efficacy of substances tested (ANOVA, $P < 0.05$). Different strains of *Escherichia coli, Enterobacter cloacae, Actinomyces naeslundii* showed different zones of inhibition. One isolate of *A. naeslundii* was completely resistant to the antibacterial substances.
Conclusions Sodium hypochlorite and chlorhexidine gluconate have an antimicrobial efficacy on microorganisms isolated from endodontic retreatment cases. Calcium hydroxide has a weak antimicrobial effect *in vitro*. Different strains of persistent endodontic microflora may have a different susceptibility to root canal irrigants and calcium hydroxide.

R37

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Ex vivo comparison of antimicrobial activity of calcium hydroxide against *Enterococcus faecalis* and *Candida albicans*

Aim To evaluate the antimicrobial efficiency of calcium hydroxide (CH) mixed with different vehicles: saline, glycerin + CMCP, CHX and proprietary brands of CH pastes: Calcicur, MM paste, Metapaste and to determine the most appropriate treatment for endodontic infections caused by *Enterococcus faecalis* and *Candida albicans.*
Methodology An agar diffusion method was used to investigate the antimicrobial activity of calcium hydroxide mixed with different vehicles, 2% water based CHX, sterile saline, Glycerin + CMCP, and proprietary brands of calcium hydroxide pastes: Metapaste, Calcicur, MM paste. *E. faecalis* and *C. albicans* were used as the standard test organisms. Standard holes in the cultivated agar plates were filled with one of the CH pastes and control agents. Zones of microbial inhibition were measured after the incubation period. Data were analyzed using the statistical software package SPSS. One way analysis of variance (ANOVA) and Duncan's test were performed for *C. albicans* whereas *t* test was performed for *E. faecalis*.
Results The CH pastes could be ranked from strongest to weakest as follows CHX-CH > Glycerin + CMCP-CH > Sterile saline-CH > Calcicur, MM paste. Metapaste study groups did not demonstrated any antibacterial effect against *E. faecalis.* The antifungal effects of the CH pastes were ranked from strongest to weakest as follows: Glycerin + CMCP-CH > CHX-CH > Calcicur > Sterile saline-CH > Metapaste > MM paste.
Conclusions In this laboratory investigation, the addition of medicaments to CH pastes appeared to increase their antimicrobial effectiveness, but this should be confirmed by *in vivo* studies.

R38

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The effect of different root canal medicaments on the elimination of *E. faecalis ex vivo*

Aim To evaluate the antimicrobial effect of chlorhexidine gel 2% (CHX-G), chlorhexidine powder (CHX-P), povidone-iodine, polyhexanide and camphorated-and-mentholated-chlorophenol (ChKM) *ex vivo*.
Methodology For every medicament group 10 root segments (15 mm long) of extracted human single-rooted anterior teeth were prepared to size 45 and sterilised after smear layer removal ($n = 50$). Root segments were then inoculated with *E. faecalis* in yeast-extract-glucose-solution McFarland 0.5 (10 μL per root). The specimens were aerobically incubated at 37°C for 8 days. Every 3 days the culture medium was replaced. After one week, ten root canals were filled with one of the medicaments, respectively, and incubated at 37°C for another week. Ten teeth served as positive controls and were filled with sterile saline solution. After 7 days the medicaments were inactivated and all root canals were instrumented with Hedström files sizes 50, 55, and 60, respectively. The dentine samples obtained were dispersed in Ringer solution followed by the preparation of serial dilutions (1 : 20 and 1 : 400). 10 μL per sample were applied to an agar plate and incubated at 37°C for 48 h. The colony forming units (CFU) were counted and the reduction factors were calculated and statistically analysed using the non-parametric Mann–Whitney *U*- and the Chi-square-test.
Results Compared to the positive controls, all medicaments exhibited an antibacterial effect against *E. faecalis*. The reduction factors for CHX-G, CHX-P, and ChKM were significantly higher compared to povidone-iodine and polyhexanide ($P < 0.05$). In contrast to povidone-iodine and polyhexanide, CHX-G, CHX-P, and ChKM were able to eliminate *E. faecalis* from all dentine samples.
Conclusions Within the limitations of this *ex vivo* investigation, 2% chlorhexidine gel and chlorhexidine powder were as effective as camphorated-and-mentholated-chlorophenol against *E. faecalis*. Thus, when choosing a root canal medicament the better biocompatibility of chlorhexidine compared to ChKM should be taken in consideration.

IRRIGANTS/DISINFECTION: CANAL CLEANING

R39

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Investigation of artificial tooth models for assessing the efficacy of root canal irrigation protocols using sodium hypochlorite

Aim To develop and test artificial tooth models, to replicate the collagen film model (Huang *et al.* 2008), for investigation of the efficacy of sodium hypochlorite (NaOCl) irrigation.
Methodology Human teeth ($n = 5$) were prepared, split, had impressions made and were then replicated with Type IV dental stone. Surface treatments were applied to the stone as follows: collagen surface coating ($n = 10$); heat treatment ($n = 10$); hydroxyapatite (HA) conversion ($n = 10$); HA conversion with collagen surface coating ($n = 10$). Four layers of stained Type I collagen were applied to the canal surface of the teeth and stone samples. Standardised static and dynamic irrigation regimens were performed on the test groups, with 2.5% NaOCl as the irrigant. Control teeth ($n = 5$) were re-used for each test group; the experiments for each of which was performed on a separate experimental day. Digital image analysis was used to quantify the area of canal surface covered with remaining stained collagen film based on pre-treatment images. Data were analysed using the paired *t*-test and binomial regression models.
Results There was no significant difference ($P > 0.05$) in the mean percentage of remaining stained collagen between the control teeth, HA converted stone and unconverted stone with a collagen pre-coat, for either static or dynamic irrigation. The surface integrity of the unconverted stone was questionable as it exhibited erosion during the irrigation tests.
Conclusion The use of HA converted Type IV dental stone as an artificial tooth model, with a stained collagen film, was validated for NaOCl irrigation.

R40

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Comparison of substrates for the fabrication of an artificial tooth-model to test endodontic disinfection protocols

Aim To evaluate substrates [Hydroxyapatite (HA), Type IV gypsum stone, HA converted gypsum stone] for fabrication of an artificial tooth model to test endodontic disinfection protocols using surface characteristics and bacterial biofilm growth as outcome measures.
Methodology Type IV stone was converted into HA. The degree of conversion as influenced by methods of mixing (hand, mechanical, vacuum) was assessed by X-ray diffraction and surface energy-dispersive X-ray microanalysis. The surface topography and energy of the substrates (untreated, Smear Clear™ treated, polished, collagen coating) were assessed using scanning electron microscopy (SEM) and fluid contact angle, respectively. Multispecies biofilms were grown on five selected substrates [dentine, HA, hand-mixed stone (HMT4) with or without HA conversion and collagen coating] in a constant depth film fermentor (CDFF) by incubation for various periods of time (2, 24 h, 7, 14 and 28 days). Bacterial colonisation and biofilm growth was assessed by culture and SEM.
Results HMT4 showed the highest level of HA conversion. None of the artificial substrate surfaces bore a physical resemblance to dentine, however HA converted stone exhibited surface features comparable to dentinal tubule openings. Fluid contact angles of all substrates except polished-HA were significantly different ($P < 0.05$) from untreated-dentine. All substrates allowed bacterial colonisation at all time points with less than 1-log difference. At week 4, HMT4 dissolved in the CDFF, and on the remaining substrates the biofilms appeared similar.
Conclusions HA converted HMT4 demonstrated a close resemblance to dentine in chemical composition, surface topography and bacterial colonisation, and was considered a suitable material for fabrication of an artificial tooth model.

R41

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The effect of ultrasonic irrigation variables on the dimensions of artificial root canals

Aim To investigate the effects of power-setting, type of irrigant and duration of ultrasonic irrigant agitation with Irrisafe™ on the mean

percentage change in the cross-sectional area and diameter of artificial root canals in an *ex vivo* model.

Methodology Twenty-five artificial canals (size 30, 0.06 taper) were created using extracted teeth. The artificial root canals were randomly assigned to five groups ($n = 5$ each). Group 1: irrigation with 2.5% NaOCl, and ultrasonic agitation at power-setting 7; Group 2: irrigation with 17% EDTA, ultrasonic agitation at power-setting 7. Groups 3, 4 (manufacturer recommended protocol), and five were irrigated sequentially with 2.5% NaOCl, 17% EDTA, 2.5% NaOCl, with ultrasonic agitation at power-setting 4, 7, or 10, respectively. Irrigant was delivered with a gauge 27 needle and ultrasonically agitated with size 25 Irrisafe™ tips and a P5 Satelec® Unit. Canal area and depth were measured at 17, 16 and 9 mm from the coronal canal orifice at baseline and after 1 (manufacturer recommendation), 2, and 5 min of ultrasonic agitation.

Results The mean increases (percentage) in cross-sectional area and diameter, respectively, from baseline at the 17 mm level, in descending order were: Group 2 [0.05 mm^2 (52.7%), 0.07 mm (26.2%)]; Group 5 [0.04 mm^2 (42.6%), 0.07 mm (25.8%)]; Group 4 [0.05 mm^2 (23.2%), 0.04 mm (9.4%)]; Group 3 [0.06 mm^2 (14.6%), 0.03 mm (5.1%)]; Group 1 [0.02 mm^2 (6.5%), 0.05 mm (3.8%)]. Linear regression analyses revealed that canal dimensional changes were significantly affected by irrigant regime ($P = 0.0001$), corono-apical level ($P = 0.009$) and duration of irrigant agitation ($P < 0.0001$).

Conclusions Ultrasonic agitation of the irrigant with Irrisafe™ tips following the recommended protocol (group 4) resulted in clinically insignificant increase in cross-sectional canal dimensions. However, the increase was clinically significant with high power-setting (10), irrigant choice and duration of agitation.

R42

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Effect of irrigation protocol on irrigant extrusion and biomolecular film removal from root canal model

Aim To integrate two previous experimental models (Huang *et al.* 2008, Alexander *et al.* 2010) to develop a root canal irrigation model sensitive enough to simultaneously detect irrigant extrusion and root canal cleaning for the assessment of clinically relevant irrigation strategies in an *ex vivo* model.

Methodology The mathematical relationship between pigmentation loss in standard volumes of dyed agarose gel and volume of 2.5% sodium hypochlorite extrusion was investigated. Simulated root canals with various dimensions were prepared in acrylic blocks and set in dyed agarose gel. The volumes of NaOCl extruded into dyed agarose gel following various irrigation protocols were calculated from a standard curve. Single root canals in extracted single rooted teeth were prepared and split before application of stained collagen to the canal walls. The root halves were then re-opposed and set in the dyed agarose. The irrigant extrusion and residual root canal collagen coating following static and manual-dynamic irrigation protocols were recorded. Multivariable regression was used to analyse the data.

Results The area of gel pigment loss followed a cubic relationship with volume of NaOCl deposition. Apical foramen size had a significant ($P < 0.05$) effect on extrusion regardless of irrigation protocol. Extrusion following manual-dynamic agitation of irrigant was significantly affected by the relative fit of the gutta-percha cone with greater extrusion when the gutta-percha cone fitted the canal tightly coronally but was loose apically. In the *ex vivo* model, manual-dynamic agitation of NaOCl was associated with significantly ($P < 0.05$) more extrusion but less residual root canal collagen coating apically compared to static irrigation.

Conclusions A clinically relevant model was successfully developed to allow simultaneous quantification of NaOCl extrusion and residual root canal collagen coating. The manipulation of manual-dynamic agitation parameters maybe optimized to produce an irrigation protocol that combines minimal extrusion with maximal canal cleaning.

R43

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Cleaning efficacy of various irrigation methods in mandibular molars with isthmi: an *ex vivo* study

Aim To evaluate cleaning efficacy of various irrigation system in the mesial root canals and isthmi of extracted human mandibular molars.

Methodology Sixty extracted mandibular molars were randomly divided to four experimental groups. Mesial root canals of each tooth were prepared with ProFile 0.06 taper instruments to apical size 35. Each prepared canal was finally irrigated for 1 min: group 1 with Maxi-probe, group 2 with ultrasonic irrigation, group 3 with RinsEndo system, and group 4 with the microcannula of the EndoVac system. After histological processing, cross sections of the specimens were evaluated at 1-, 3-, 5-mm level from the working length using an optical microscope. The cleanliness values of canals and isthmi were evaluated separately. Statistical analysis was completed by using Kruskal–Wallis and Mann–Whitney *U* tests. The significance level was set at $P < 0.05$.

Results For canal cleanliness, there was no statistical difference among groups, except at the 1 mm level between group 1 and 4 ($P = 0.002$). For isthmus cleanliness, group 4 had significantly higher values than other groups at all levels. At the 3 mm level of the isthmi, group 3 ($P = 0.021$) and group 4 ($P = 0.000$) were significantly different from group 1, and group 4 had significantly higher values than group 3 ($P = 0.004$). Group 1 had significantly lower values at 5 mm level of isthmi ($P = 0.000$).

Conclusions On the basis of this *ex vivo* investigation, EndoVac irrigation has the potential to achieve significantly better canal and isthmus cleanliness than the other methods tested in the mesial root canals and isthmi of mandibular molars.

R44

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Tissue dissolution and related physicochemical properties of Chlor-XTRA™ compared to conventional NaOCl solutions

Aim To compare commercially available household bleach to a recently marketed sodium hypochlorite product (Chlor-XTRA™; Vista Dental, USA), which, according to its manufacturer, should

have lower surface tension and be more digestive on necrotic soft tissues than comparable NaOCl solutions.
Methodology Six different brands of household bleach were bought from local supermarkets in San Francisco, CA, USA. These were compared to Chlor-XTRA and technical NaOCl solutions produced from a 9% stock solution regarding their chlorine content (wt % NaOCl), initial pH, alkaline capacity, osmolarity, surface tension (Wilhelmy plate method), and price. Bovine pulp tissue (N = 10 specimens per group) dissolution at 37°C effected by test and control solutions adjusted to 1% NaOCl using ultrapure water was assessed. Reduction in tissue weight was compared between groups by one-way ANOVA followed by Bonferroni correction. The alpha-type error was set at 0.05.
Results The pH of undiluted solutions ranged between 11.1 and 12.7. Batches of the same product differed in NaOCl content. No product contained more than 0.1 M NaOH. One household bleach (Safeway Bleach Summit Fresh) was slightly alkalized, the others were not. Osmolarity was similar between products. The surface tension of Chlor-XTRA and Safeway Bleach Summit Fresh was approximately half that of the other solutions. Tissue dissolution was statistically similar ($P > 0.05$) among all solutions.
Conclusions There was no notable difference between the Chlor-XTRA and the household bleach brands under investigation, other than the price, which was about 100-fold higher per litre. In contrast to earlier reports, which did not adjust for chlorine, reduced surface tension did not result in greater soft tissue dissolution by NaOCl.

R45

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Endodontic irrigation with two different needles: computational fluid dynamics analysis based on an *ex vivo* model

Aim To analyze the irrigation efficiency of two needle types in a realistic root canal model using Computational fluid dynamics (CFD).
Methodology An extracted human maxillary canine was decoronated at the enamel – cement junction. After cleaning and shaping, the prepared root canal was scanned by computed tomography (Siemens Somathom® DRH Sensation 16; Siemens, Germany). A 3D object representing the prepared root canal was obtained using Scan IP® (Simpleware, UK) and computer-assisted design (CAD) software Catia® (Dassault Systèmes, France). An open-end notched 27G irrigation needle (Appli-Vac®, Vista, USA) was measured and a CAD model created, providing a plotting template for a virtual side-vented irrigation needle with closed tip (based on 30G KerrHawe Irrigation Probe®). Both 27G, virtual needles were placed at 0.9, 4.5 and 9 mm (95, 75, 50% of working length respectively) from the apical terminus of the prepared root canal model and, after merging with root canal, models were exported to commercial CFD solvent software Star-CCM+® (CD-adapco, USA). Four different irrigant inlet velocity values were set, determined by clinicaly reproducible flow rates (0.1, 0.2, 0.3 and 0.4 mL s^{-1}), for each needle type and position. CFD analysis of irrigant flow pattern, velocity and pressure was performed.
Results Open-ended needles had satisfactory flow patterns at 0.9 and 4.5 mm for all flow rates analyzed, while side-vented needles were effective at 4.5 mm distance only with 0.4 mL s^{-1}, and at 0.9 mm with 0.2–0.4 mL s^{-1} flow rates. Irrigant pressure in apical portions of canals was significantly increased in the case of open-ended comparing to side-vented needle.
Conclusions On the basis of this *ex vivo* investigation, open-ended irrigation needles had efficient irrigation at lower flow rates compared to side-vented closed-ended needle of the same diameter, though the latter provides decreased irrigant pressure, and safer irrigation.

R46

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Vibringe®, an *ex vivo* study of a sonic irrigation technique

Aim To evaluate an irrigation method with a sonic vibrating syringe (Vibringe®; Vibringe B.V., Holland) in its efficiency of cleaning root canals by measuring the capability of Vibringe to remove a calcium hydroxide paste from the apical part of simulated canals in plastic blocks.
Methodology Twenty four plastic training blocks for System B (SybronEndo, USA) were filled with a mixture of calcium hydroxide powder (Acros Organics, USA) and physiologic saline with a lentulo spiral filler until complete filling of the canal was obtained. After 1 h, 12 canals were rinsed with the activated Vibringe syringe and 12 canals with the inactivated Vibringe syringe. In all cases, new endoneedles (VMK endoneedle, Vedefar, Belgium) were mounted on the syringes. Five millilitres of a 3% NaOCl (Vista Dental, USA) solution was used with the needle at the same depth and for the same period of time. The length of uncleared canal after the irrigation was measured with the microsope (Opmi-Pico, Zeiss, Germany) and compared with a Mann–Whitney test.
Results The length of uncleared canal was not significantly different between the activated (2.4 ± 0.5 mm) and non-activated (2.3 ± 0.5 mm) syringe. None of the irrigation systems was capable of completely cleaning the apical part of the simulated root canals.
Conclusions In cleaning artificial root canals filled with a calcium hydroxide paste, no difference was found with or without activating the Vibringe syringe; it is possible that the low frequency (150 Hz) did not lead to the desired effect.

R47

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Efficacy of the EndoVac system in the removal of calcium hydroxide from root canals *ex vivo*

Aim To compare different irrigation techniques for the removal of $Ca(OH)_2$ from root canals *ex vivo*.
Methodology Forty palatal roots of maxillary molars were used. The roots were stored in distilled water throughout the study. The canals were prepared to size 45 with a step-back technique. Root canals were filled with a radiopaque $Ca(OH)_2$ paste (MicroMega, France) using a lentulo spiral filler. Roots were divided randomly into three groups (n = 10) and two groups (n = 5) served as negative and positive controls. For each sample 10 mL of 5.25% NaOCl was used for irrigation. In group 1, K-files with the same size

as the master apical file (MAF) were used with 5.25% NaOCl delivered by syringe injection. Group 2 was irrigated with passive ultrasonic irrigation (PUI) (EMS, Switzerland) and 5.25% NaOCl. Group 3 was irrigated with EndoVac (Discus Dental, USA) and 5.25% NaOCl. Radiographs were taken before and after the application of the three irrigation techniques. Remaining $Ca(OH)_2$ was compared with ImageJ Software. Data were statistically evaluated using Wilcoxon and Kruskal–Wallis tests.

Results Group 2 had significantly less remaining $Ca(OH)_2$ than groups 1 and 3 ($P < 0.05$). The manual technique was more effective in removing $Ca(OH)_2$ than EndoVac, but there was no significant difference between group 1 and 3 in removing $Ca(OH)_2$ paste. In all groups, the amount of remaining $Ca(OH)_2$ in the apical part of the samples was significantly greater than in the coronal and middle parts ($P < 0.05$).

Conclusions On the basis of this *ex vivo* investigation, none of the techniques removed the $Ca(OH)_2$ dressing completely. The EndoVac system was no more effective in removing $Ca(OH)_2$ from root canals than other techniques.

R48

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Effects of volume and application time of EDTA for removing calcium hydroxide from root canals *ex vivo*

Aim To evaluate the effect of changing application time and volume of EDTA for removing $Ca(OH)_2$ medicament from root canals *ex vivo.*

Methodology Fifty teeth with single root canals were sectioned to give standardised 12 mm roots. Canals were enlarged to size 50 with a step-back technique and smear removed with 17% EDTA (5 mL, 2 min). $Ca(OH)_2$ mixed with saline was placed into the root canals of 45 teeth with a lentulo spiral filler and plugger. Five teeth served as positive controls without any attempt to remove the paste. Five negative control teeth received no calcium hydroxide. Forty teeth were stored at 37°C – 100% humidity for 1 week. Teeth were divided into four groups ($n = 10$) that received different EDTA irrigation regimes: Group 1: NaOCl (2 mL, 1 min) – EDTA (2 mL, 1 min) – NaOCl (2 mL, 1 min); Group 2: NaOCl (2 mL, 1 min) – EDTA (2 mL, 3 min) – NaOCl (2 mL, 1 min); Group 3: NaOCl (2 mL, 1 min) – EDTA (5 mL, 1 min) – NaOCl (2 mL, 1 min); Group 4: NaOCl (2 mL, 1 min) – EDTA (5 mL, 3 min) – NaOCl (2 mL, 1 min). The irrigation needle was placed in the middle thirds of roots. Teeth were bisected longitudinally and each half examined by SEM. Three observers scored all samples. Data were analyzed using Kruskall–Wallis and Mann–Whitney *U* tests.

Results No difference was observed among experimental groups. All teeth demonstrated $Ca(OH)_2$ plugs in the apical third. Only one sample was free of an apical plug but dentinal tubules in the apical third were clogged with paste remnants.

Conclusions Within the limitations of the study, it can be concluded that complete removal of $Ca(OH)_2$ paste from the root canal is difficult to achieve with the combined use of NaOCl and EDTA.

R49

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Effects of different irrigation techniques on smear layer, debris and erosion scores: a SEM study

Aim To evaluate with SEM analysis the effects of different irrigation techniques (EndoVac, RinsEndo, Cleanmax, Sonicmax, Ultrasonic, and Manual) on smear layer, debris and erosion scores.

Methodology Thirty-nine freshly extracted single-rooted teeth were instrumented with ProTaper rotary instruments to a master apical file of F4. The roots were randomly divided into six experimental groups ($n = 6$). Group 1 (Endovac), Group 2 (Rins-Endo), Group 3 (Cleanmax), Group 4 (Sonicmax), Group 5 (Ultrasonic), Group 6 (Manual). Final rinsing was performedwith 5 mL 2.5 % NaOCl followed by 5 mL 17% EDTA and 5 mL 2.5% NaOCl for 2 min in all groups. Three roots served as control and were irrigated with only NaOCl. Roots were split longitudinally and prepared for SEM examination. Smear layer, debris and erosion scores were recorded in cervical, middle and apical thirds by two blinded examiners. Statistical analysis was performed using Kruskal–Wallis tests.

Results There was no statistically significant difference among the experimental groups in the middle and coronal thirds in terms of smear layer and the root canals were clean, with no or only small areas of smear layer. In the apical region, there was a higher score of smear layer in manual and ultrasonic groups. In the middle third, higher debris scores were observed in the ultrasonic group. There was a significant difference in the coronal thirds ($P = 0.009$) in terms of erosion score. Whereas Cleanmax, RinsEndo, Ultrasonic and Endovac demonstrated similar, moderate erosion, manual and Sonicmax produced severe erosion on the root canal walls.

Conclusions All irrigation techniques removed smear layer and debris efficiently in the middle and coronal thirds, but in the apical third, manual and ultrasonic groups were not effective. All the irrigation systems with final irrigation (EDTA and NaOCl) caused erosion especially in the coronal part of the roots.

R50

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Influence of different final irrigation regimes and various endodontic filling materials on root fracture resistance *ex vivo*

Aim To evaluate the influence of different endodontic materials and final irrigation regimes on root fracture susceptibility in an *ex vivo* model.

Methodology The crowns of 90 similarly sized, recently extracted single-rooted human teeth were removed leaving 12 mm roots. The canals were shaped using ProTaper rotary files (Dentsply Maillefer) to size F4. Irrigation with 2 mL of 5.25% sodium hypochlorite (NaOCl) was performed between each file. Following instrumentation, 80 teeth were divided randomly into two groups according to final irrigation regimens: Group 1: 2 mL 5.25% NaOCl, Group 2: 2 mL 2% chlorhexidine gluconate (CHX). Each group was further subdivided into four subgroups ($n = 10$) according to the obturation system used: (i) single gutta-percha (GP) cone and bioceramic-based sealer (i-RootSP, Veriodent, Canada). (ii) only bioseramic-based sealer; (iii) single GP cone and MTA-based sealer (Fillapex, Angelus, Brasil); (iv) single GP cone and resin-based sealer (AH26, Dentsply, Germany). The remaining 10 teeth were divided into two ($n = 5$),

as negative (no instrumentation or filling) and positive (instrumentation but no filling) controls groups. After setting of sealers, the roots were embedded in acrylic moulds and subjected to compressive loading at a rate of 1 mm min^{-1}. Fracture load was recorded and statistically analyzed using Kruskal–Wallis one-way analysis of variance and chi-square tests.

Results The fracture values of negative control group were higher than those of experimental groups. Regarding final irrigation solutions, fracture values of the roots irrigated with NaOCl were significantly lower than roots irrigated with CHX ($P = 0.039$). Fracture values of roots filled with single-GP-cones and MTA-based sealer after NaOCl irrigation were significantly lower than other groups ($P < 0.05$).

Conclusions Roots were significantly weakened by root canal instrumentation. Susceptibility to fracture was lowest following filling with AH 26 and iRootSP after CHX irrigation.

SESSION 2: FRIDAY 16 SEPTEMBER 2011

PREPARATION: CLEANING ABILITY

R51

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Apical box and cone preparations in bicuspids with apical periodontitis *in vivo*: microbiological considerations

Aim To study the microbial growth reduction in premolars with necrotic pulps and apical periodontitis *in vivo*, after root canal instrumentation with either apical box preparation or apical cone preparation.
Methodology The study included 24 premolars in 24 patients randomized to apical box preparation (size 60) or cone preparation (apical size 30). The two groups were comparable regarding primary caries, type of restoration, and size of the apical radiolucency. The box preparation group was instrumented using RaCe (FKG Dentaire, Switzerland) and NiTi-flex instruments (Dentsply Maillefer, Switzerland). In the apical cone preparation group ProTaper SX, GT size 20, 0.06 taper (Dentsply Maillefer) and NiTi-flex was used. In the course of canal preparation each tooth was irrigated with 2.5% NaOCl (12 mL). Finally, the canals were flooded with 17% EDTA (2 × 30 s) and with 5% iodine potassium iodide (IKI) for 10 min. The canals were sampled for microorganisms on four occasions: before and after instrumentation, 10 min after application of the IKI dressing, and at the beginning of the second appointment 1 week later. Between the two treatments sessions, the root canals were sealed with a temporary restoration of IRM cement. In the laboratory a cultivation technique was used to measure microbial growth, quantified as none, very sparse, sparse, moderate, heavy or very heavy.
Results Initially, microbes were recovered from 88% of the teeth. Growth was classified as none or very sparse in 43% of the teeth following instrumentation and in 75% after the application of IKI. Irrespective of time of sampling, no significant difference in terms of microbial growth reduction was observed between the two types of apical preparation.
Conclusions No difference in microbiological growth reduction was found when root canal preparation ended as an apical box or as an apical cone in teeth with necrotic pulps and apical periodontitis.

R52

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Comparison of the cleaning efficiency of three NiTI rotary systems in extracted teeth

Aim To compare the cleaning efficiency of: Endoflare/Revo-S (Micro-Mega, France), Endoflare/Hero Shaper (Micro-Mega) and ProTaper (Dentsply Maillefer, France) in extracted teeth.
Methodology Fifty one root canals with curvatures ranging between 10 and 80° were divided into three homogeneous groups (n = 17 canals per group). Root canals in Group 1 were shaped with Endoflare/Revo-S; Group 2 with Endoflare/Hero Shaper, and Group 3 with ProTaper according to the manufacturers' instructions. The root canals were irrigated with 3% stabilized sodium hypochlorite (32 mL total) followed by a 14% EDTA flush and a 3 mL distilled water final rinse. Four root canals served as controls: one was uninstrumented and three were shaped with each system without irrigation. After root canal preparation and SEM observation, the amount of debris and smear layer was scored on scales from 0 to 3 by two investigators. Debris and smear layer were scored for each sample in the coronal, middle and apical thirds. Statistical analysis was carried out by the non-parametric Kruskal–Wallis test ($P < 0.05$).
Results Although the amount of debris and smear layer was low, none of the systems completely cleaned the root canal walls. For all systems, the cleanliness of the root canal walls was better in the coronal and middle thirds than in the apical third. Nevertheless, the use of Endoflare/Revo-S resulted in significantly ($P < 0.04$) less smear layer than the other systems in the apical third.
Conclusions Under these experimental conditions, Endoflare/Revo-S was associated with less smear layer in the apical portion of the root canal, although no system was able to completely eliminate debris and smear.

R53

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SEM investigations of root canal preparations with different rotary NiTi systems

Aim To compare several parameters of root canals prepared with two rotary nickel-titanium systems ProTaper (Dentsply Maillefer, Switzerland) and Hero-Shaper (Micro Mega, France).
Methodology The study was performed *ex vivo* on 12 maxillary premolars heaving the same mean canal curvature. Selected teeth were embedded into a muffle system. The root canals were divided in two groups of six teeth and prepared to size 30 using ProTaper or Hero-Shaper rotary instruments. The following parameters were evaluated: cleaning ability, working time, working safety (instrument fracture, lateral perforation). Cleanliness of the root canal walls was investigated under the SEM, in the middle third of the canal, using a five-score system for debris and smear layer. Parallel cross sections were photographed under standardized conditions. The difference between the root canal diameters pre- and post operatively was evaluated by superposition photographs. Data were analysed by the χ^2 test ($P = 0.05$).
Results In terms of debris and smear layer removal, the results were similar ($P = 0.211$). Both systems achieved a large number of score 2, showing good cleaning ability. Mean working time was

shorter for Hero-Shaper (124s) than for ProTaper (184s). No lateral perforations and no instrument fractures were recorded. Cross-section analysis revealed that for the coronal third of the root canals, the Hero-Shaper performed in a superior manner (67% sections having a superimposition of 75–100%) and for the apical third, ProTaper was superior (67% sections having a superimposition higher of 50%). Evaluating the form of the postoperative cross sections, both systems had a high number of regular cross sections.
Conclusions Within the limits of this study, both systems had almost the same cleaning ability and excellent centring capacity.

R54

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Cleaning efficiency and straightening of curved canals instrumented with four rotary NiTi instruments *ex vivo*

Aim To assess cleaning efficiency and canal straightening after instrumentation of curved root canals in extracted teeth with four different NiTi rotary instruments.
Methodology Forty curved root canals (degree of curvature: 25–50°) of human molars were randomly assigned to one of the following four groups ($n = 10$): group 1: K3; group 2: Liberator; group 3: LightSpeed LSX; group 4: ProTaper Universal. Standard access was prepared and digital radiographs were taken to assess pre-operative canal curvature. The root canals were instrumented to size 40, 0.04 taper according to the manufacturers' instructions and instrumentation time was recorded. Irrigation of the root canals was performed with distilled water in order to exclude an interference of irrigants on the cleaning efficiency of the instruments. Radiographs were taken to assess the amount of canal straightening. To assess debris, smear layer and root canal surface, each root half was imaged under SEM in the apical, middle, and coronal sections and images scored for according to Hülsmann *et al.* (1997). Statistics were computed using SPSS Win 17.0 (Kruskal–Wallis test, Dunn's multiple comparison test, ANOVA, and Friedman test).
Results All instruments produced substantial amounts of smear layer. Debridement with water irrigation was efficient. Debris and smear layer scores decreased from coronal to apical ($P < 0.05$). Kruskal–Wallis test revealed significant differences for root canal surface cleanliness (ANOVA, $P < 0.001$). LightSpeed LSX (0.1 degrees) and K3 instruments (0.4 degrees) showed significantly less canal straightening when compared with Liberator (3.7 degrees) and ProTaper instruments (5.7 degrees) (ANOVA; $P < 0.001$). Root canal instrumentation revealed significant differences between groups (Kruskal–Wallis test, $P < 0.001$). Instrumentation with K3 instruments required significantly more time compared than Liberator and ProTaper instruments (Dunn's multiple comparison test).
Conclusions Severely curved root canals in extracted teeth were sufficiently debrided by NiTi rotary instruments. Canal straightening was dependent on instrument type.

R55

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Evaluation of canal cleanliness and tubular penetration of sealer in extracted primary second molars

Aim To evaluate canal surface changes and debris/smear remaining after canal preparation, and the subsequent penetration of root canal sealers into dentinal tubules in extracted primary second molars, using scanning electron microscopy (SEM).
Methodology The widest roots of 120 recently extracted human primary second molars were used. Roots were randomly distributed into four groups according to instrumentation techniques [conventional stainless-steel hand files/nickel-titanium (Ni-Ti) rotary files] and irrigation solutions [0.5% sodium hypochlorite (NaOCl) and 0.9% saline solution/0.4% chlorhexidine gluconate (CHX)] employed. Surface change and debris/smear layer remaining after instrumentation/irrigation and tubular penetration of root canal sealers [zinc oxide and eugenol (ZOE)/Apexit Plus (AP)] were evaluated using SEM. All data were analyzed using the Kruskal–Wallis test.
Results No significant difference was found between the surface changes and debris/smear layer scores according to root canal preparation technique and irrigant solution ($P > 0.05$). ZOE cement was unable to enter dentinal tubules, while AP was able to gain limited entry to the tubules of some roots, but not others.
Conclusions No differences in canal cleanliness were noted among the instrumentation and irrigant protocols evaluated. AP was found in this *ex vivo* study to penetrate the dentinal tubules of prepared primary molar root canals more effectively than ZOE, though penetration with AP was not noted in every case.

R56

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Modification of the tip enhances apical cleaning ability of the Roeko CanalBrush *ex vivo*

Aim To evaluate the cleaning ability of Roeko CanalBrushes with different tip variations within the apical 2 mm of the root canal in extracted teeth.
Methodology Root canals of sixty extracted straight single rooted human teeth were instrumented to size 45, 0.04 taper. Teeth were split into two halves, reassembled, and embedded into model resin, to form separable tooth models. Artificially manufactured dentine debris was packed into the apical 2 mm of teeth to form a relatively consistent debris challenge to be assessed. Then, debris score was evaluated using a modified Hülsmann-score (0 = clean to 5 = debris > 50%) for the apical 2 mm. After remounting the teeth, canals were irrigated with NaOCl 3% (2 mL), in group 1 with needle irrigation only, and in groups 2–4 with additional activation with different versions of the CanalBrush (Coltène Whaledent, Germany) for 30 s in a slow-speed angle piece at 300 rpm. In group 2, the tip had one straight bristle (old version); in group 3 the tip had two additional diagonal bristles (new version). In group 4 the central straight bristle of the new version was removed. After finishing the

irrigation protocol, the apical 2 mm of canals were evaluated again. Statistical tests were performed using PASW Win 18.0 ($\alpha = 0.05$).
Results Scores were significantly lowered in all groups due to irrigation (Wilcoxon-test, $P < 0.001$). The scores were lowered in average as follows: group 1: −1.3; group 2: −1.8; group 3: −2.6; group 4: −3.0. Groups 3 and 4 showed significantly cleaner apices than groups 1 and 2 (Mann–Whitney-tests, $P < 0.05$).
Conclusions Using a new or experimental version of the Roeko CanalBrush resulted in significantly improved apical canal cleanliness compared to the old brush or needle irrigation alone.

R57

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Efficacy of self-adjusting file in C-shaped canals during root canal preparation *ex vivo*

Aim To assess the efficacy of instrumentation of C-shaped canals with Self-Adjusting File (SAF) *ex vivo*.
Methodology Ten mandibular molars with C-shaped canals were identified by cone beam computed tomography and decoronated. After root canal enlargement with hand files to size 20, light green dye was injected into the root canals and the roots were kept in a vacuum container. Following dye colouring, the C-shaped root canals were prepared using the SAF for 4 min. Roots were split into two halves using the clear-cut technique and images were recorded using a stereomicroscope. Images were then transferred into Adobe Photoshop and Image J to calculate the areas of dentine touched or untouched by SAF. Arithmetical means were calculated and recorded.
Results Overall, 66% of the total root canal area was touched by SAF. However, 34% of the root canal dentine walls remained untouched in these complex, C shaped canals.
Conclusions Under the conditions of this study, the SAF system was unable to contact all walls of C shaped canal systems during 4 min of instrumentation.

R58

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Hard tissue debris accumulation during conventional rotary versus self-adjusting file instrumentation *ex vivo*

Aim To compare accumulated hard tissue debris (AHTD) levels in complex canal systems generated by the self-adjusting file (SAF) system compared to counterparts affected by conventional rotary instrumentation.
Methodology Based on pre-scans in a micro-computed tomography system, 40 extracted human mandibular molars with joining mesial root canals and an isthmus between these were selected. Root canals in group A ($N = 20$) were instrumented using the ProTaper rotary system until the F3 instrument reached working length. One millilitre of a 3% NaOCl solution was applied during instrumentation after each instrument. Root canals in group B ($N = 20$) were prepared using the SAF as recommended by the manufacturer. Continuous irrigation was applied throughout the procedure at 5 mL min^{-1} using 3% NaOCl. Percent values of total canal system volume filled with AHTD were calculated based on high-resolution scans. Obtained data were skewed; AHTD levels were compared between groups using the Mann–Whitney *U*-test. The alpha-type error was set at 1%.
Results Instrumentation of the root canals with ProTaper left 10.12% (IQR 5.15) of the total canal system volume filled with AHTD. After preparation with the SAF 1.65% (IQR 1.61) of the total canal volume was found to be filled with AHTD. This difference was statistically highly significant (Mann–Whitney *U*-test, $P < 0.001$).
Conclusions The SAF produced considerably less accumulated debris than conventional rotary instrumentation in extracted human teeth.

PREPARATION: SHAPING ABILITY

R59

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Shaping ability of three RaCe sequences in simulated resin root canals

Aim To compare the shaping ability of three sequences of RaCe rotary instruments in simulated root canals.
Methodology Sixty simulated canals consisting of two different shapes in terms of curvature were prepared with RaCe (FKG, Switzerland) instruments. Canals were instrumented according to the two manufacturer's sequences and the author's sequence to an apical size of 40. In the author's sequence canals were prepared with all files to the full working length, from size 25, 0.02 taper to size 40, 0.04 taper. Pre-and post-operative pictures of the canals, recorded using a digital camera, were superimposed with an image analysis program. Canal transportation was measured at 18 measuring points. Incidence of canal aberrations and change of working length were recorded. The data were analysed statistically using Student's *t*-test.
Results In both canal types, the author's technique maintained working length significantly more accurately in groups of S-shaped canals ($P < 0.05$). In L-shaped, canals no significant difference was observed between the three groups. All of the instrumentation techniques resulted in transportation towards the outer aspect of the L-shaped curved canals in the apical part and the inner aspect of the S-shaped canals at the curve. The author's sequences had a better centring ability in the apical part of the canal. Canal aberrations were associated with all instrumentation techniques.
Conclusions According to the results of this laboratory study the author's sequence respected original root canal curvature well. None of the technique showed a completely optimal shaping ability.

R60

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Evaluation of the efficacy and safety of two nickel-titanium instrumentation systems *ex vivo*

Aim To compare the efficacy and safety of two Nickel-Titanium rotary systems – ProTaper® (Dentsply Maillefer, Switzerland) and Revo-S™ (Micromega, France) in extracted teeth.
Methodology A sample of 36 human premolar teeth, with intact roots, was assembled. Curvature angles were measured according to Schneider's method and two homogeneous groups of 18 premolar teeth with five straight ($\alpha \leq 10°$), 12 moderate ($10° > \alpha < 25°$) and one severe ($\alpha \geq 25°$) root curvatures were established. Teeth were prepared according to the manufacturer's instructions and each instrument was used six times. Canal patency was maintained using a size 10 K-file and a total volume of 50 mL

of sodium hypochlorite (0.5%) irrigation was used per canal. The entire sample was radiographed pre and postoperatively. Alteration of the working length, curvature and conformation of the canal were studied. All instruments were carefully examined in order to register possible morphological changes and fractures. Statistical analysis was undertaken in SPSS, applying the Wilcoxon and Mann–Whitney tests, at a confidence interval of 95% ($P < 0.05$).

Results Canal preparation led to small changes of the WL. The initial and final values for ProTaper® were 21.28 (±1.82)/20.61 (±2.12) and for Revo-S™ 19.81 (±2.71)/19.50 (±2.52). Changes in working length for ProTaper® ($WL_{initial}$–WL_{final} = −2.214) were statistically significant ($P = 0.027$), but no statistically significant difference was found for Revo-S™ ($WL_{initial}$–WL_{final} = −1.294). A decrease of canal curvature was registered with both systems, although with no statistical significance. The two nickel-titanium systems maintained the canal shape; only one iatrogenic injury (apical perforation) was observed with Revo-S™. No instrument fracture was recorded. Only one instrument deformation occurred (SC2 file from the Revo-S™ system).

Conclusions Both systems maintained the canal anatomy of extracted teeth and the preparation proved to be effective and safe.

R61

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Root canal preparation with two different rotary systems: comparative study assessed by micro-computed tomography

Aim To evaluate root canal transportation using micro-computed tomography (micro-CT) after canal preparation *ex vivo* with two nickel-titanium rotary instruments.

Methodology Mesiobuccal roots of 20 maxillary first molars with similar angles of curvature were divided randomly into two groups and scanned using a SkyScan 1172 micro-CT scanner (SkyScan, Belgium). Root canals were then prepared with AlphaKite (Komet, Germany) or BioRaCe instruments (FKG Dentaire, Switzerland). The prepared roots were re-scanned and cross-sectional images of the pre- and post- instrumentation scans were reconstructed using the Skyscan reconstruction software. Cross-sectional images were compared and the amount of transportation was assessed quantitatively by measuring the shortest distance from the edge of the uninstrumented canal to the periphery of the root (mesial and distal) and then comparing this with the same measurements obtained from the instrumented images. A negative result indicated transportation toward the distal surface of the root (inner curvature), a positive result toward the mesial surface (outer curvature), and nil, the absence of transportation. Student *t*-tests and ANOVA tests were used for statistical analysis.

Results The average canal transportation with BioRaCe instruments in the apical, middle and coronal thirds were 0.063 ± 0.016, −0.010 ± 0.027 and −0.121 ± 0.060 mm, respectively, with a significant difference ($P < 0.05$) in the amount of transportation between the apical and coronal levels. Average canal transportation with AlphaKite instruments in the apical, middle and coronal thirds were 0.032 ± 0.010, −0.042 ± 0.032 and −0.052 ± 0.075 mm, respectively, with no significant difference between the groups ($P > 0.05$). There was no statistically significant difference between the systems in regard to canal transportation across all levels ($P > 0.05$).

Conclusions Within the limitations of this *ex vivo* study, there were few differences between the two instrumentation techniques used. Both techniques produced minimal canal transportation.

R62

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Curved root canals: comparison of three contemporary rotary NiTi-instruments in extracted teeth

Aim To compare NiTi rotary instrument systems with a shaft design of progressive (ProTaper Universal) and with constant taper (Mtwo, RaCe) in shaping curved human root canals under simulated clinical conditions. To investigate (i) loss of working length, (ii) root canal transportation, (iii) prepared root canal outline and (iv) time for complete root canal treatment.

Methodology Sixty curved root canals from extracted molars were divided into three groups and shaped by three different rotary instrument systems (Mtwo, ProTaper Universal or RaCe) to an apical preparation size of 40. Root canal instrumentation was carried out in a phantom head under simulated clinical conditions. Cross sectional areas of canals 2.5 mm short of working length were evaluated before and after complete root canal treatment. Loss of working length, file fracture rate and time spent on root canal preparation were also recorded.

Results Instrument type had no significant influence on root canal transportation and prepared root canal outline with mean values exceeding 80%. Loss of working length was recorded in half of the cases in the ProTaper Universal group. Mean time for complete root canal preparation ranged from 5:32 min (RaCe) to 7:22 min (Mtwo).

Conclusions Under the conditions of this study, root canal preparation with good clinical and morphological results was possible with different rotary root canal instruments. Reaching a satisfactory apical size with rotary instruments of increased taper is limited by the flexibility of the instruments and depends on the design of instruments, which varies between the systems.

R63

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Evaluation of centering ability and canal transportationof NiTi instruments, by CBCT and subtraction imaging

Aim To evaluate the root canal transportation and centering ability of Twisted Files (SybronEndo, USA) by a combination of Cone-Beam-Computed-Tomography (CBCT) and digital subtraction radiography *ex vivo*.

Methodology Six mesial roots of mandibular molars were selected. Canal curvatures ranged from 35° to 55° (Schneider's classification). Root canals were submitted to CBCT before and after preparation with the Twisted Files. Serial cross-sectional images were obtained at 14.16 μm intervals. Cross-sectional images of the specimens at two root levels, in the middle and apical third respectively were chosen to evaluate canal transportation and centering ratio. The two CBCT images of each root at each of these levels were subjected to digital subtraction. Canal transportation and centering ability were measured with reference to the distance between the non-instrumented portion of the root canals and the mesial and distal periphery of the roots, as visualized on the final subtractive images, using digital image processing and analysis software.

Results The Twisted File system was not sufficiently effective in performing biomechanical preparation of the root canals. There was significant buccolingual root canal transportation. The centering ability of these instruments proved to be low.
Conclusions Digital subtraction of CBCT images appeared to be reproducible and reliable for evaluating certain aspects of endodontic instrumentation. Observation of the subtraction images enabled direct visual evaluation of the above parameters. Within the limitations of this *ex vivo* investigation, the Twisted File system did not seem to be effective enough to produce centered root canal preparations. The evaluation of other file systems by similar methodology is warranted.

R64

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Micro-CT analysis of root canal preparation using rotary and reciprocating NiTi instruments

Aim To evaluate root canal morphology after instrumentation in extracted teeth with the Endo-Eze Tilos (Ultradent Inc., USA) and ProFile Ni-Ti rotary systems (Dentsply Maillefer, Switzerland) using micro-computed tomography.
Methodology A micro-computed tomography scanner (SkyScan 1072, Belgium) was used to analyze 10 maxillary and 10 mandibular first molars. Specimens were scanned before and after root canal preparation using Endo-Eze Tilos reciprocating system (Group 1) and ProFile NiTi rotary files (Group 2). Each system was used to prepare five maxillary and five mandibular molars. After pre-processing of the images acquired by the scanner, data were processed using software specifically designed for endodontic analysis developed by the authors. The software consists of different modules that permit different operations: Fine superimposition of different scans on the same specimens, establishing the root canal axis before and after the treatment, and recognizing the root canal surface modified by the endodontic instruments. After these steps, the changes in root canal diameters (D), surface area (A), and buccolingual and mesio-distal root canal ratio (R) were performed. Moreover, a qualitative evaluation of root canal preparation was performed. Student *t*-test was used to determine significant differences between the two experimental groups ($P < 0.05$).
Results No instruments fractured. No statistically significant differences were noted between the groups in the mean changes of the D, A and R values, except for the apical third of the mesiobuccal canal and for the distal canal of mandibular molars, where in Group 2 instruments produced significantly less change in canal diameters than in Group 1 ($P = 0.04$).
Conclusions Both the systems analyzed were able to prepare molar teeth in extracted teeth with similar distribution of dentine removal and low risk of procedural errors. Reciprocating Ni-Ti instruments are a novel and efficient system for preparing root canal systems.

R65

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Micro CT analyses of apical enlargement and molar root canal complexity in a simulated general practice environment

Aim To compare the effectiveness of two rotary hybrid instrumentation techniques with focus on apical enlargement in molar teeth as performed in a simulated general practice environment, and to quantify and visualize spatial details of instrumentation efficacy in root canals of varying complexity including intraradicular connections and consequences of unprepared portions of narrow root canals.
Methodology Eighteen molar teeth were scanned using X-ray Micro-computed tomography (SCANCO Medical AG, Switzerland), with a pixel size, slice thickness, and slice increment of 30.7 μm. Root canals were prepared either using a RaCe/NiTi protocol (FKG Dentaire, Switzerland) or a GT/Profile protocol (Dentsply Maillefer, Switzerland). Variables used for evaluation were: distance between root canal surfaces before and after preparation (DAP), percentage of root canal area remaining unprepared, and increase in volume after preparation. Root canals were classified according to size and complexity.
Results No difference was found between the two techniques: $DAP_{apical\text{-}third}$ ($P = 0.5904$), area $unprepared_{apical\text{-}third}$ ($P = 0.1258$) and volume $increase_{apical\text{-}third}$ ($P = 0.8214$). Root canal area unprepared became larger in relation to root canal size and complexity, irrespective of the techniques used. Percentage of root canal area remaining unprepared was significantly lower in small root canals compared with large ($P < 0.0001$) and in complex compared with large ($P < 0.0001$). The intraradicular connection area *per se* contributed, with a mean of 18%, and 26%, when a narrow root canal remained unprepared.
Conclusions The addition of intraradicular connections or isthmuses did not particularly alter the ratio of instrumented and unprepared areas within a root. Distal and palatal root canals had the highest level of unprepared area irrespective of the two instrumentation techniques examined.

R66

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Training required for different rotary nickel-titanium systems and its influence on root canal preparation

Aim To evaluate the influence of training on the quality of root canal preparation with NiTi systems *in vitro*.
Methodology One hundred and five simulated root canals in resin blocks, with a curvature of 35° were prepared with seven different rotary NiTi systems: MT- Mtwo, FM- FlexMaster (VDW, Germany), RS- Revo-S (Micro-Mega, France), ES- EasyShape and AK- AlphaKite (Gebr. Brasseler, Germany), GT- GT Series X (Dentsply, Germany), TF- Twisted File (SybronEndo, USA). The first five blocks

were prepared within the initial training period with each file system. Second and third sets of five blocks were then prepared with each instrumentation system during intermediate (application) and final phases of training respectively. Pre and post instrumentation images were recorded and assessment of canal shaping and material removed was carried out with an Image analysis program (GSA, Germany). Preparation time, the total active instrumentation and procedural errors were recorded and statistically assessed for the different periods.

Results In the inner curvature of canals, the difference between the materials removed in the three periods of canal preparation was significant ($P < 0.05$) with all systems except MT, while in the outer curvature the difference was statistically significant ($P < 0.05$) for MT and FM only. Preparation time significantly decreased ($P < 0.05$) in the final training period for MT, RS, and FM systems. Morphological changes and loss of working length were observed with MT, ES, GT and RS in initial training and application periods and were continued with ES in the final period.

Procedural errors were observed with TF frequently in all training periods, with GT only in the initial training period and with RS, ES and AK seldom at different periods.

Conclusions Under the conditions of this study, RS, ES and GT require more training than MT, FM, TF and AK to reduce the chance of canal aberration and to increase preparation safety.

R67

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Evaluation of reciproc versus twisted file preparations by experienced and inexperienced operators *ex vivo*

Aim To evaluate two different instrumentation techniques, Reciproc (VDW, Germany) and Twisted File (SybronEndo, USA) in simulated curved root canals, with the same master apical file (MAF) size 25, 08 taper, performed by experienced and inexperienced operators.

Methodology Twenty simulated canals in resin blocks were used. Ten dentists with no experience of either preparation technique where divided into two groups. Group A ($n = 5$) general dentists, with no experience of rotary instrumentation techniques, group B ($n = 5$) endodontists, all with experience of rotary instrumentation techniques. All operators were provided with the manufacturer's instructions prior to instrumentation. Time for the MAF to attain working length was measured. Ledge formation, canal patency and file deformation were investigated using an OPMI Pico Dental Microscope (Carl Zeiss, Germany).

Results Mean time for group A using Reciproc was 103 ± 16 s and 270 ± 94 s using Twisted Files. Mean time for group B using Reciproc was 88 ± 31 s and 181 ± 50 s for Twisted Files. The Kruskal–Wallis test was significant ($P = 0.004$). A subsequently performed Dunn's test showed significant ($P < 0.05$) differences between general dentists working with Twisted Files and endodontists working with Reciproc. One file separation and one file block occurred in group A using Reciproc. All other operators were able to maintain canal patency and prevent ledge formation. File deformation occurred twice using Reciproc whereas all used Twisted Files ($n = 30$) were deformed regardless of the rotary instrumentation experience of the operator.

Conclusion Within the limits of this laboratory study, Reciproc was faster than Twisted Files regardless of the operator's rotary experience and less file deformation was reported with Reciproc than with Twisted File.

PREPARATION: FRACTURE RESISTANCE

R68

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Numerical comparison of torsional resistance of nickel-titanium rotary files with various geometries

Aim To compare the effect of instrument geometric characteristics on the torsional resistance of nickel-titanium (NiTi) instruments.

Methodology Various finite-element (FE) models of NiTi rotary instruments with different cross-sectional geometries and different numbers of threads were made for numerical comparison of torsional resistance. Four cross-sectional shapes were given to the instrument models – triangular, rectangular and square. The same taper and radius of external periphery were given for all models, while different cross-sectional areas and/or centre-core areas were given as variables. Different pitch values (5-, 10-, and 15- threads) were incorporated into each model with the same cross-sectional geometry as another variable. The twelve FE models were then compared for torsional resistance by twisting the file shafts 20-degrees with restriction of the apical file tip at 4 mm.

Results The files with bigger pitch (fewer threads) showed the effects of decreasing torsional resistance. Files with rectangular cross-section had higher torsional resistances than files with triangular cross-section even with the same cross-sectional area. Files with bigger cross-sectional area had increased torsional resistance regardless of cross-sectional geometry.

Conclusions Under the limitations of the present study, torsional deformation and/or fracture of NiTi rotary files would be reduced by designing the files with reducing the pitch (increasing the number of threads) and increasing the cross-sectional areas rather than the centre-core area.

R69

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Cyclic fatigue resistance of ProFile versus Revo-S in a range of simulated curvatures

Aim To evaluate the cyclic fatigue resistance of new (Revo-S; MicroMega, France) versus traditional ground (ProFile; Dentsply Maillefer, Switzerland) NiTi rotary instruments in a range of simulated curvatures.

Methodology Simulated root canals were milled in a stainless steel block with a tapered shape corresponding to the dimensions of the instruments tested. ProFile ($n = 24$) and Revo-S ($n = 24$) instruments size 25, 0.06 taper were tested in two simulated root canals with an angle of curvature of 60°. One had the curvature centre at 2 mm with a radius of curvature of 2.5 mm. The centre of curvature of the other was at 5 mm from the apex, with a radius of curvature of 5 mm. The instruments were then rotated until fracture at a constant speed of 300 rpm and a maximum torque of 5.2 N cm^{-1}. Recorded working time was used to calculate the number of cycles to failure (NCF). Length of the fractured tip was also recorded. Data were analyzed by completely randomized factorial experimental design (ANOVA) to determine any statistical difference among the groups ($P = 0.05$).

Results In both of the curvature positions, ProFile instruments displayed better cyclic fatigue resistance than Revo-S ($P < 0.001$). ProFile (774.16 and 475.41 cycles) was more resistant in 5 mm

than in 2 mm curvature position in contrast to Revo-S (158.75 versus 199.58 cycles). Length of the fractured tip was significantly affected by the position of the curvature ($P < 0.001$). The lengths of the fractured tips were 1.87 ± 0.37 and 6.54 ± 1.28 mm for ProFile, 2.25 ± 0.45 and 5.75 ± 0.81 mm for Revo-S.
Conclusions Lifespan of Profile instruments was superior to Revo-S in cyclic fatigue tests regardless of the position of the curvature centre.

R70

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Cyclic fatigue resistance of three rotary Nickel-Titanium systems: Endosequence, K3 and twisted file

Aim To evaluate the fatigue resistance for three types of nickel-titanium rotary files, comparing instruments produced by a traditional NiTi grinding process with those produced by twisting and to examine their fracture characteristics.
Methodology K3 (K3; SybronEndo, USA), EndoSequence (ES; Brasseler, USA) and Twisted Files (TF; SybronEndo, USA) size 25 with 0.06 taper ($n = 5$ each) were examined by SEM for surface characteristics prior to use. The cyclic fatigue testing was performed by rotating instruments until failure inside stainless steel artificial canals with a 2 and 5 mm radius and 45° of curvature. The time to fracture was recorded and the total number of cycles was calculated for each instrument. Surfaces of fractured instruments were observed under SEM. The number of rotations to failure among all test groups was compared by one-way ANOVA and the Tukey test for individual comparisons with a confidence level of $P < 0.05$.
Results Among both curvatures (2 and 5 mm), TF instruments size 25, 0.06 taper performed significantly better than the other instruments ($P < 0.05$), showing a higher resistance to cyclic fatigue than all other files tested. No significant difference in the number of cycles to failure was noted between EndoSequence and K3 instruments ($P > 0.05$). SEM evaluation showed fracture cross sections of all brands to have a similar fractographic appearance.
Conclusions Within the constraints of this labaratory study, the Twisted File manufacturing process (R-phase technology) appeared to improve the resistance of instruments to fatigue compared with instruments manufactured by the traditional NiTi grinding process.

R71

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Fatigue resistance of traditional solid rotational endodontic instruments and a prototype non-solid instrument

Aim To compare the cyclic fatigue resistance of traditional solid rotational nickel-titanium endodontic instruments and a prototype steel super-flexible non-solid endodontic instrument.
Methodology Four types of solid rotational nickel-titanium endodontic instruments: LightSpeed size 35 (LightSpeed Technology, USA), RaCe size 20 0.02 (FKG Dentaire, Switzerland), FlexMaster size 20, 0.02 taper (VDW, Germany), PathFile 019 (Dentsply Maillefer, Switzerland) and a prototype non-solid rotational steel instrument were investigated. Fatigue testing was carried out in specially prepared, transparent glass tubes. Tubes were bent in such a way that the instrument deflection angle was 90°, and radius of curvature was 5 mm. Endodontic instruments were placed in the tubes so that their diameter at the point of maximum curvature was 0.3 mm. Rotation speed was 300 rpm. Time to fracture was recorded and converted to number of cycles for each instrument.
Results There was no significant difference in the mean number of cycles before fracture between PathFiles (198 cycles), FlexMaster (197 cycles) and Race (232 cycles). LightSpeed showed a significant increase in the mean number of cycles to failure (608 cycles) when compared to the tapered instruments ($P < 0.05$). The prototype steel non-solid endodontic instrument reached significantly higher number of cycles before fracture (1315 cycles) when compared to all instruments ($P < 0.05$).
Conclusions The new prototype non-solid steel endodontic instrument had better fatigue characteristics than several traditional solid nickel-titanium endodontic instruments. In addition, non-tapered solid nickel-titanium instrument had significantly higher fatigue resistance than tapered solid nickel-titanium instruments of the same diameter.

R72

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Effect of sterilization on the surface of rotary NiTi instruments evaluated by SEM and EDS

Aim To evaluate the effects of repeated dry heat sterilization on the surface characteristics of NiTi rotary instruments by SEM and energy dispersive x-ray spectroscopy (EDS).
Methodology Thirty-two new instruments, ProTaper (Dentsply Maillefer, Switzerland) and AlphaKite (Komet, Italy) with a coating of TiN were evaluated. The instruments were divided into four groups: control, 1, 5 and 10 cycles of sterilization. Each group included four ProTaper (S1, S2, F1, F2) and four AlphaKite (15, 20, 30, 40) instruments. After sterilization, the instruments were observed by a low-vacuum SEM (FEI, Netherlands), and surface analysis was performed on each instrument by EDS. Measurements were performed on the active part and on the shank.
Results SEM observations indicated the presence of debris, pitting and deep milling marks in both new and sterilized files. After 5 and 10 sterilization cycles, surface roughness increased significantly ($P < 0.05$) for ProTaper, while AlphaKite (TiN-coated) instruments showed no significant differences compared to the controls ($P < 0.05$). EDS analysis showed that ProTaper instruments were composed mainly of nickel 54.15%, titanium 43.66% and aluminum 2.19%. In addition to titanium (46.24%) and nickel (30.38%), AlphaKite showed the presence of nitrogen as high as 21.08%. These relations changed after dry heat sterilization, with alterations of the coating surface; in particular ProTaper had a decrease in Nickel and Titanium percentages and an increase of Aluminum, while Alphakite had a reduction of TiN and an increase in Nickel.
Conclusions Dry heat sterilization might modify the surface morphology and chemical composition of NiTi instruments and that TiN coatings can reduce these phenomena.

R73

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Influence of thermal treatment on the flexural fatigue resistance of NiTi alloys

Aim To evaluate the influence of specific thermal treatment for the manufacturing of a novel NiTi alloy, named CM-wire™ (Coltene-Whaledent, Germany) on the flexural fatigue resistance of NiTi rotary instruments.
Methodology Two groups of 20 instruments each of the same size 25, 0.06 taper were tested for cyclic fatigue resistance under continuous rotation at 300 rpm. Tests were performed using a cyclic fatigue testing device to evaluate the number of cycles to failure (NCF) of a first group of instruments manufactured without thermal treatment (group A) and of a second group manufactured with the CM-wire™ thermal treatment (group B). The artificial canal used had a 60° angle of curvature and a 5 mm radius of curvature. Means and standard deviations of the NCF were calculated for the two groups and student *t*-test was performed to evaluate any statistically significant difference between the two groups. Significance was set at $P < 0.05$.
Results Mean NCF values (standard deviation) for group A were 173 (±25) and for group B, 1331 (±95). The difference between the two groups was highly significant ($P < 0.001$).
Conclusions Since instrument design and dimensions were the same, it may be concluded that the thermal CM-wire specific thermal treatment and manufacturing process influenced the *in vitro* cyclic fatigue resistance of NTR instruments significantly when compared with traditional NiTi alloy.

R74

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Evaluation of deformation and fracture rate of ProTaper nickel-titanium rotary instruments after clinical use

Aim To evaluate the deformation and fracture rate of ProTaper nickel-titanium rotary instruments in relation to the number of clinical uses.
Methodology A total of 619 ProTaper (Dentsply Maillefer, Switzerland) instruments (S1, S2, F1, F2, F3) were collected after each clinical use by one endodontist over a period of 4 years. These instruments were grouped following 1–3, 4–6 and 7–9 clinical uses, the decision on the number of re-uses being at the discretion of the endodontist. All instruments were ultrasonically cleaned and autoclaved in standardized conditions following clinical uses. The instruments were then evaluated blindly by one investigator under a stereomicroscope for deformation and fracture. The data were statistically analyzed using Fisher's exact test ($P < 0.05$).
Results The overall proportion of instrument deformation was found to be 10%, and instrument fracture amounted to 1%. There was no statistically significant difference among groups of instruments used 1–3, 4–6 or 7–9 times for each of S2, F1 and F2 instruments with regard to deformation and fracture incidence ($P > 0.05$). However, S1 showed higher incidence of fracture when used 1–3 times as compared to 4–6 ($P = 0.025$) and 7–9 ($P = 0.004$) times whereas no significant difference was detected in deformation incidence for S1 ($P > 0.05$). When all instruments were compared to each other with regard to number of uses, there was no significant difference among the instruments except for S1 which showed higher incidence of deformation than S2 ($P = 0.04$), F1 ($P = 0.008$) and F2 ($P = 0.049$) when used 7–9 times. Since F3 was not preferred for use more than three times clinically, some sub-groups could not be included within the statistical analysis.
Conclusions In the hands of an experienced endodontist, ProTaper rotary files could be safely used on occasions up to nine times.

FILLING: LEAKAGE

R75

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The effect of sodium hypochlorite solutions on sealing ability of two different root canal filling systems

Aim To evaluate *ex vivo* the effect of sodium hypochlorite (NaOCl) solutions with different compositions and concentrations on the sealing ability of gutta-percha/AHPlus (Dentsply DeTrey, Germany) and Resilon/Epiphany SE (Pentron Clinical Technologies, USA).
Methodology Crowns of ninety extracted human mandibular premolar teeth were removed. The roots were randomly assigned into four experimental groups according to the sodium hypochlorite irrigation solutions used: (i) 5% ACE (Procter & Gamble, Turkey), (ii) 1% ACE, (iii) 5% Domestos (Unilever, Turkey), (iv) 1% Domestos. Root canals were instrumented using RaCe instruments to size 40, 0.04 taper and 5% EDTA was used to remove the smear layer. In all groups, the specimens were randomly assigned into two subgroups according to the filling system used: (i) gutta-percha/AH Plus and (ii) Resilon/Epiphany SE, both using the lateral compaction technique. The remaining 10 teeth were used as controls. Leakage was measured one week after filling by the fluid filtration method.
Results Mann–Whitney analysis indicated significantly less leakage ($P < 0.001$) using gutta-percha/AHPlus compared to Resilon/Epiphany SE. NaOCl solutions with 5% concentration resulted in higher leakage values than NaOCl solutions with 1% concentration (Kruskal–Wallis, $P < 0.001$). For gutta-percha/AHPlus groups, while the difference between brands (ACE versus Domestos) was not statistically significant ($P > 0.01$), the difference within brands with varying concentrations was statistically significant ($P < 0.01$). Teeth irrigated with 5% Domestos leaked more than the other gutta-percha groups. For Resilon/Epiphany SE, the difference between all groups was statistically significant with the following ranking: 5% ACE > 5% Domestos > 1% ACE > 1% Domestos ($P < 0.01$).
Conclusions Both gutta-percha and Resilon groups were significantly more resistant to fluid movement when NaOCl was used at lower concentrations. The compositions of NaOCl solutions adversely affected the sealing ability of each filling system *ex vivo*.

R76

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The effect of chlorhexidine on the microleakage of three different sealers in root canals

Aim To determine the long term effect of chlorhexidine on microleakage of root fillings with three different sealers.
Methodology Sixty individual roots from extracted third molars were filled with three different sealers and gutta-percha cones. The root canals were irrigated with 3% NaOCl (Chlorcid; Ultradent, USA) and 18% EDTA (Ultradent) during preparation. The roots were divided into six groups ($n = 10$). Before filling, the root canals were irrigated either with CHX (Consepsis 2%; Ultradent) or with physiological saline for 60 s. The three different sealers: (i) RealSeal SE (SybronEndo, USA), (ii) Topseal (Dentsply Maillefer, Switzerland) and (iii) EndoREZ (Ultradent) were applied into root canals according to the manufacturer's instructions. The roots were filled with a master cone and 1–3 accessory points. The roots were stored in artificial saliva for 18 months. Microleakage was measured by the fluid filtration method (Flodec; De Marco Engineering, Switzerland) with a pressure of 6.89 kPa (10 psi). Kruskal–Wallis and Mann–Whitney *post-hoc* tests were used for statistical analysis.
Results EndoREZ demonstrated the highest leakage both with and without CHX: the differences between RealSeal and Topseal were minor. CHX improved the sealing ability only with EndoREZ. With RealSeal ($P = 0.011$) and Topseal ($P = 0.089$) the microleakage was slightly higher with CHX.
Conclusions Chlorhexidine had a small detrimental effect over a relatively long time period with RealSeal SE and Topseal, but improved that with EndoREZ.

R77

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The quality of root fillings created by three root filling techniques when evaluated by bacterial leakage and micro-computed tomography

Aim To compare the quality of root fillings produced by three root filling techniques (lateral compaction, thermoplasticized ProTaper technique and ProTaper single-cone technique) using a bacterial leakage model and micro-computed tomography (micro-CT) and to determine the correlation between the two testing methods.
Methodology The study sample consisted of 51 extracted human single-rooted teeth, which were instrumented using the conventional sequence of ProTaper to a final size of F2. The teeth were randomly divided into three experimental groups of 15 roots each according to the root canal filling technique; group 1, lateral compaction using gutta-percha cones; group 2, thermoplasticized ProTaper technique; group 3, ProTaper single-cone technique. AHPlus was used as a root canal sealer in all groups. Positive controls ($n = 3$) were left without filling, and negative controls ($n = 3$) were filled as in the lateral compaction group and covered with three layers of nail varnish. The quality of the root fillings was evaluated using a bacterial leakage model, and the volume of voids and gaps in root canal fillings was measured using micro-CT.
Results After the period of 30 days, 13 samples in the Thermafil and single cone groups leaked bacteria. In the lateral compaction group, 11 samples leaked bacteria. There was no statistically significant difference between three groups using a bacterial leakage test and micro-CT ($P > 0.05$). The results obtained by bacterial leakage test and micro-CT correlated well.
Conclusions No differences in the quality of canal filling between three techniques was identified using a bacterial leakage test or micro-CT. Correlation between bacterial leakage testing and micro-CT revealed that both methods can be used effectively for the evaluation of root canal filling *ex vivo*.

R78

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An *ex vivo* quantitative evaluation of the sealing ability of two canal filling techniques using a glucose leakage test

Aim To evaluate the sealing ability of two different canal filling techniques in a glucose leakage test.
Methodology Forty eight extracted single-rooted teeth were decoronated and the root canals prepared using ProTaper® rotary instruments to an apical dimension of F3. The specimens were then randomly divided into two experimental groups ($n = 16$) and two control groups ($n = 8$). The root canals were filled with gutta-percha and AH Plus® sealer by cold lateral compaction (group A) and combined warm vertical compaction using the Elements Obturation Unit® (group B). For the negative control group, eight roots were filled using lateral compaction as in group A. Another eight roots were filled using lateral compaction, but without any sealer, as the positive control. A glucose leakage model was used for quantitative evaluation of the coronal-to-apical microleakage at 24 h, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 weeks. Data were recorded as mM and analyzed statistically with a Mann–Whitney *U*-test. The significance level was set at $P < 0.05$
Results The positive control group gave significantly more glucose penetration values than experimental groups throughout the experimental period ($P < 0.05$). For the positive controls, gross leakage was seen on the first day and increased rapidly over time. In the negative control group, no glucose was detected throughout the experimental period. For the two experimental groups, the amount of leakage increased gradually throughout the test period. No significant difference in the cumulative amount of leakage was found among the A and B groups at all observation times.
Conclusions Cold lateral compaction and combined warm vertical compaction technique with the Elements Obturation Unit® resulted in similar leakage. The glucose leakage method used in this study was able to provide a nondestructive, quantitative, long-term evaluation of the sealing ability of root canal fillings.

R79

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Sealing ability of different root canal sealers

Aim To compare apical microleakage of canals filled with six different root canal sealers: I Root SP (Verio Dental Co. Ltd., Canada), MTA Fill-Apex (Angelus, Brazil), AH Plus (DeTrey, Germany), Sealapex (Kerr, USA), Roekoseal (Coltène/Whaledent, Germany) and Acroseal (Fisher Scientific, Canada) using a fluid filtration system.
Methodology Ninety-four single rooted teeth were used. Six groups of 15 teeth received root canal fillings of gutta-percha and

one of the following sealers: I-Root SP, MTA Fill-Apex, AH-Plus, Sealapex, Roekoseal, Acroseal (randomised) using the lateral condensation technique. Four teeth were used as negative controls. After 1 week of storage in 100% humidity, a computerized fluid filtration system was used to evaluate apical leakage. Filtration rate was measured by movement of an air bubble in a micropipette at 2 min intervals under a pressure of 120 kPa (1.2 atm). Measurement was performed four times for each specimen. The mean values were determined and expressed as μL cm H_2O^{-1} min^{-1}. All data were fed into PC compatible software and analyzed statistically using ANOVA followed by Tukey HSD test ($P < 0.05$). The negative control group was tested with a root of which the apex was covered with three coats of nail polish to ensure that was no leakage in the device.

Results Acroseal had the most and AH-Plus the least leakage, but there was no statistical significant difference amongst groups ($P < 0.05$).

Conclusions All sealers were associated with minimal leakage; there was no significant difference between the materials.

R80

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Interface porosity of human root canals sealed with a new flowable MTA-based sealer: a high-resolution micro-computed tomography study

Aim To develop a novel endodontic sealer based on a flowable calcium-silicate MTA cement and to measure the 3D volume of voids (pores/gaps) at the interface of filled root canals using a high-resolution micro-computed tomography (micro-CT).

Methodology An experimental hydraulic hydrophilic flowable root-canal sealer (FMTA endo-flow) composed of a fluoride-doped tricalcium-silicate/dicalcium-silicate mineral powder and a specific hydrophilic polymer was designed (Gandolfi MG, University of Bologna, Italy). Sixteen single root-canal from extracted teeth were prepared with ProTaper rotary instruments (Dentsply Maillefer, Switzerland), randomly allocated into two groups ($n = 8$) and filled with size 30 Thermafil Obturators (Dentsply Maillefer) in association with FMTA endo-flow or AH Plus (Dentsply Maillefer) sealers. The filled roots were stored at 37°C in 5 mL of simulated body fluid HBSS (Hanks's Balanced Salt Solution, Lonza, Belgium) and scanned using a high-resolution micro-CT (SkyScan1172, Belgium) after 7 days and 6 months. The image datasets included 3000 images (in 8-bit jpg format) that were analyzed in 3D and binarized (software CTAnalyser version 1.10.1.0; Skyscan). The 3D distribution of the voids (porosity and marginal gaps) at the gutta-percha-sealer-dentine interface were detected through a threshold grey level and expressed as percentage of a predefined interface volume of interest (20 microns of interface dentine + 20 microns of gutta-percha-sealer). A method of analysis based on the root-canal segmentation was used and coronal, middle and apical thirds considered separately. The results (3D void volume) were compared statistically by two-way ANOVA (statistical significance for $P < 0.05$).

Results Micro-CT detected gaps at the dentine-sealer interface in both groups. The apical thirds showed a significantly lower 3D void volume ($P < 0.05$) than the middle and coronal thirds. The 3D void volume reduced significantly ($P < 0.05$) with time. The preliminary analysis of the micro-CT data showed the following 3D void volume values: at 7 days 0.17 ± 0.06 apical, 0.56 ± 0.16 middle, 1.63 ± 0.39 coronal third for FMTA endo-flow sealer and 1.47 ± 0.46 apical, 2.01 ± 0.50 middle, 4.30 ± 0.87 coronal thirds for AH Plus. At 6 months 0.18 ± 0.07 apical, 0.32 ± 0.09 middle, 1.40 ± 0.34 coronal third for FMTA endo-flow sealer and 0.30 ± 0.09 apical, 0.56 ± 0.14 middle, 1.30 ± 0.37 coronal thirds for AH Plus.

Conclusions (i) apical thirds were relatively void/gap free compared with middle and coronal thirds; (ii) the experimental flowable MTA-based sealer may be used in association with Thermafil; (iii) smaller voids/gaps were detected in roots filled with the flowable MTA-based sealer than with AH Plus; (iv) micro-CT was a suitable technique to estimate the presence of voids/gaps at the dentine-sealer-gutta-percha interface.

R81

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Sealing ability of calcium-containing endodontic sealers TechBiosealer Endo, CRCS and Sealapex in teeth with open apices

Aim To evaluate the sealing ability of three commercial Ca-containing endodontic sealers in teeth with artificial wide-open apices *ex vivo*.

Methodology Thirty human single-rooted teeth were shaped with ProTaper instruments (Dentsply Maillefer, Switzerland) and then enlarged using a no. 4 Gates Glidden drill (Dentsply Maillefer) to obtain an apical root canal diameter of size 110. The canals were irrigated between each instrument with 0.5 mL of EDTA and 1 mL of NaOCl, and finally rinsed with deionized water. A tailored device was designed (*Prati periapical chamber*): a special silicone (Optosil) support was prepared for each root and 0.02 mL of HBSS (Hank's Balanced Salt Solution) was added at the bottom of the support to simulate the presence of periapical fluid. The samples were randomly divided into three groups and filled with a hydraulic hydrophilic bioactive calcium-silicate sealer (TechBiosealer Endo, Italy), a calcium-hydroxide eucalyptol-&-eugenol-containing sealer (CRCS – Calciobiotic Root Canal Sealer; Hygenic, USA) or a non-eugenol calcium-hydroxide polymeric root-canal sealer (Sealapex; Kerr, USA). Each sealer was prepared according to the manufacturers instruction and gently compacted with endodontic pluggers sizes 3 and 4 (LM-Dental, Finland) in the apical 5 mm. No back-filling was performed in order to leave a fluid reservoir for fluid-flow testing. All the samples were stored at 37°C in 10 mL of HBSS and the sealing ability was assessed after 24 h, 7 days, 1 and 3 months using a micro-infiltration measurer (*Gandolfi digital fluid flow-meter*) working at 1 psi hydraulic pressure. The data were statistically analyzed by two-way ANOVA (Student-Newman-Keuls test, significant differences at $P < 0.05$).

Results TechBiosealer Endo maintained a stable seal from 24 h to 3 months showing values significantly lower than the other groups ($P < 0.05$). The fluid filtration values of TechBiosealer Endo (≈0.045 μL min^{-1}) and Sealapex (≈0284 μL min^{-1}) groups were significantly lower ($P < 0.05$) than CRCS group (≈1.765 μL min^{-1}).

Conclusions The use of the hydraulic Ca-containing sealer TechBiosealers Endo provided the most stable seal compared to the other commercial Ca-containing sealers up to 3 months. The use of hydraulic hydrophilic bioactive sealers is recommendable for treatment of teeth with open apices.

R82

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The use of high performance liquid chromatography – tandem mass spectrometry for endodontic nanoleakage studies

Aim To introduce a new high performance method for nanoleakage quantitative analysis of endodontic sealing materials.
Methodology Thirty-five single-rooted extracted teeth were randomly divided into three experimental groups ($n = 10$) and one negative control group ($n = 5$). The experimental groups were prepared using a rotary crown-down technique and filled by lateral compaction with: Resilon + Epiphany (G1), gutta-percha + Sealapex (G2) or gutta-percha + AH Plus (G3). The negative control group was filled using lateral compaction with gutta-percha and AH Plus sealer, and was subsequently completely coated with two layers of nail varnish, including root canal orifices and apical foramina. Each root was mounted in a specific system apparatus, using caffeine solution as the tracing agent (2000 ng mL^{-1}, pH 6.0). The tracing agent was placed over the coronal surface of the root with a hydrostatic pressure of 2.55 kPa towards the apical foramen. Nanoleakage of caffeine was measured by a type of high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS), measuring the presence of the caffeine in a receiving sterile solution into a glass bottle at intervals of 10, 30, and 60 days.
Results The negative control group had no leakage in all periods. For all experimental groups no nanoleakage was observed at the 10 day analysis. After 30 days, G2 and G3 groups showed similar nanoleakage patterns with mean values of 16.0 and 13.9 ng mL^{-1}, respectively ($P = 0.9981$). G1 group had higher leakage than G2 and G3 with a mean value of 105.2 ng mL^{-1}, that was significantly different from the other two groups ($P = 0.0001$). After 60 days, a significant difference on infiltration was observed among the groups ($P < 0.0001$). The maximum values were 182.6 ± 14.85 ng mL^{-1} for G1, 139.0 ± 10.83 ng mL^{-1} for G2 and 53.5 ± 3.87 ng mL^{-1} for G3.
Conclusions AH Plus had better sealing ability than the other groups at 60 days. HPLC-MS/MS demonstrated high sensitivity and specificity for the quantification of the tracing agent, with potential as a reliable tool for nanoleakage studies.

R83

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Effect of smear layer on the apical seal of MTA using radioisotope methods

Aim To evaluate the effect of smear layer on the apical microleakage of MTA placed as an apical barrier in permanent teeth with simulated immature apices.
Methodology Thirty-four single-rooted extracted teeth were selected according to predetermined criteria and kept in saline solution. After the crowns were sectioned to obtain 17 mm-long root segments, 2 mm of the apex of each root was resected and an open apex model created. The root canal of each tooth was prepared by the same operator using rotary ProFile™ instruments. The root segments were randomly assigned to four groups: G1 ($n = 12$) – group smear layer (+), G2 ($n = 12$) – group smear layer (–), G3 ($n = 5$)- (+) control group and G4 ($n = 5$) – (–) control group. In all groups a 4 mm apical barrier of MTA was placed with the exception of group G3 (positive control group).
Two coats of nail polish were applied to the external surface of each root except for the resected apical root-end, unlike the negative control group, where the root ends were also sealed with nail polish. On the 7th and on the 28th day the apices of the teeth were submerged in a solution of ^{99m}Tc-Pertechnetate for 3 h. The radioactivity was counted using a gamma counter and a gamma camera.
Results The results revealed statistically significant differences between the control group and the experimental group smear layer (–), ($P = 0.031$). Within the experimental groups there were no statistically significant differences.
Conclusions Within the limits of this *ex vivo* study it may be concluded that there is no particular advantage in removing smear layer to improve the sealing capacity of MTA in apexification procedures.

FILLING: MTA

R84

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Cellular response of human pulp-derived cells to MTA and a novel calcium phosphate-containing silica xerogel

Aim To evaluate and compare the cellular response of human, pulp-derived, fibroblast-like cells to MTA (ProRoot™MTA; Dentsply Tulsa, USA) and a novel calcium phosphate/silica xerogel composite (BONITmatrix®; DOT GmbH, Germany).
Methodology Eluates of the tested materials were prepared at three time points: 1, 5 and 7 days after mixing. Cells were seeded into 96-well plates at 2×10^4 cell/well incubated with 100 μL eluates from each group. Cells cultured with culture medium only served as control. After 24 h incubation, cell viability in response to eluates of the three time points was assessed using MTT assay. The effect of 5-day eluates of the tested materials on cell number was assessed by the crystal violet proliferation assay. The effect of direct contact of specimens of materials on cells in culture was observed using inverted microscopy after 5 and 9 days. Statistical analysis involved one-way and two-way ANOVA followed by Tukey *post hoc* for multiple comparisons.
Results One- and 5-day eluates of MTA and BONITmatrix® resulted in significantly higher cell viability than the control ($P < 0.05$); however, with the 7 day eluates, MTA resulted in significantly higher cell viability than both BONITmatrix® and the control ($P < 0.05$). Cell number with the material eluates was similar to the control ($P > 0.05$). For MTA, three zones could be identified around specimens: zone of lysis, cell-free zone and zone of normal cell morphology after 5 days; a decrease in the zone of lysis and appearance of a few cells in the cell-free zone occurred after 9 days. Cells showed direct contact with the BONITmatrix® particles with wider intercellular spaces than in the control after 5 and 9 days.
Conclusions MTA and BONITmatrix® showed biocompatibility to human, pulp-derived cells. BONITmatrix® might be considered as a potential pulp capping agent.

R85

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Histocompatability of bioceramic nanoparticulate bioaggregate and mineral trioxide aggregate

Aim To investigate and compare the systemic toxic effect of DiaRoot BioAggregate which is composed of ceramic nano-particles and ProRoot MTA on liver, kidney, spleen, and lymph nodes after 7 and 30 days.
Methodology Thirty-six white albino rats were divided into three groups. Group (1) received saline as a control group; Group (2) received DiaRoot BioAggregate (Diadent, Canada); Group (3) received ProRoot MTA (Dentsply, Tulsa Dental Specialty, USA). The Teflon tubes were filled with freshly mixed BioAggregate and MTA and implanted subcutaneously in the dorsal side of the rats. Blood samples were taken to investigate the change of kidney and liver functions on day 7 and day 30. Then, these organs were sampled for histopathological examination.
Results On day 7 a significant severe inflammatory reaction was observed in all tested organs in the experimental groups (2) & (3) (P-value < 0.05), compared to the control. The severity of reaction decreased over time to 30 days. However, some specimens exhibited a slight persistent inflammatory cell infiltration especially around the hepatic portal vein and areas of degeneration in lymph nodes .The kidney functions were not affected after 7 days but increased after 30 days. Liver functions increased after 7 days and decreased in the BioAggregate group after 30 days while in the MTA group a continuous increase in liver function was observed.
Conclusions DiaRoot BioAggregate and ProRootMTA showed almost similar systemic reaction of initial inflammation that subsided over time without permanent injury of organs with acceptable tissue toxicity. Increased liver function in the MTA group requires further investigation.

R86

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Cytotoxicity of MTA and Portland cement prepared with different mixing vehicles on L929 fibroblasts

Aim To evaluate the cytotoxicity of ProRoot MTA (Dentsply Maillefer, Switzerland) and Portland cement (PC) mixed with sterile water (SW) or an experimental liquid (EL) (Kollicoat SR 30 D; BASF, Germany), using cell culture techniques.
Methodology L929 fibroblast cells were seeded in six-well plates and allowed to attach overnight. The cells were exposed to the freshly mixed specimens of ProRoot MTA with SW (MTA + SW) or with EL (MTA + EL) and Portland cement with SW (PC + SW) or with EL (PC + EL), which were placed over permeable membrane inserts. Untreated cells served as controls. Cell proliferation after incubation periods of 24 and 48 h were determined by using MTT assay. Cytotoxicity was rated based on the cell viability relative to control. The absorbance data were analysed statistically by two-way ANOVA and *post hoc*-test at a significance level of $\alpha = 0.05$.
Results No significant difference was observed between control and experimental groups ($P > 0.05$). After 24 h incubation period PC + EL was significantly less toxic ($P < 0.05$), however after 48 h incubation period PC + EL was significantly more toxic than the other experimental groups ($P < 0.05$). Only MTA + SW after 24 h incubation (89.47%) and PC + EL after 48 h incubation (80.26%) were found to be slightly cytotoxic to L929 cells, others were found to be non-cytotoxic.
Conclusions All the tested mixtures of MTA and PC were non or only slightly cytotoxic to the L929 fibroblasts. This does not necessarily reflect the long-term *in vivo* risk for adverse effects as vital tissue is generally more resistant to toxic substances than a cell culture.

R87

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Calcium and hydroxyl ion release and apatite-precipitation triggering by TheraCal, a new light-curable MTA-containing material for pulp-capping

Aim To evaluate the ion-release and the apatite-forming ability (i.e. bioactivity) of a novel, light-curable MTA-containing pulp capping material (TheraCal; Bisco Inc, USA) when immersed in phosphate-containing solution.
Methodology Sample disks (8 mm dia × 1.6 mm) of TheraCal, Dycal and ProRoot MTA (Dentsply, USA) and Vitrebond (3M; USA) were prepared. TheraCal and Vitrebond samples were light-cured on both surfaces for 20 s using a LED light after application of a transparent polyester strip (Directa Matrix Strips; Directa AB, Sweden). Dycal and ProRoot MTA samples were cured at 37°C, 98% relative humidity for a time 50% longer than the final setting time (ISO6876), i.e. 2 min for Dycal, 117 min for ProRoot MTA. The discs were immersed in 10 mL deionized water and calcium and hydroxyl ion release analyzed using ion-selective electrodes after 3 and 24 h and 3, 7, 14, 28 days at 37°C. For bioactivity evaluation, discs were immersed in 10 mL phosphate-containing solution DPBS (Dulbecco's Phosphate Buffered Saline) at 37°C. The surface chemistry, morphology and formation of apatite on materials surface after 1, 7, 14, 28 days in DPBS was assessed by ESEM-EDX, micro-Raman and FT-IR techniques.
Results TheraCal released the most calcium throughout the test period and alkalinized the surrounding fluid initially to pH 10–11 (3 h–3 day) and subsequently to pH 8 (7–28 day). Amorphous apatite (952 cm^{-1} Raman band) was detected within the first 24 h, while a more crystalline apatite (960 cm^{-1} Raman band) was seen at 7 days. A layer of apatite entirely covered the surface of TheraCal after 28 days.
Conclusions TheraCal and ProRoot MTA demonstrated the capacity to form apatite after just 24 h in DPBS. Dycal showed limited, and Vitrebond no bioactivity. In this laboratory setting, TheraCal acted as a calcium-releasing material that induced the formation of apatite when in contact with biological fluids. It may have potential to promote new dentine formation and cell proliferation.

R88

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The effect of various mixing and placement techniques on the compressive strength of mineral trioxide aggregate

Aim To evaluate the effect of trituration of encapsulated MTA and conventional condensation as mixing techniques and indirect ultrasonic activation as a placement technique on compressive strength of mineral trioxide aggregate.
Methodology White ProRoot MTA (Dentsply Maillefer, Switzerland) and white MTA Angelus (Angelus Solucoes Odontologicas, Brazil) were used. An aliquot of 0.34 g of distilled water was added to the MTA powder and left until it was absorbed. It was then mixed either by trituration of a capsule for 30 s at 4500 rpm or compacted by application of condensation pressure of 3.22 MPa for 1 min. The material was placed into stainless steel cylindrical moulds 6 mm high and 4 mm in diameter. Half of the samples were then treated with ultrasonic vibration for 30 s at scale five using a Suprasson P5 Ultrasonic Booster (Satelec, France). All samples were incubated after preparation and subjected to compressive strength testing after 4 days. Tukey HDS and independent *t*-tests were carried out to compare the means at a significance level of $P < 0.05$.
Results Compressive strength values of ProRoot MTA were significantly higher than those of MTA Angelus ($P < 0.05$). The highest compressive strength values were recorded in samples of ProRoot MTA that were mixed by trituration and ultrasonically activated (mean = 101.71 MPa) whereas the lowest values were recorded for MTA Angelus samples that were mixed by conventional condensation and placed without ultrasonic activation (mean = 53.47 MPa). Regardless of the MTA type and the placement method, a statistically significant difference was found between the mixing techniques ($P < 0.05$); the samples mixed by trituration had higher compressive strength values.
Conclusions Irrespective of mixing and placement techniques, ProRoot MTA had higher compressive strength values than MTA Angelus. Trituration of capsules containing premeasured MTA and water enhanced the compressive strength of the material and provided a standardized way of mixing.

R89

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Antibacterial activity of MTA and Portland cement against three bacterial species

Aim To evaluate and compare the antibacterial activity of mineral trioxide aggregate (ProRoot ™MTA; Dentsply) and Portland cement (PC) using the direct contact test (DCT).
Methodology Antibacterial activity was examined against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, which were placed on the materials immediately, 3 days, or 7 days after mixing. Materials were placed at the bottom of wells in 96-well plates in triplicate. Empty wells served as controls. Aliquots of each bacterial suspension [10 μL, optical density (OD) = 0.35 at 540 nm] were placed in the test and control wells for 1 h at 37°C. Fresh media were then added to each well, after which a 15 μL quantity of bacterial suspension was transferred to corresponding wells in new plates with fresh media (215 μL). Growth of surviving bacteria was then measured by microplate spectrophotometry every 2 h for 6 h at 620 nm. Each experiment was repeated three times ($n = 9$/gp). Data for each bacterial species were statistically analyzed using three-way mixed ANOVA followed by Tukey *post hoc* test for multiple comparisons.
Results The type of material (control, MTA, PC) and setting condition significantly affected OD ($P < 0.05$). MTA and PC had lower OD than the control ($P < 0.05$), yet no difference was found between MTA and PC ($P > 0.05$). When the control was excluded, MTA and PC had similar OD with *P. aeruginosa* and *E. faecalis* ($P > 0.05$), but MTA had lower OD than PC with *S. aureus* ($P < 0.05$). Fresh and 3-day-old mixes had significantly lower OD than 7-day-old mixes for the three species.
Conclusions MTA and PC showed antibacterial activity against the three tested bacterial species. The antibacterial activity was similar for both materials against *P. aeruginosa* and *E. faecalis*, while MTA had more antibacterial activity than PC against *S. aureus*. Fresh and 3-day-old mixes had more antibacterial activity than 7-day-old mixes.

R90

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A 3D μ-CT evaluation of root canal filling with MTA

Aim To assess the effect of indirect ultrasonic application on the incidence of voids within MTA root fillings and at the interface with the root canal walls *ex vivo*.
Methodology Human teeth with single canals and minimal curvature were collected. Canal instrumentation was carried out to size 50, 0.05 taper apically and teeth were randomly allocated into two groups ($n = 12$). In group A (MC group); manual condensation of MTA was carried out using hand pluggers. In group B (UC group); indirect ultrasonic activation was applied for 1 s to each increment of manually compacted MTA. Filled roots were scanned using an X-ray Micro-CT device to produce 2D images. Those images were reconstructed using computer software to obtain cross-sectional 2D slices which were integrated into 3D models and analyzed for the percentage volume of voids in relation to the total filled canal volume. Data were analysed using the Mann–Whitney test at $P < 0.05$.
Results Ultrasonic condensation of MTA produced a mean volume of voids of 3.8% compared to 0.7% voids obtained by manual condensation. The difference was statistically significant ($P < 0.001$).
Conclusions One second of indirect ultrasonic activation following manual condensation of MTA increments resulted in more voids than manual condensation alone. It remains to be determined whether different ultrasonic application times would have a different effect and whether the observed effects could have any clinical significance.

FILLING: OTHER

R91

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Cytotoxicity comparison of four root canal sealers on human gingival fibroblasts

Aim To investigate the cytotoxic effects of methacrylate resin (EndoREZ; Ultradent, USA), epoxy resin (AH Plus; Dentsply Maillefer, Switzerland), calcium hydroxide (Apexit; Ivoclar Vivadent, Liechtenstein) and silicone based (GuttaFlow; Roeko/Coltene/Whaledent, Germany) root canal sealers on human gingival fibroblasts at different times after mixing.
Methodology Sealer specimens were fabricated in sterile cylindrical Teflon moulds (4 mm diameter × 2 mm high) and divided into groups: fresh and aged specimens. Fresh specimens were immersed in extraction media immediately while aged specimens were stored in a humid environment at 37°C in a 5% CO_2 incubator for 24, 48 h and 7 days before extraction in cell culture medium using the ratio 1.25 cm^2 mL^{-1}. Human gingival fibroblasts were then exposed to the eluates. The cytotoxic effects of root canal sealer eluates on human gingival fibroblasts were evaluated by WST-1 assay (Roche). The results were analyzed using ANOVA single factor test.
Results Eluates of all four sealers expressed cytotoxicity. The effects of the different root canal sealers on cell viability were deemed significant ($P < 0.05$). While eluates of AH Plus and EndoREZ showed slight cytotoxicity for all time periods, GuttaFlow and Apexit showed slight cytotoxicity only for 24 h. GuttaFlow and Apexit eluates were not cytotoxic for other time periods.
Conclusions AH Plus and EndoREZ expressed slight cytotoxicity. GuttaFlow and Apexit were considered as biocompatible root canal sealers.

R92

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Physicochemical properties of AH Plus® taken from differet parts of the tube

Aim To analyse several physicochemical properties of AH Plus® (Dentsply, Germany), including setting time, flow and the setting reaction *in vitro* and to correlate the results with the source of the material: from the beginning, middle or end of the tubes in which they were supplied.
Methodology Three experimental groups were established for each property investigated. Group 1: corresponded to material taken from the beginning of tubes A & B, Group 2: corresponded to material taken from the middle of each tube, and Group 3: from the end of each tube. The setting time was recorded according to ASTM International-C 266-08. The flowability test was performed according the ANSI/ADA Specification No. 57. The Degree of Conversion (DC) was determined from infrared spectra, which were recorded at 0, 1, 2, 3, 4, 5, 6, 8, 10, 16, 18, 20, 24 and 30 h. Data were analyzed statistically by analysis of variance (ANOVA) and the Tukey test, with a significance level of 5%.
Results With the exception of the DC, the performance of the Group 1 samples was significantly different ($P < 0.05$) from middle (Group 2) and end (Group 3) samples, displaying higher values.
Conclusions Segregation appears to occur between the organic and inorganic components of AH Plus®, especially at the beginning of the catalyst paste (paste B), changing the setting time and flow.

R93

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Apical sealer placement into simulated canal irregularities using different placement techniques *ex vivo*

Aim To evaluate the quality of sealer placement into apical simulated canal irregularities using different sealer placement techniques.
Methodology Root canals of 120 straight single rooted human teeth were instrumented to size 80, 0.02 taper. Teeth were split longitudinally into two halves, reassembled, and embedded into model resin, to form separable models of teeth. Canal irregularities were ground into different halves of the teeth: one side: groove of 0.3 (width) × 2 × 6 mm, beginning from 2 mm distance from the apex; other side: four standardized holes of 0.3 mm in diameter and 2 mm depth, in distances of 2, 4, 6, and 8 mm from the apex. After remounting the teeth, root canals were filled with AH plus (DeTrey) using one of the following sealer placement methods: master point, paper point, EZfil bidirectional spiral, K-reamer size 70, 0.02 taper, lentulo spiral size 40, Roeko CanalBrush (Coltène Whaledent) in rotary motion, CanalBrush with sonic activation, ultrasonic sealer placement. Filling of the root canal irregularities was evaluated using a scoring system (0 = no filling; 1 = incomplete filling, 2 = complete filling) and measured in mm using radiographic images and Digora-software (Gendex Digora, Germany). Statistical tests were performed using PASWR Win 18.0 ($\alpha = 0.05$).
Results Scores as well as depth of filling were best for the ultrasonically or sonically activated techniques, followed by rotary placement. Passive placement techniques did not deliver the sealer effectively into root canal irregularities. Ultrasound managed to place the sealer into nearly the whole depths of the irregularities, followed by sonically activated CanalBrush, followed by different rotary placement techniques, which were able to fill approximately 50% of depths of irregularities. Differences were statistically significant (Kruskal–Wallis-test, $P < 0.001$; ANOVA, $P < 0.001$).
Conclusions Activated sealer placement enhances filling of simulated root canal irregularities. Ultrasonic sealer placement performed best, followed by sonically activated CanalBrush.

SESSION 3: SATURDAY 17 SEPTEMBER 2011

RESTORATION

R94

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An examination of the heat-conductive properties of restorative lining materials *ex vivo*

Aim To examine the heat conductive properties of five different cavity lining materials *ex vivo*.
Methodology Zinc oxide eugenol cement, zinc phosphate cement, Calcimol, Ionoseal and Calcimol LC were examined. These materials were prepared according to the manufacturer's instructions and were applied to standard molds. Three samples of each material were prepared. Measurements were taken using a Heat Conduction Unit (P.A. Hilton Ltd, UK). Heat conducting coefficient was calculated for each sample using the Fourier equation. Coefficients were statistically analyzed by the Kruskal–Wallis test.
Results Significant differences were found between some materials ($P < 0.05$). The conducting coefficient of zinc oxide eugenol cement was found to be larger than that of the other materials ($P < 0.05$). The heat conducting coefficient of zinc phosphate cement was found to be smaller than zinc oxide eugenol cement and larger than those of Calcimol, Ionoseal and Calcimol LC ($P < 0.05$). No statistically significant differences were found between Calcimol, Ionoseal and Calcimol LC ($P > 0.05$).
Conclusions This laboratory study showed that restorative lining materials functioned as thermal insulators with various degrees.

R95

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An *ex vivo* evaluation of the shear bond strength of three adhesive systems to ozonated dentine

Aim To evaluate the effect of gaseous ozone treatment on the shear bond strength of adhesive systems to dentine.
Methodology The coronal two thirds of 78 extracted sound molars were removed to expose the dentine surfaces. The exposed surfaces were conditioned with 17% EDTA followed by 5.25% NaOCl. The teeth were randomly divided into six groups ($n = 13$). The first three groups received ozone application: 40% for 50 s (CR probe, Biozonix; High-Frequency Ozone Generator, Germany). The other three groups were not treated with ozone. One of the following adhesive systems: (Group 1) Adper Single Bond (3M ESPE; USA); (Group 2) ozone/Adper Single Bond; (Group 3) Futura Bond (Voco, USA); (Group 4) ozone/Futura Bond; (Group 5) G Bond (GC, Japan); (Group 6) ozone/G Bond was applied to the dentine surface. After bonding, 4 × 3 mm core build-ups were created with composite resin (Clearfil AP-X; Kuraray, Japan). Specimens were stored in distilled water at 37°C for 24 h and then tested to failure for shear bond strength (MPa) at a crosshead speed of 0.5 mm min^{-1}. Data were analyzed using one-way ANOVA and Kruskal–Wallis tests, with significance set at $P < 0.05$.
Results Ozone application reduced the bond strengths of Adper Single Bond, Futura Bond and G Bond adhesive systems to dentine ($P > 0.05$).
Conclusions On the basis of this laboratory investigation, ozone application prior to bonding procedures may reduce the bond strength of self-etch and total-etch adhesives to dentine.

R96

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Micro tensile bond strength of resin based cements to root dentine with and without ozone treatment *ex vivo*

Aim To compare the micro tensile (μTBS) bond strength of four resin based cements to root dentine surfaces with or without ozone treatment.
Methodology The crowns of 24 extracted human single rooted premolar teeth were removed and sectioned longitudinally into two halves. Each half was prepared to expose flat dentine surfaces with 800 grit SIC paper and eight groups ($n = 3$) were created. The first four groups (G1-O, G2-O, G3-O, G4-O), were exposed to gaseous ozone (80%,120 s, KP probe, Biozonix; Dental high-frequency ozone generator, Germany). The other four groups were not treated with ozone. Three × four build-ups were created with one of the following resin cements: Variolink II (Ivoclar) (G1, G1-O), Secure (Sun Medical) (G2, G2-O), Clearfil Esthetic Cement (Kuraray) (G3, G3-O) and Super Bond C&B (Sun Medical) (G4, G4-O). After storage (37°C, 24 h) the roots were sectioned for μTBS test. Specimens were attached to the microtensile tester with a cyanoacrylate adhesive for μTBS testing at (1 mm min^{-1}). Mean bond strength values were analyzed using two-way ANOVA and Tukey HSD tests ($P < 0.05$).
Results Bond strength values were not significantly influenced by the ozone treatment ($P > 0.05$). regardless of adhesive systems used.
Conclusions On the basis of this laboratory investigation, the treatment of root canals with ozone did not alter the bond strengths of Variolink II, Secure, Clearfil Esthetic Cement, and Super Bond C&B cements to root dentine.

R97

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Effect of post-space disinfection with various irrigants on push-out bond strength of a glass-fibre post

Aim To investigate and compare the displacement resistance of glass-fibre reinforced composite posts (FRC Postec Plus; Ivoclar

Vivadent, Liechtenstein) when cemented with Clearfil Estetic Cement/ED Primer II (Kuraray, Japan) to root dentine after post-space disinfection with various irrigants alternated with 17% ethylenediamintetraacetic acid (EDTA) (Ultradent, USA).

Methodology A total of 30 non-carious extracted human mandibular premolars were decoronated, instrumented, and filled with gutta-percha and AH Plus sealer and prepared for post-cementation using the FRC Postec Plus system's drill and divided into five groups ($n = 6$). The post spaces were irrigated with 2 mL of (i) octenisept dihydrochloride (Octenisept®; Schülke&Mayr, Germany), (ii) 5.25% sodium hypochlorite (NaOCL) (Caglayan Kimya, Turkey), (iii) 2% chlorhexidine (CHX) (Imıcryl, Turkey), (iv) 10% iodine potassium iodide (Batticon; Adeka, Turkey) or (v) 0.9% saline (İ.E Uragay, Turkey) as control. Specimens were sectioned to obtain 1 mm thick slices perpendicularly to their long axes. The displacement resistance was measured using a Universal Testing Machine at a crosshead speed of 1 mm per minute. Push-out bond strength (MPa) was calculated. Data were statistically analysed with one-way ANOVA and *post hoc* Tukey's test ($\alpha = 0.05$).

Results Statistical analyses demonstrated that the disinfection of post-spaces with 2% chlorhexidine and 10% iodine potassium iodide had a negative effect on bond strength of FRC Postec Plus posts to dentine. A new irrigant, octenisept dihydrochloride resulted in comparable bond strength values to 5.25% NaOCl and 0.9% saline groups.

Conclusions *Ex vivo* data suggest that the disinfection of post spaces with a new irrigant, octenisept dihydochloride has no detrimental effect on the bond-strength of fibre posts luted with a self-etch resin cement.

Results Were comparable to irrigation with 5.25% NaOCl alternated with 17% EDTA.

R98

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Discolouration potential of recent endodontic materials

Aim To investigate a new bovine tooth model to assess tooth discolouration by endodontic materials.

Methodology Two hundred and ten quadratic blocks ($10 \times 10 \times 3.5$ mm) were prepared from bovine incisors. Standardized cavities were prepared and filled as follows ($N = 15$ per group): group A: left empty, group B: blood, group C: calcium hydroxide, group D: ApexCal, group E: Ultracal XS, group F: Ledermix, group G: triple antibiotic paste (3Mix-MP), group H: MTA grey, group I: MTA grey + blood, group J: MTA white, group K: MTA white + blood, group L: Portland cement (MED-PZ), group M: MED-PZ + blood and group N: AH Plus. The cavities were sealed with composite and stored in water. Colour measurements (VITA Easyshade compact device) were made at seven time points: prior to (T0) and immediately after placement of the filling (T1), after 1 week (T2), 1 month (T3), 3 months (T4), 6 months (T5) and 1 year (T6). Colour change (ΔE) values were calculated. A two-way analysis of variance was used to assess significant differences between the tested endodontic materials. The means of all groups were compared using the Tukey multiple comparison test ($\alpha = 0.05$).

Results Significant differences were detected among the experimental groups after 12 month ($P < 0.0001$). The lowest colour change values were observed in the groups N (3.2 ± 1.5), A (3.8 ± 1.4), L (4.1 ± 1.7), C (4.7 ± 1.5), E (5.1 ± 1.9) and J (7.9 ± 6.7). The most severe discoloration was measured in groups G (66.2 ± 9.9) an F (46.2 ± 11.6). When portland cement was contaminated with blood (group M) a significantly higher ΔE value (13.6 ± 4.2) was detected compared to group L ($P = 0.032$).

Conclusions Materials used in endodontic therapy may stain teeth. Therefore, the material choices should not rely solely on biocompatibility and physicochemical properties, but consider the risk of discolouration as well.

R99

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The influence of the post-endodontic restoration on the fracture resistance and the failure mode of teeth *ex vivo*

Aim To compare different post-endodontic restorations according to fracture resistance, failure mode and to the possibility of repair *ex vivo*.

Methodology Eighty-eight human maxillary incisors were root filled and decoronated. The groups were restored with composite pin and direct composite crown (B, $n = 16$); composite pin, direct core build-up and cast crown (C, $n = 16$); fibre post and direct composite crown (D, $n = 16$); fibre post, direct core build-up and cast crown (E, $n = 16$) and metal post and core build-up and cast crown (F, $n = 16$). Eight teeth remained intact and served as a control group (A). All teeth were artificially aged (load: 50N/1.2 million. cycles and 5500 thermo-cycles). Facture load was evaluated and analyzed using the Tukey-Kramer-Test. The failure mode and the possibility to repair the teeth with regard to the failure mode were evaluated.

Results Facture load was significantly lower in group C (546N; SE ± 196N) compared to group F (820N; SE ± 330N), group B (900N; SE ± 315N), group E (985N; SE ± 470N), group D (925N; SE ± 310N) and group A (1033N; SE ± 176N). Only one vertical fracture occurred in group F, oblique fractures were found in group B (1), group D (1), group E (2) and in group F (6). All other fractures were located within the restoration or horizontally beneath the restoration. The possibility to repair the restoration with regard to the failure mode was assessed to be positive in 63% of the cases in group F, 88% in groups E and D, 94% in group B and 100% in group C.

Conclusions Based on the results of this *ex vivo* study, it is suggested that fibre posts should be used when cast crowns are planned for the restoration of severely compromised incisors. Even direct composite restorations exhibited a reasonable fracture resistance.

R100

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Effects of ultrasonic and canal brush systems on push-out bond strength of translucent posts *ex vivo*

Aim To evaluate the effects of active irrigation using ultrasonics and canal brush on the push-out strength of a fibre post system.

Methodology Sixteen maxillary single-rooted, human teeth were decoronated to obtain roots of standard length. The root canals were instrumented (ProTaper; Dentsply Maillefer, Switzerland) and filled with gutta-percha and AH-Plus sealer (Dentsply Maillefer). Standardised post space preparation was performed with the FRC Postec Plus System's drill (Size 1) and the teeth randomly divided into four groups. The root canals in group 1 were irrigated only

with 5 mL of 5.25% NaOCl and served as controls. In the other groups the root canals were irrigated with 5 mL of 5.25% NaOCl and 5 mL of 17% EDTA. In group 2, the smear layer was removed passively. Ultrasonic and canal brush systems were used during irrigation of the root canals respectively in group 3 and 4. After the root canals were dried with paper points, fibre posts were luted with Panavia-F-2.0 (Kuraray, Japan) according to manufacturer's instructions. Specimens were sectioned to obtain 1 mm thick slices perpendicularly to their long axes. Push-out tests were performed using a Universal Testing Machine at a crosshead speed of 1 mm min^{-1}. Push-out bond strengths (MPa) were calculated. Data were analysed using one-way ANOVA and Tukey test.

Results The control group showed the lowest average scores. Removal of the smear layer was associated with significantly ($P < 0.05$) increased push-out bond strength of fibre posts. There was no significant difference between the passive group and canal brush group ($P > 0.05$). Use of ultrasonics for removal of the smear layer resulted in the highest push-out bond strength ($P < 0.05$).

Conclusions On the basis of this *ex vivo* investigation, the use of ultrasonics for activation of irrigation solution was associated with increased push-out bond strength of translucent fibre posts.

R101

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Test-device to produce changes in flexural properties of fibre posts

Aim To develop a test-device, capable of exerting stress and causing changes in flexural properties of different types of fibre posts.

Methodology Fourteen fibre posts (RelyX Fiber Post, Metalor Stylepost, Ena Post, ER Dentin Post, Radix Fiber Post, NTI Head Master System, FibreKleer parallel, FibreKleer serrated, FibreKleer conical, FRC Postec Plus, Twin Luscent Anchors, Bonafit, VDW DTLight SL, Mirafit 3 in 1) were selected and embedded up to 8 mm in resin blocks (PMMA), using a luting material (RelyX Unicem). An especially-developed device was used for exerting stress by deflecting the fibre posts. To simulate the effect of the forces during chewing, the posts were exposed to 5000 deflections of 0.5 mm, each for 0.5 s. Before this treatment and afterwards, the modulus of elasticity of the fibre posts was measured with a universal testing machine with a deflection of 1 mm (UPM 1425; Zwick). Possible changes of the fibre posts were visualized by scanning electron microscopy.

Results Most of the fibre posts (RelyX Fiber Post, Metalor Stylepost, Ena Post, ER Dentin Post, NTI Head Master System, FibreKleer conical, FRC Postec Plus, Twin Luscent Anchors, Bonafit, VDW DTLight SL, Mirafit 3 in 1) showed a loss of elasticity (modulus of elasticity up to 25 kN mm^{-2}) as a consequence of fatigue of the fibre system. For three posts (Radix Fiber Post, FibreKleer serrated, FibreKleer parallel), stiffening was observed. In no case was a fracture of the posts observed.

Conclusions The study demonstrated that the applied stress caused a loss of elasticity to all fibre posts and that this special test-device was able to exert defined stress conditions to fibre posts.

R102

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Suitability of an intra-canal composite anchorage compared to cemented titanium or fibre posts in root filled premolars *ex vivo*

Aim To compare the fracture resistance of severely decayed root filled premolars restored with different types of intraradicular anchorage and composite crowns *ex vivo*.

Methodology Ninety-six extracted mandibular premolars were root filled and randomly assigned to four groups ($n = 24$). Post space preparations were performed to a depth of 6 mm (group 1, 2) or 3 mm (group 3, 4). In group 1, titanium posts were cemented with zinc phosphate cement. Glass fibre posts were adhesively cemented in groups 2 and 3 using a dual cure resin after post and dentine pre-treatment. In group 4, intra-canal anchorage was solely performed with a dual cure composite. All teeth were restored with standardized direct composite crowns without a ferrule. After thermo-mechanical loading in a computer-controlled masticator (1 200 000×, 5–50°C), static load was applied until failure. Loads-to-failure [in *N*] were statistically analyzed by ANOVA and Tukey's test ($\alpha = 0.05$).

Results Group 2 revealed significantly higher fracture values (394N ± 99) than all other groups. No significant difference was detected between group 1 (295N ± 82), group 3 (275N ± 76), and group 4 (241N ± 87).

Conclusions Within the limitations of this laboratory study, it was concluded that adhesive intra-canal anchorage to a depth of 3 mm using glass fibre posts or solely resin composite had the same fracture resistance as titanium posts conventionally cemented to a depth of 6 mm.

R103

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Do oval posts improve fracture resistance of teeth with oval-shaped root canals?

Aim To determine *ex vivo* whether oval posts increase fracture resistance of restored root canal treated teeth with oval-shaped canals compared to circular posts with and without foregoing post space preparation, respectively.

Methodology Extracted mandibular premolars with similar dimensions were decoronated, leaving a root length of 12 mm. Only teeth with a maximal root canal diameter of 1.7 mm at the level of the amputation were included. After root canal treatment root fillings were removed to a depth of 6 mm. To simulate the presence of oval-shaped root canal morphology, standardized preparations were performed with a conical diamond bur. Specimens were randomly assigned to three groups ($n = 24$).

In Group SFP (small fibre post), circular posts with a cervical diameter of 1.7 mm were used without foregoing post space preparation. In group LFP (large fibre post) a circular post space preparation using a form congruent drill was necessary to facilitate the use of posts with a cervical diameter of 1.9 mm. In group OFP (oval fibre post) oval posts with a long cervical diameter of 1.9 mm and short cervical diameter of 1.3 mm were utilized. No post space preparation was necessary in this group. Posts were cemented with dual cure resin. All specimens were restored with standardized direct composite crowns. Thermal cycling and mechanical loading was performed using a computer-controlled masticator. Finally, the

specimens underwent fracture resistance testing. Means and standard deviations were calculated. The data were analysed using ANOVA ($\alpha = 0.05$).

Results The highest mean fracture load was observed in group OFP (273N ± 51), the lowest in group LFP (258N ± 72). One-way ANOVA indicated no significant differences in fracture resistance among the three experimental groups.

Conclusions Within the limitations of this laboratory study, oval posts failed to increase fracture resistance of RCT premolars with oval shaped root canals compared to circular posts.

R104

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Post space preparation and post cementation: apical seal in teeth treated with Thermafil

Aim To investigate by means of a fluid filtration whether immediate or delayed post space preparation and post cementation may influence the apical seal associated with Thermafil Obturators (Dentsply Maillefer, Switzerland).

Methodology Fifty-four single-rooted teeth were randomly divided into five experimental ($n = 10$) and two control ($n = 2$) groups. Crowns were removed with a diamond bur at the cemento-enamel junction. Canals were shaped with Mtwo (Sweden & Martina, Italy), smeared with Pulp Canal Sealer (SybronEndo, USA) and filled with Thermafil Obturators (Dentsply Maillefer). Filled canals were subjected to the following protocols: G1, no other treatment; G2, post space preparation with Thermafil Post Space Bur (Dentsply Maillefer); G3, post space preparation with Thermafil Post Space Bur and D.T. light post (RTD, France) cementation; G4 and G5, as G2 and G3, the same respectively, after one week saline storage. Specimens underwent fluid filtration testing in accordance with the Çobankara *et al.* model (2002) after 7 and 30 days.

Results Mean microleakage values ± SD (μL) after 7 days were: G1, 0.22 ± 0.22; G2, 0.56 ± 0.89; G3, 0.52 ± 0.76; G4, 0.30 ± 0.38; G5, 0.26 ± 0.46; and after 30 days: G1, 0.03 ± 0.03; G2, 0.06 ± 0.07; G3, 0.02 ± 0.04; G4, 0.01 ± 0.02; G5, 0.04 ± 0.12. Positive controls leaked respectively 75 and 88 μL in a minute; negative controls did not show filtration. There was no significant difference amongst the groups.

Conclusions Under the conditions of the present *ex vivo* study, immediate or delayed post space preparation and post cementation did not influence apical seal.

RETREATMENT

R105

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Efficacy of rotary Ni-Ti retreatment systems in root canals filled with different techniques

Aim To compare the effectiveness and working time of Mtwo, ProTaper and Hedström files for removing gutta-percha (GP) placed with warm vertical (BeeFill 2 in 1; VDW, Germany) or lateral compaction techniques from extracted teeth.

Methodology Forty two freshly extracted single-rooted human teeth were selected. All canals were prepared with Mtwo Ni-Ti rotary instruments (VDW) to size 35, 0.04 taper. The prepared canals were divided randomly into two groups: The first was root filled by the BeeFill 2 in 1 warm vertical compaction technique ($n = 21$) and the second was root filled by the lateral compaction technique ($n = 21$). AH26 sealer was used in both of the groups. The filled root canals were divided randomly into three groups ($n = 7$) according to retreatment techniques; Group 1: GP removal with Hedström files (VDW), Group 2: GP removal with ProTaper Universal files (Dentsply Maillefer, Switzerland), Group 3: GP removal with Mtwo files (Sweden and Martina, Italy). The time required for retreatment was recorded. The roots were then split longitudinally and each half was examined under an operation microscope and photographed. The areas of remaining obturation materials were measured using an image analysis program (COMEF 4.3; OEG Messtechnik, Germany). The area of residual root filling material was measured and the time required for root canal filling removal was evaluated statistically.

Results In groups filled with Beefill 2 in1, the retreatment time for ProTaper was significantly shorter than Mtwo and Hedström files ($P < 0.05$). In groups filled with lateral compaction, Hedström files required the longest time ($P < 0.05$). All retreatment techniques failed to remove GP completely. The maximum amount of remnant was observed when Mtwo files were used for removing GP placed with the Beefill 2 in 1 technique ($P = 0.020$).

Conclusions According to the results of this study, manual and rotary instrumentation failed to remove GP completely.

R106

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Efficacy of different nickel-titanium rotary instruments during retreatment in curved root canals: an *ex-vivo* study

Aim To compare the efficacy of hand files and different Ni-Ti rotary systems in removing gutta-percha and maintaining the original canal shape during retreatment *ex vivo* in curved root canals.

Methodology One-hundred mandibular molars with mesio-buccal canals having a curvature ≥15° were included. All canals were prepared manually with K-files and filled with System B (SybronEndo, USA) + AH26 (Dentsply Maillefer, Switzerland). The teeth were then randomly divided into five groups of 20 specimens each. Retreatment was done with Hedström files in the control group and with either ProTaper (Dentsply Maillefer), RaCe (FKG Dentaire, Switzerland), HeroShaper (Micro–Mega, France) or R-Endo (Micro–Mega, France) in test groups. Digital pre- and post-retreatment radiographs taken in clinical and proximal views with a standardized technique were superimposed, and the width of dentine removal was measured. Total amount of removed material, amount and direction of transportation and centering ratios were determined. Data were analyzed using one-way ANOVA and *post hoc* Tukey comparison tests. Roots were divided into apical, middle and coronal parts and scored on the post-retreatment radiographs with a four-grade scale to evaluate the root canal cleanliness by trained observers. Intra-observer reliability was tested using Kappa test. The Kruskal–Wallis test was used to compare the difference between retreatment groups at the three parts.

Results There was no significant difference among any of the groups with regard to canal cleanliness ($P > 0.05$). Significant differences were found among the groups for maintaining the original canal shape ($P < 0.05$). R-Endo gave the best results throughout almost the entire canal.

Conclusions R-Endo was found to be more effective than the other systems tested in maintaining the original canal shape, however there were no differences among the systems in regard to canal cleanliness during simulated retreatment.

R107

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ProTaper and Mtwo retreatment systems for removing resin-based root filling materials *ex vivo*

Aim To evaluate the effectiveness of ProTaper and Mtwo retreatment systems for removal of three different resin-based filling materials during simulated retreatment.
Methodology One-hundred-and-sixty extracted single-rooted maxillary anterior teeth were enlarged to size 30 using two different rotary systems, 80 teeth with ProTaper (Dentsply Maillefer) 80 teeth with Mtwo (Sweden and Martina) instruments. Each group of 80 teeth were randomly divided into four groups of 20 specimens each. Groups 1 and 5 were root filled using Resilon-Epiphany (Pentron Clinical Technologies, USA). Groups 2 and 6 were filled with gutta-percha (DiaDent Group International Inc., Korea) and Epiphany. Groups 3 and 7 were filled with gutta-percha and AH Plus (Dentsply De Trey GmbH, Germany). Groups 4 and 8 were filled using gutta-percha and an eugenol-based paste (Kerr, USA). In all groups, a vertical compaction technique was used. Groups 4 and 8 served as control groups. In groups instrumented by ProTaper files, ProTaper Retreatment files were used for removal of root canal fillings. In groups instrumented by Mtwo files, Mtwo retreatment files were used for removal of root canal fillings. After clearing the roots, the teeth were split vertically into halves and the cleanliness of canal walls was determined by SEM at three canal levels.
Results Retreatment of specimens filled with gutta-percha and Kerr Pulp Canal Sealer showed significantly more remaining filling material than specimens filled with resin-based materials. No significant difference was found between ProTaper and Mtwo retreatment files. Both resulted in cleaner canal walls in the apical third of teeth filled with Resilon + Epiphany and AH Plus + gutta-percha when compared to Epiphany + gutta-percha and Kerr Pulp Canal Sealer + gutta-percha. In all the filling techniques most remaining material was observed in the coronal third of the teeth.
Conclusions Resilon + Epiphany and AH Plus + gutta-percha were removed more effectively than Epiphany + gutta-percha and Kerr Pulp Canal Sealer + gutta-percha. ProTaper retreatment files were more effective than Mtwo retreatment files in removing root canal filling materials.

R108

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Efficacy of ProTaper retreatment system for removing gutta-percha from root canals filled with three sealers

Aim To evaluate the cleaning efficacy of ProTaper Universal rotary retreatment system (Dentsply Maillefer, Switzerland) for removing gutta-percha (GP) placed with three different endodontic sealers *ex vivo*. Time required for removal of root canal fillings was also evaluated and compared.
Methodology Forty teeth with a single canal were sectioned to standardize working length at 13 mm. Teeth were prepared with ProTaper® files up to size F4 with 2 mL of 5.25% NaOCl irrigation between the files. Teeth were randomly divided into four subgroups: Gr1: single cone GP and bioceramic-based sealer (i-RootSP; Veriodent, Canada), Gr2: single cone GP and MTA-based sealer (Fillapex; Angelus, Brazil), Gr3: single cone GP and resin-based sealer (AH26; Dentsply, USA), Gr4: lateral condensation of GP and resin-based sealer (AH26). All teeth were reinstrumented using ProTaper Universal retreatment instruments until D3 reached working length. Elapsed time was recorded, roots were cut longitudinally, and each half was examined under a stereomicroscope at ×500 magnification and photographed with a digital camera. Three observers scored each third of both halves of each tooth. Data were analyzed using Kruskall–Wallis and Mann–Whitney *U*-tests.
Results Filling material removal in Gr2 was significantly faster than in other groups. In all groups, some debris was observed after reinstrumentation. There were no differences between the groups among apical and middle thirds in terms of filling remnants whereas the coronal third of the i-RootSP group showed significantly more debris compared to the other groups. Within-group comparisons demonstrated a significant difference only among coronal and apical thirds of the lateral condensation and AH26 group, while no significant difference was observed among different thirds of each group.
Conclusions Within limitations of this *ex vivo* study, it can be concluded that irrespective of sealer type, complete removal of root fillings could not be attained with ProTaper Universal retreatment instruments.

R109

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Efficacy of four different irrigation techniques in the removal of root canal sealer

Aim To evaluate the efficacy of four different irrigation techniques in removing root canal sealer from simulated root canal irregularities.
Methodology Fifty-three single-rooted extracted human teeth were instrumented, split longitudinally and standardized lateral grooves were prepared into the dentine (apical and coronal). The root canal surface and lateral grooves were covered with sealer (AH Plus; Dentsply De Trey, Germany). After re-assembling of the roots the removal of sealer was performed with RinsEndo (Dürr Dental, Germany), ultrasonic irrigation (Piezon Master 400; EMS, Switzerland), EndoActivator® (Dentsply Tulsa Dental Specialties, USA), CanalBrush™ (Coltène/Whaledent, Switzerland) ($n = 12$) and NaOCl. The amount of remaining sealer in the lateral grooves was evaluated using a four grade scoring system. For evaluation of the root canal surface a seven grade scoring system was used.
Results No irrigation technique was able to remove the sealer completely either from the coronal or the apical groove. No statistically significant difference could be observed between the coronal and apical groove ($P \geq 0.05$). On the root canal wall, ultrasonic irrigation resulted in significantly less remaining sealer compared to all other irrigation techniques ($P < 0.005$).
Conclusions None of the irrigation techniques was able to completely remove root canal sealer from lateral grooves. Ultrasonic irrigation showed a superior effect in removing sealer from the root canal surface during simulated endodontic retreatment compared to the other irrigation techniques.

R110

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Removal of gutta-percha fillings *ex vivo* using Nd:YAG laser

Aim To investigate the ability of Nd:YAG laser for removing gutta-percha fillings when used in combination with hand files.

Methodology The root canals of 30 extracted single-rooted teeth were enlarged and filled with lateral condensation of gutta-percha. The teeth were divided into two groups; Group 1: the material was removed with Hedström files and NaOCl irrigation, without solvents; Group 2: the material was removed with a Nd-YAG Laser (10 Hz, 100 mj, 10 s) after each Hedström file. The teeth were split longitudinally, and the area of remaining gutta-percha was determined with the aid of a computer programme. The results were analysed using the Kruskal–Wallis and the Mann–Whitney tests.

Results The percentage area coverage with remaining gutta-percha was the largest in Group 1 (3.6 ± 3.9%), followed by Group 2 (0.3 ± 0.7%). The difference between Groups 1 and 2 was significant ($P < 0.05$). In both groups, the cervical and middle parts of the root canals were significantly cleaner than the apical part. Regarding the time required for gutta-percha removal, the combination Nd-YAG laser with Hedström files did not differ significantly from the Hedström files alone ($P = 0.67$)

Conclusions The use of Nd-YAG has been shown to facilitate the ability of Hedström files in removing gutta-percha fillings from root canals *ex vivo*.

R111

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Deformation in three Nickel-Titanium rotary retreatment systems after simulated clinical use: an SEM study

Aim To evaluate deformation and microfractures of ProTaper retreatment, D-Race, and R-Endo systems after single and multiple clinical uses to remove gutta-percha (GP) from straight canals by scanning electron microscopy (SEM).

Methodology Root canals of ninety extracted human single rooted teeth were chemomechanically prepared and filled with laterally compacted GP and AH26 sealer. Teeth were divided after 2 weeks into three groups A, B and C. Group A: GP removal was completed with ProTaper universal retreatment instruments (Dentsply Tulsa, USA); Group B: D-Race (FKG, Switzerland) was used; Group C:R-Endo (Micro-Mega, France) was used. Five kits of each system were evaluated for deformation and microfractures before use and after the completion of one, two, and three canals by using SEM.

Results All the instruments of the three tested rotary systems had at least one of defect (tip fracture, micro crack, micro fracture, pitting, fretting, unwinding, and fold fracture) after a single clinical use. These deformations increased by increasing the number of simulated clinical uses. ProTaper instruments revealed the least amount of deformations followed by D-Race and R-Endo. The mean deformation depth in the instrument tip was 2.1 mm for ProTaper, 2.21 mm for D-Race, and 3.06 mm for R-Endo.

Conclusions A high rate of deformation was observed for all three rotary systems with undesirable outcomes during simulated clinical use .These systems should be considered as single–use instruments. The variation of deformation rate between the three tested systems may be due to the inherent weakness of the alloys themselves or variation in instrument design.

R112

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Basic studies on the removal of broken NiTi endodontic files from root canals by chemical corrosion

Aim The primary purpose of this *in vitro* study was to clarify whether subjecting broken instruments to corrosion facilitates their removal or not. The second purpose was to investigate the influence of solution temperature on the corrosion of broken instruments.

Methodology The composition of the two solutions, NCN and APF were 10% NaClO + 19% NaCl and 2% NaF, (pH of 4.5) + phosphoric acid, respectively. The temperature of NCN and APF were set to 37, 45 and 60°C. NiTi files were made to rotate and broken at a point 5 mm from the tip. The weight change of the file was measured after immersion in NCN or APF. The morphologic changes of the file were also observed with an X-ray micro analyzer and 3-D laser measuring microscope.

Results Broken NiTi files immersed in NCN demonstrated corrosion progressing from the broken edge resulting in rugged borders. In APF, pitting corrosion had progressed gradually and uniformly all over the surface. For both NCN and APF, an increase in solution temperature increased the rate of corrosion of NiTi files.

Conclusions The higher the solution temperature, the faster the corrosion of NiTi files. The results suggest that the clinical time of this removal method can be shortened. Therefore, this method may be clinically useful in facilitating the removal of broken NiTi files.

R113

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Efficiency of three different instruments for removal of various fibre posts – an fpVCT study

Aim To examine the efficiency of three different instruments for removal of different types of fibre posts *ex vivo*.

Methodology The root canals of one-hundred and fifty-three extracted single-rooted human teeth were prepared to size 35, 0.02 taper and filled. Post space was prepared at a standardised length of 10 mm. The following three types of posts were cemented with Prime and Bond NT and Dyract Cem plus (both Dentsply Maillefer, Switzerland) ($n = 51$ per group): glass-fibre posts: Easy Post (Dentsply Maillefer); quartz-fibre posts: DT White Post (VDW, Germany); carbon-fibre posts: Cytec posts HT (Hahnenkratt, Germany). Posts in each subgroup ($n = 17$) were removed using the SonicFlex handpiece with tip size67 (KaVo, Germany), a long-shaft round bur (size 12; Komet, Germany) or a post removal kit (DT-Post removal kit; VDW). Time for removal was recorded. Following post removal the teeth were examined using flat panel volumetric computed tomography (fpVCT; GE Medical Systems, USA) to evaluate remnants of posts or adhesive, perforations, and smoothness of the root canal surface. Statistical analysis was performed by SAS using two-factional balance sheet analysis of variance ($P < 0.05$).

Results Significant ($P < 0.05$) differences were found between the different removal techniques and between the types of posts. The DT-Post removal kit removed all posts significantly faster than the other techniques but left the most remnants. Long-shaft burs left significantly less remnants than the DT-Kit but caused a rough surface with a high loss of tooth substance, and even perforations.

Removal using the SonicFlex device took the most time but was more effective than the DT-Kit, leaving a clean and smooth surface.
Conclusions The DT-Post removal kit was most efficient in removing all tested posts in the shortest time while SonicFlex left cleaner and smoother surfaces.

SURGERY

R114

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The effect of ultrasonic retro-tips on the removal of different root filling materials *ex vivo*

Aim To compare residual root filling materials in apical root-end cavities following their removal with ultrasonic retro-tips *ex vivo*.
Methodology Thirty-six freshly extracted single-rooted teeth were used. Preparation of all roots was performed with Ni-Ti rotary instruments (ProTaper; Dentsply Maillefer, Switzerland), followed by irrigation with 2.5% sodium hypochlorite. Two experimental groups were formed according to the filling materials. Group A: 15 canals filled with single gutta-percha cone and epoxy resin based sealer (AH 26; Dentsply Maillefer), Group B: 15 canals filled with single gutta-percha cone and zinc oxide-eugenol sealer (Roth 811; Roth International, USA). Three teeth served as positive and three teeth as negative controls. Teeth were immersed in distilled water for 48 h until the sealer was set. Root-ends were resected, 3 mm from apex, perpendicular to the long axis of the tooth. All root-end cavities were prepared with diamond coated ultrasonic retro-tips (P14D; Satelec Acteon Group, France), using an ultrasonic unit (mode E, medium power, Pmax Suprasson; Satelec Acteon Group). The ultrasonic application time was fixed at 60 s. Teeth were split longitudinally, dehydrated and sputter coated with gold. Remnants of residual materials were evaluated by SEM. A region in the wall of the root end cavity was marked as a reference point in all specimens
Results Zinc oxide-eugenol sealer was removed more quickly from the dentinal walls than the epoxy resin based sealer. However, this difference was not significant.
Conclusions Ultrasonic diamond-coated retro-tips were effective in the removal of root canal filing materials during root-end cavity preparation *ex vivo* regardless of the type of sealer. The clinical relevance of the removal of root canal filling materials in periapical surgery should be further investigated.

R115

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Evaluation of haemostatic efficacy and histologic tissue responses to Expasyl, Viscostat, Astringedent and Astringedent-X

Aim To evaluate the haemostatic efficacy and the histologic tissue reactions to four haemostatic agents (Expasyl, Viscostat, Astringedent, Astringedent-X) in standardized bone defects in the jaws of rabbits.
Methodology Five male rabbits were used. The haemostatic agents tested were Expasyl (Kerr), Viscostat (Ultradent), Astringedent (Ultradent) and Astringedent-X (Ultradent). Standardized circular bone defects (diameter 2 mm, depth 2 mm) were prepared, initial photographs taken and haemostatic agents applied for 3 min. Expasyl paste was removed and the sites were photographed at 10, 30 s, 1, 3 and 5 min. All the agents were removed by curettage under saline irrigation. The bleeding was measured using a special architectonic program (autocad 2007) and the results compared using Wilcoxon's matched-pairs signed-rank test. Bleeding scores after the removal of the haemostatic agents were compared using the Kruskal–Wallis test. The animals were killed after 5 weeks and transverse sections of the jaws were stained with haematoxylin and eosin and subjected to histological analysis. The histological results were compared using Fisher's exact and Kruskal–Wallis tests.
Results Expasyl presented the most efficient haemorrhage control whereas Astringedent had the weakest bleeding reduction effect. The histological analysis after 5 weeks demonstrated remnants of the haemostatic agents in 26% of the cases. Foreign body reaction was evident in 9% of cases and with just two of the haemostatic agents, Expasyl and Viscostat. None of the materials caused inflammatory tissue reactions while new bone formation could be estimated to cover 77% of the area of the bony crypt in the control group, 53% in Expasyl, 49% in Viscostat and 39% and 42% in Astringedent and Astringedent-X respectively.
Conclusions Expasyl appeared to be the most efficient agent to control the bleeding within the bony defects created in the jaws of rabbits.

R116

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Sealing ability of two root-end filling materials: *ex vivo* analysis by non-destructive methods

Aim To assess the apical sealing ability of two root-end filling materials in extracted teeth by means of a fluid filtration system and a preliminary microtomographic analysis.
Methodology The crowns of twenty-four single-rooted freshly extracted caries-free teeth were removed. Electronic working length was determined making use of an alginate-based model. Canals were instrumented with Mtwo rotary files (Sweden & Martina, Italy), irrigated with 5% NaOCl, dried and filled with thermoplasticized gutta-percha (System B; SybronEndo, USA). Under constant water irrigation, three mm of the root apices were resected and root-end cavities were prepared with ultrasonic tips. Two experimental groups ($n = 10$) were defined: root-end cavities were filled with: Group 1 (G1) – SuperEBA (Bosworth, USA); Group 2 (G2) – MTA (Dentsply Maillefer, Switzerland). Four roots served as positive and negative controls. The specimens underwent a fluid filtration test by the method of Çobankara *et al.* (2002), during which leakage was measured at multiple time intervals. Collected data were subjected to non-parametric analysis ($P < 0.05$). Microtomographic scans of two specimens per group were acquired.
Results Final filtration values (μL) were: G1, 0.087 ± 0.052; G2, 0.083 ± 0.054. The difference in terms of leakage was not statistically significant. As a general trend, an extended exposure to fluid pressure led to significantly increased leakage values, although this increase tended to diminish with time. Limited void volumes were detected in both experimental groups through microtomographic analysis; they were distributed inside the filling materials and along the canal walls.
Conclusions Within the limitations of this *ex vivo* study, root-end cavities filled with SuperEBA and MTA exhibited similar apical seal. The root canal filling materials could not entirely fill the canal space in any scanned specimen. The relation between voids distribution/amount and sealing ability is still unclear.

R117

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A clinical study of periradicular surgery using Mineral Trioxide Aggregate as a root-end filling material

Aim The aim of this prospective clinical study was to evaluate the success rate of periradicular surgery using MTA as a root-end filling material.
Methodology A total number of 30 teeth from 25 patients requiring periradicular surgery were included. Patients were instructed about the surgical procedure, postoperative care, follow-up examinations and alternative treatment options. All patients were placed on a preoperative regimen of antibiotics and anti-inflammatory drugs. Apical surgery was performed under local anaesthesia. After reflecting a mucoperiosteal flap, affected roots were resected approximately 3 mm from the apex. Root-end cavities were prepared to a depth of 3 mm. MTA was mixed according to the manufacturer's instructions and placed into the root-end cavities. Patients were seen 7 days after surgery for suture removal. Recalls were scheduled at 3, 6 and 12 months and then yearly. Success was classified as complete healing, incomplete healing, uncertain healing and unsatisfactory healing.
Results Overall, 26 out of 30 teeth had complete healing radiographically with no other signs and symptoms. One tooth had no symptoms but incomplete radiographic healing. Three teeth had uncertain healing. The overall success rate including complete and incomplete teeth was 27 out of 30 teeth (90%).
Conclusions MTA can be recommended as a root-end filling material in apical surgery.

R118

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Amalgam versus MTA as root-end filling materials in endodontic surgery: a long term longitudinal study

Aim To compare the long-term outcomes of endodontic surgery with root-end fillings of amalgam or MTA.
Methodology Two cohorts of consecutive patients (42 subjects 55 teeth- for amalgam and 136 subjects -181 teeth – for MTA) were followed for 13 years after endodontic surgery. All the surgeries were performed by the same operator with the same instruments and technique; the only difference was the filling material. The group treated with amalgam was treated from 1997 to 2002 and the group treated with MTA from 2002 to 2010. For each patient, recalls were scheduled at 3, 6 months, and then every year. During each recall, a radiographic ad clinical examination was performed. Two-tailed Fisher exact and $\chi 2$ tests were used to compare outcomes between the two groups at each recall time ($\alpha = 0.05$).
Results No significant difference was observed between amalgam and MTA at each recall except at 6 m ($P = 0.041$). Only two unfavourable outcomes were observed with MTA (success = 98.9%) and three with amalgam (success = 94.5%)
Conclusions Within the limitations of this study, clinical outcomes were comparable for cases managed with amalgam and MTA. A significant difference was detected only at 6 months, where the results for MTA were more favourable. The compliance of the patients to recall was low, with more than half of the subjects lost to follow up at each recall time.

BASIC SCIENCE: PULP

R119

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Vascular and angiogenic markers in the dentine-pulp complex of immature permanent teeth

Aim To investigate the topographical distribution of VEGF and CD34, CD146 positive cells in the dentine-pulp complex of immature permanent teeth using immunohistochemistry.
Methodology Ten unerupted human third molar teeth at a similar stage of root development were decalcified in 10% EDTA and paraffin embedded. Sections were stained with haematoxylin and eosin and by immunohistochemistry using mouse monoclonal antibodies to CD34, CD146 and VEGF. A pyogenic granuloma served as a positive control and a monoclonal nonspecific mouse IgG was an isotype negative control. Slides were evaluated using light microscopy and the distribution of markers analysed in the coronal, middle and apical regions of each specimen.
Results The expression of $CD34^+$ and $CD146^+$ endothelial cells within pulp tissue indicated a topographical difference in the distribution and size of blood vessels. The coronal pulp was most vascular with small vessels located sub-odontoblastically, especially in the pulp horns. A similar pattern was found in the mid-root region although blood vessels located centrally appeared larger and ran consistently in an axial direction. In contrast, the apical papilla region was largely avascular with a small number of $CD34^+$ and $CD146^+$ cells located at the periphery of the tissue. VEGF expression was observed in the dentine matrix adjacent to odontoblasts in all three regions. Apically VEGF was widespread within the stroma and on stromal fibroblasts.
Conclusions The difference in distribution of vascular and angiogenic factors in the dentine-pulp complex suggests variability in the healing potential of different regions within the pulp.

R120

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The expression and role of lysyl oxidase (LOX) in dentinogenesis

Aim Lysyl oxidase (LOX) is an enzyme with a major role in collagen cross-linking and extracellular organic matrix (ECM) stabilization. High LOX concentration in odontoblasts and its role as major dissociative protein in dentine matrix indicate that LOX is essential in dentine ECM organization. The hypothesis was set that eliminating LOX gene would affect dentine formation.
Methodology Human odontoblasts and pulp tissue were used to study the expression of LOX and its four isoenzymes (LOXL1-4) with Affymetrix cDNA microarray. Wildtype (*wt*) and LOX knock-out ($Lox^{-/-}$) mouse E18.5 tissues were used to study dentine formation by histochemical staining, and adult *wt* and $Lox^{+/-}$ mouse teeth were examined with field emission scanning electron microscopy (FESEM).
Results Native human odontoblast and pulp tissue expressed LOX and its isoenzymes, with minor effects observed after culturing with and without TGF-β1. No differences between knock-out and

wildtype mouse tooth morphology were seen in light microscopy or FESEM.
Conclusions The data indicates that LOX is not essential in dentinogenesis. The absence of functional LOX may be compensated by LOX isoenzymes.

R121

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Is the concept of a marker gene sufficient for cell line characterization?

Aim To evaluate gene expression profiles of the *in vivo* state of odontoblasts and pulp tissue in comparison with the corresponding *in vitro* pulp-tissue derived cell culture.
Methology Total RNA was isolated from the odontoblast layer of six caries free impacted wisdom teeth of three donors (14–16 years old). Three pulp tissues were used for isolation of total RNA and the other three pulp tissues for establishing pulp derived cell cultures. Prior to RNA isolation the odontoblast layer and the pulp tissue were stabilised with RNA later (Qiagen, Germany). Total RNA was obtained from cells of the third passage cultivated in DMEM supplemented with 8% (v/v) foetal calf serum and 1% (v/v) penicillin-streptomycin (Invitrogen, Germany). RT-qPCR of 84 genes relevant for biomineralisation was performed using the iCycler (BioRad, USA) and RT2 Profiler PCR Arrays (Qiagen). Relative gene expression levels were calculated according to the $\Delta\Delta C_T$ method. Normalisation was achieved by averaging the data of three reference genes.
Results Except for DMP1, the markers usually used for odontoblasts or biomineralisation (ALPL, AMBN, AMELY, BGLAP, DSPP, ENAM) were either not able to distinguish odontoblasts from pulp tissue or from the cell culture or from both. Nevertheless, sets of genes were identified which were significant for the *in vivo* state of odontoblasts (CD36, COL11A1, DMP1, ITGA2, PDGFA, PHEX, RUNX2, TFIP11), pulp tissue (COL14A1, COL15A1, FLT1, ITGA1, ITGAM) and the corresponding *in vivo* pulp derived cell culture (BMP2, CDH11, CTSK, FN1, ICAM1, IGF1R, IGF2, SMAD4, TGFBR1, TWIST1, VEGFA & -B).
Conclusion Qualitative analysis of a single or two marker genes was not sufficient for the characterization of a cell line. A quantitative analysis of multiple genes gives more information about the properties of cell culture and is therefore more reliable.

HISTOPATHOLOGY: PERIAPICAL LESIONS

R122

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Angiogenic factor expression in endothelial cells in experimentally-induced rat periapical lesions

Aim Angiogenic factors in endothelial cells, such as VEGFR2 (vascular endothelial cell growth factor receptor 2), Bcl-2 (a proangiogenic signaling molecule), and CXCL8 (a proangiogenic chemokine) related molecules such as CXCL1 and CXCR2, may have critical roles in enhancing the establishment of periapical lesions. To understand the roles of these factors in the pathogenesis of the lesions, we conducted immunohistochemical and molecular biological analysis.
Methodology Unsealed pulp exposure was made in mandibular first molars of Wistar rats. Observation periods were set at 0 (normal), 14, 21, and 28 days after pulp exposure. After given periods, the molars were retrieved, embedded as frozen sample blocks, and cut in a cryostat. Immunostaining for CD31 (a marker for endothelial cells) and Bcl-2, and real-time PCR analysis of VEGFR2, Bcl-2, CXCL1, and CXCR2 mRNA were then performed. In the real time PCR analysis, mRNA was extracted from CD31-stained endothelial cells that were retrieved with laser capture microdissection. For statistical analysis of immunohistochemistry, the immuno-stained area was plotted, and pixel counts were determined. The percentage of the immuno-stained area in the total area was then calculated.
Results The density of CD31-stained area increased until 21 days after pulp exposure. On the other hand, Bcl-2-stained area showed the highest density at 14 days (active lesion expanding phase), and then decreased until 28 days (lesion stability phase). VEGFR2, Bcl-2, CXCL1, and CXCR2 mRNA expression in endothelial cells showed highest levels at 14 days and then decreased until 28 days.
Conclusions The upregulation of VEGFR2, Bcl-2 and rat CXCL8 related molecules (CXCL1, and CXCR2) mRNAs in endothelial cells at the lesion-expanding phase suggests that these angiogenic factors play some role in lesion progression.

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Phenotypic identification of TLR2$^+$ cells in refractory periapical granuloma of endodontic origin

Aim To phenotypically characterize TLR2 expressing cells within refractory periapical granuloma by use of a double immunofluorescence (DIF) technique.
Methodology Eight samples of periapical granuloma from refractory periapical lesions were obtained by apical surgery. Samples were fixed in formalin and embedded in paraffin using conventional techniques. Periapical scar from the diagnostic archives was used as negative tissue control while tonsil and oral ulcer tissue acted as positive controls. Histological serial sections, 4 μm thick, were prepared. The expression of TLR2, CD38, CD68 or CD83 in the lesions was first confirmed with immunohistochemistry (IHC) followed by DIF staining of TLR2 expression with respect to CD38, CD68 or CD83 cells. All stained slides were digitally photographed under light and fluorescence microscopes at ×20, ×40 and ×100 objective magnifications. Images were analysed with Adobe® Photoshop® CS2 software to determine the co-localization of markers.
Results TLR2 was expressed in all lesions, while there was no expression in periapical scar tissue. Furthermore, TLR2 was expressed on the surfaces of CD38$^+$, CD68$^+$ and CD83$^+$ cells. Among the three inflammatory cell markers, CD38$^+$ were the most frequently identified cells expressing TLR2 in all lesions.
Conclusions Lymphocytes (CD38$^+$), macrophages (CD68$^+$) and dendritic cells (CD83$^+$) express TLR2 in the periapical granuloma. Lymphocytes appear to be the dominant inflammatory cells expressing TLR2 in the periapical granuloma.

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Immunohistochemical analysis of vascular endothelial growth factors and receptors in human periapical lesions

Aim To identify vascular endothelial growth factors (VEGFs) and receptors (VEGFRs) in human periapical lesions.
Methodology Patients diagnosed with chronic apical periodontitis were subjected to endodontic surgery at the Department of Maxillofacial Surgery, Haukeland Hospital, Bergen. The periapical lesions were collected and kept frozen until further handling. Histological evaluation was performed in representative paraffin embedded Hematoxylin and Eosin stained sections (5 μm). Furthermore, serial cryosections (16 μm thickness) of the lesions were subjected to immunohistochemical analysis. Antibodies against VEGF-A, VEGF-C, VEGF-D, VEGFR-2 and VEGFR-3 were used and identified thereafter with the Avidin-Biotin Complex (ABC) method and 3,3-diaminobenzidine (DAB) as the chromogen. The histology and immunohistochemical labeling of VEGFs and VEGFRs were evaluated using light microscopy connected to a digital camera using Lucia imaging software. Double immunofluorescence was used for labelling of macrophage lineage marker CD68 and VEGFs, and evaluated with Zeiss Axio Imager for fluorescence microscopy.
Results The histological evaluation revealed heterogeneous morphology of the periapical lesions with various degrees of inflammatory infiltrates. All samples were immunopositive for VEGFs and VEGFRs. VEGFR-2 was observed mainly on vascular structures, while VEGF-A, VEGF-D and VEGFR-3 were found in both cells and vessel-like structures. VEGF-C positive macrophages were observed in the cell rich part of the lesioned tissue.
Conclusions The presence of VEGFs and their receptors in cells and vessels in chronic apical periodontitis suggests their involvement in cell signalling and vasculogenic activities and can be important mediators in disease progression.

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Expression and localisation of vascular endothelial growth factors and receptors in rat apical periodontitis

Aim To quantitate gene expression and localise vascular endothelial growth factors (VEGFs) and receptors (VEGFRs) in periapical lesions in rats.
Methodology Unilateral pulp exposures of first right maxillary or mandibular molars were created in female Wistar rats (n = 26) and left open to the oral cavity for either 10 or 21 days. The contralateral sides served as controls. Block specimens of the induced periapical lesions were subjected to gene expression analysis with q-PCR using TaqMan® gene expression assays (Applied Biosystems, USA) for VEGF-A, VEGF-C, VEGFR-3 and β-actin as reference gene. Furthermore, protein localisation of VEGF-A, VEGF-C, VEGF-D, VEGFR-2 and VEGFR-3 in jaw cryosections was performed with immunohistochemistry and double immunofluorescence was used for labelling of macrophage lineage marker CD68 and VEGFs/VEGFRs.
Results VEGF-A and VEGF-R3 gene expression was up-regulated throughout the experimental period ($P < 0.05$), whereas VEGF-C gene expression was increased at day 10 but not significantly compared to controls. VEGF-C and -D were observed in $CD68^+$ cells and in vessels in the lesions at days 10 and 21, but were absent in control periapical tissues. VEGF-A, VEGFR-2 and VEGFR-3 were found on vascular structures as well as on cells in the lesioned area. VEGFR-2 was also observed in $CD68^+$ osteoclasts lining the bone in the periphery of the lesion from day 10. The immunohistochemical expression of all VEGFs and their receptors was increased at 3 weeks in comparison to the 10-day period.
Conclusions The gene analysis and up-regulation of protein expression for VEGF-A, VEGF-C, VEGF-D, VEGFR-2 and VEGFR-3 in apical periodontitis indicated that the VEGF family and receptors were involved in vasculogenesis and immune functions during disease development. The finding of VEGFR-2 on osteoclasts might indicate that bone resorbing activity in the lesions was influenced by VEGFs.

R126

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T helper cell function in mice periradicular inflammation

Aim To determine the role of IL-17 in bone resorption stimulated by dento-alveolar infections.
Methodology Infrabony resorptive lesions were induced in IL-17 receptor A (RA) knockout mice (KO) by surgical pulp exposure and microbial infection of lower 1st molar teeth. The extent of periapical bone destruction was evaluated using micro-computed tomography (μCT), ELISA was used to quantify cytokine expression in periapical lesion extracts and histology was used to evaluate the neutrophils and osteoclasts expression.
Results IL-17 was strongly induced in periapical tissues by 7 days after infection but was not expressed in uninfected mice. Dento-alveolar infections of IL-17 RA knockout mice (KO) demonstrated significantly increased bone destruction as well as IL-17 neutralized animals and more abscess formation in the apical area compared to wild type mice. More macrophages and B-cells and higher densities of neutrophils were found in the lesioned area in the KO mice compared to the littermate at day 21. The expression of IL-1 (α and β), and MIP2 were significantly upregulated in the IL-17 RA^{KO} mice at the same time interval.
Conclusions These results demonstrate that IL-17RA signalling down-regulates macrophage and mononuclear inflammation elicited by endodontic pathogens and is protective against infection-induced bone destruction in dento-alveolar infections.

R127

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Evaluation of IL-6 and PGE2 levels in GCF of the teeth with periapical diseases

Aim To evaluate any differences of prostaglandin-E_2 (PGE_2) and interleukin-(IL-6) levels in gingival crevicular fluid (GCF) of teeth with periapical diseases compared with healthy teeth.
Methodology Twenty healthy patients with teeth diagnosed with acute (AAP) or chronic apical periodontitis (CAP) having periapical

index (PAI) scores 3–5 were included in the study, following ethical committee approval and patients' consents. GCF samples were obtained with periopaper strips from the diseased tooth and from a healthy contra-lateral tooth on the first and seventh days. Enzyme-linked immunosorbent assay was used to analyze the concentration of IL-6 and PGE_2 in the samples.

Results IL-6 levels in GCF of the teeth with CAP were significantly higher in both of the first and seventh day samples, compared with the corresponding control teeth ($P < 0.05$). There was no significant difference in the PGE_2 levels between the diseased and control teeth in the CAP group ($P > 0.05$). In AAP cases, the first day PGE_2 level decreased significantly by the seventh day and was significantly higher than that of the control groups ($P < 0.05$). There were no significant differences in the IL-6 levels between the diseased and control teeth in the AAP group ($P > 0.05$).

Conclusions Within the limitations of this study, IL-6 and PGE_2 levels in GCF of teeth with CAP and AAP were higher when compared with healthy teeth. These findings might help to better understand the pathophysiology of the diseases with endodontic origin. The IL-6 and PGE_2 levels in GCF may be used as potential diagnostic or prognostic markers of the healing or disease.

TRAUMA/REGENERATION

R128

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Validation of three dentinsialophosphoprotein (DSPP) antibodies from various sources on six different cell types

Aim To validate various antibodies raised against DSPP for immunohistochemical analysis of DSPP expression.

Methodology As potentially DSPP-forming cells, human pulp derived cells (hpdcs) and human pulp derived stem cells (hpdscs) were used. Both primary cell lines were derived from third molar teeth of three donors aged 15–19 years with incomplete root development. Experiments were done in transfer (T)-phase 2 or 3. As putatively DSPP-negative cell lines U-2OS human osteosarcoma cells (A), NIH/3T3 mice fibroblasts (B), HEK293 human embryonic kidney cells (C) and PC12E2 pheochromocytoma cells (D) were used. Trials were conducted in triplicate on four-well glass Chamber Slides (Lab-Tek; Nalgene Nunc, Denmark). Each cell type was cultivated using the adequate specific medium. After 7 days, immunohistochemical staining was performed with the rabbit polyclonal antibody LF-154 (L. Fisher, NIH, USA) taken as a reference. Two additional polyclonal antibodies (antibody 1, 2) were obtained by rabbit immunisation with a peptide sequence of DSPP (Sigma Genosys, UK). Negative primary and secondary antibody controls were included. Analysis was performed by two independent observers under fluorescent light microscopy (Cell Observer; Zeiss, Germany).

Results Cell line A showed in one sample a positive staining of anti-DSPP (antibody 2). Cell lines B-D showed no positive signalling for any of the tested anti-DSPP antibodies. The hpdcs and the hpdscs showed positive signals with all tested antibodies. The cell morphology and pattern of the DSPP-positive signals were different between the hpdcs and the hpdscs. There was no difference between the two observers and no apparent difference between the three types of antibodies used. No signals were detected for the primary and secondary antibody controls.

Conclusions The tested DSPP antibodies showed conformity, they are a valid adequate tool for detecting DSPP protein expression in cell cultures.

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R129

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Knowledge in managing avulsed teeth among general dental practitioners in Malaysia

Aim To investigate the knowledge of general dental practitioners (GDPs) and their clinical practice in managing avulsed teeth.

Methodology Malaysia's National Oral Health Plan 2010 stated that one of the key goals was to reduce dental injuries and improve on the management of the traumatised teeth. Thus, a questionnaire was constructed consisting of Part I – Demographic data (age, gender, main practice, years of practicing dentistry and number of trauma cases managed), Part II – 10 questions on knowledge in managing avulsed teeth. Random convenient sampling was used. Questionnaires were distributed to the GDPs who attended the Malaysian Dental Association's conference in January 2010. Data were entered and analysed using PASW Version 18.

Results A total of 182 GDPs participated in the study, consisting of 75% ($n = 136$) female, with a mean age of 35 years. Practice duration varied from 0.5 to 33 years (mean duration of 10 years). Number of avulsed teeth treated in the past 2 months ranged from 0 to 12 teeth. Fifty eight percent ($n = 105$) of the GDPs were from the government clinics. Forty six percent of the respondents achieved a knowledge score of 80 and above out of a total score of 100. Those who practiced in government clinics had significantly higher scores compared to private practitioners ($P = 0.01$). Logistic regression analysis was able to predict correctly with overall success rate of 64% with the odds ratio of 3.615 for the place of practice, which means that those working in government clinics were 1.44 more likely to score more than 80.

Conclusions Knowledge levels on the management of avulsed teeth among GDPs in Malaysia could be improved.

R130

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Production and characterization of biodegradable scaffolds for pulp regeneration

Aim To evaluate the biocompatibility of two biodegradable scaffolds, based on pectin/chitosan (PEC/CH) and hyaluronan/chitosan (HA/CH), designed for pulp regeneration.

Methodology One percent (w/v) solutions of pectin and chitosan were prepared by dissolving each polysaccharide in a 0.01 M acetate buffer (pH = 4.5). Hyaluronan solution was prepared in a 2% (w/v) concentration using the same buffer solution. The polyelectrolyte complexes (PECs) between the polymers were formed by mixing the solution of the two chosen polymers at room temperature (PEC/CH and HA/CH) under homogeniser agitation. After PECs formation and precipitation, they were isolated by centrifugation, frozen under liquid nitrogen and finally freeze-dried

for 8 h. The samples were prepared in a cylindrical mould (4 mm high and 2 mm diameter) in order to fit the tooth cavity. Scaffold biocompatibility was assessed through *in vitro* studies, using mouse mesenchymal stem cells. Cells were seeded in the presence or absence of each scaffold. Optical microscopy and a non-radioactive assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) – MTS) were used to characterize cell viability.

Results Cell adhesion and proliferation was observed. MTS assay results showed a significant difference between cells exposed to the two scaffolds (PEC/CH and HA/CH) and the positive control (dead cells) ($P < 0.05$), and between cells exposed to the scaffolds and the negative control (live cells) ($P < 0.05$), after 24, 48 and 72 h of incubation. The results obtained for both scaffolds were not statistically different.

Conclusions *In vitro* studies showed that cells adhere and proliferate in the presence of the scaffolds, which is indicative of the biocompatibility of the scaffolds herein produced. Further *in vivo* studies are currently being performed in order to characterise the applicability of these constructs.

R131

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Low level laser therapy effects on the proliferation of primary tooth dental pulp stem cells

Aim To evaluate the influence of different energy densities (3 and 5 Jcm^{-2}) of low level laser therapy (LLLT) on the growth of primary tooth dental pulp stem cells (DPSC) cultured under conditions of nutritional deficiency.

Methodology Primary tooth dental pulp stem cells (8th passage) were seeded in 96-well cell culture plates (1×10^3 cells/well) and allowed to attach in culture medium supplemented with 15% Hyclone defined foetal bovine serum (FBS) for 12 h. To promote a situation of nutritional deficiency, the FBS percentage was reduced to 5%. Cells were assigned in four groups: G1 (15% FBS) (control), G2 (5% FBS), G3 (5% FBS + LLLT 3 J cm^{-2}), and G4 (5% FBS + LLLT 5 Jcm^{-2}). For LLLT groups, two laser irradiations with 6 h-interval were performed using a diode laser (660 nm-InGaAlP, 0.028 cm^2, 10 mW). Cell viability was assessed by MTT assay immediately after the second laser irradiation (0 h) and 24, 48 and 72 h after first laser irradiation. Data were analysed by ANOVA ($P < 0.05$).

Results The control group (15% FBS) had a significantly higher rate of cell growth compared to all other groups at all experimental times ($P < 0.01$), except for LLLT 5 Jcm^{-2} at 48 and 72 h ($P > 0.05$). The group of LLLT 5 Jcm^{-2} presented a significant increase in cell growth at 48 and 72 h compared to 5% FBS and LLLT 3 Jcm^{-2} groups ($P < 0.01$). LLLT 3 Jcm^{-2} group was similar to 5% FBS group in all periods studied ($P > 0.05$)

Conclusions LLLT with 5 Jcm^{-2} irradiation can enhance the growth of DPSC even in conditions of nutritional deficiency, having similar growth rate to ideal nutritional conditions. Therefore, LLLT could be a valuable adjunct treatment on tissue engineering using stem cells obtained from primary tooth dental pulp.

OUTCOME STUDIES

R132

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Undergraduate root canal treatment outcomes for students at the University of Coimbra, Portugal

Aim To evaluate root canal treatment outcomes for treatment performed by undergraduate dental students at the University of Coimbra, Portugal.

Methodology A random sample of 596 reports of patients treated at the University of Coimbra between 1994 and 2010 was evaluated using strict criteria based on the European Society of Endodontology quality guidelines for root canal treatment. The length of the root filling and the presence of voids or unfilled spaces was evaluated. Chi-square and McNemar-Bowker statistical tests were conducted as to determine the significance of the results.

Results Of the 596 treatments evaluated, 63% were in maxillary teeth and 37% in mandibular teeth. With regard to length of root fillings, 85% were 1–2 mm short of radiographic root-length, while 9% were overfilled and 5% underfilled. Dense root fillings without voids were observed in 85% of the cases. All cases were reviewed within an average of 15 months, revealing 91% success rates, with a statistically significant remission of symptoms ($P < 0.05$).

Conclusions Within the limitations of the short recall times (average 15 months), it can be concluded that root canal treatment provided by undergraduate students at the University of Coimbra is of good quality. Longer – term studies are needed to confirm success rates.

R133

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The success and quality of root canal treatment under NHS regulations (UK): a retrospective study

Aim To assess the success of root canal treatment carried out under National Health Service (NHS, UK) contract by one dentist. To assess the quality of root fillings against ESE Guidelines and highlight the most common reasons of categorising root fillings as unsatisfactory.

Methodology All root canal treatments carried out by one dentist (PJS) between July 2007 and December 2009 were reviewed. Treatment outcome and quality of root fillings were assessed on the basis of clinical notes and radiographs. Treatments carried out under the NHS contract with minimum 12 months observation were included. Failure was ascribed to teeth which were extracted, had patient-reported symptoms, clinical signs (swelling, erythema, tooth mobility, tenderness to percussion, sinus tract or pus discharge) or periapical radiolucency equal or larger in size than the original lesion. Success was classified either as definite or probable. The quality of root fillings was classified as satisfactory or unsatisfactory.

Results Out of 186 root canal treatments carried out, 99 procedures on 97 teeth qualified for further assessment. Out of the 99 root fillings 49 (49%) were classified as satisfactory quality and 50 (51%) as unsatisfactory. The most common faults in terms of quality of root filling were incomplete apical filling and overfilling. Most failures were associated with incomplete apical

filling and/or missed canals. Eighteen treatments were classified as failures, sixteen had unsatisfactory fillings and two were satisfactory. Out of 97 teeth, nine were or are going to be extracted (9%), five teeth (5%) had re-treatment either in the surgery (4) or by a specialist (1). Three teeth (3%) are under observation and one awaits re-treatment.

Conclusions Most of the failures were associated with unsatisfactory root fillings. Incomplete apical filling and missing a canal were most likely to have serious consequences.

R134

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The status of periapical tissues of root filled teeth in Croatian adult subjects

Aim To determine the status of periapical tissues of root filled teeth in Croatian adult subjects in relation to age, gender, position of teeth and length of root filling.

Methodology This study involved 163 Croatian adult patients (413 teeth). All subjects presented as new patients seeking routine dental care in a university dental clinic within a 2 year period. Data collection included questionnaires and examination of digital orthopantomograms. Patients younger than 18 years, patients having less than eight teeth, and patients who had root canal treatment within the previous 2 year period were excluded. The periapical status of root-filled teeth was assessed using the periapical index system (PAI), described by Ørstavik *et al.* (1986). The periapical status of root filled teeth was evaluated in relation to age, gender, position of teeth and length of root filling. Raw data were entered to an Excel database and statistical tests were carried out using the Statistica 8.1 package. One-way ANOVA and *post hoc* tests were used at the *P*-level of 0.05.

Results Subjects of the age group under 30 years had significantly higher PAI values than age groups from 31–40 ($P = 0.018$), 41–50 ($P = 0.003$) and 51–60 ($P = 0.012$). There was no significant gender difference in periapical status ($P = 0.117$). Molar teeth had considerably higher PAI values in comparison to premolar ($P = 0.014$) and anterior ($P > 0.001$) teeth. Teeth with adequate length of root fillings had considerably lower PAI scores in comparison to underfilled ($P < 0.001$) and overfilled ($P = 0.017$) teeth.

Conclusions The status of the periapical tissues in root filled teeth was affected mostly by the position of teeth and the length of the root filling. Higher PAI values are found in molar teeth as well as in root filled teeth with inadequate length of root filling.

R135

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Treatment outcome determined with periapical radiographs (PA) and CBCT

Aim To evaluate the presence and absence of periapical lesions after root canal treatment, and to compare the endodontic outcome predictors identified with periapical radiographs (PA) and cone beam computed tomography (CBCT).

Methodology In total, 119 teeth (125 roots) with apical periodontitis (AP) were initially root filled and followed-up for 2 years. Multivariate logistic regression was performed on the data from PA or CBCT to analyze outcome predictors.

Results At recall, PA detected the absence of periapical lesions in 73 (58.4%) roots, as compared with 58 (46.4%) on CBCT images. PA also detected 33 (26.4%) shrinking lesions. The apical extent of root fillings determined by PA and CBCT was different ($P < 0.001$). Overall, 9 (69%) out of the 13 short-root fillings diagnosed by PA appeared as flush fillings on CBCT images. PA revealed 11 (8.8%) root fillings with voids, as compared with 36 (28.8%) on CBCT images. When findings from PA were analyzed, the apical extent of root filling and coronal restoration were identified as predictors ($P < 0.05$). When findings from CBCT were analyzed, density and apical extent of root filling, and quality of coronal restoration influenced the outcome significantly ($P < 0.05$), while gender, tooth type, root curvature, size of preoperative periapical lesion, number of visits, and PA-determined density of root-filling and use as abutment did not ($P > 0.1$).

Conclusions In teeth with AP, treatment outcome, length of root fillings, and outcome predictors as determined with CBCT scans may not be the same as corresponding values determined with PA.

R136

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An *in vivo* comparison of two rotary systems: periapical healing after 6 months

Aim This prospective study on teeth with necrotic pulps evaluated the clinical outcome of root canal treatments performed with two full-length Ni-Ti rotary file systems with different cross-sectional design and canal-cleaning ability.

Methodology Ninety-four patients with necrotic pulps, and periapical lesions needing root canal treatment were included and randomly divided into two groups. At baseline, a preliminary periapical radiograph was taken and the presence of pain was registered according to the Verbal Rating Scale. A single operator performed all single-visit root canal treatments under rubber dam isolation; when necessary, pre-endodontic restorations were provided. Canals were instrumented with Mtwo (Sweden & Martina, Italy) (Group 1, $n = 55$) or Alpha Kite (Komet, Germany) (Group 2, $n = 39$), irrigated with 2.5% NaOCl and filled by the continuous wave of condensation technique (System B; SybronEndo, USA). No chelating agents were used during irrigation. Coronal seal was assured by immediate direct composite restorations. Patients were recalled after 6 months to repeat clinical and radiographic evaluations. Statistical analysis was performed using chi-square, McNemar, Mann–Whitney, Wilcoxon tests (SPSS Inc., USA) ($P < 0.05$).

Results Complete periapical healing was observed in 55% and 51% of cases in Group 1 and Group 2 respectively. Subtotal reduction of periapical radiolucencies occurred in 40% of patients in Group 1 and 49% in Group 2. Almost all symptoms disappeared at 6-month follow-up in both groups. No significant differences were found between the groups in any of the parameters considered ($P > 0.05$).

Conclusions Within the limits of the present study, the use of M two and Alpha Kite instruments produced similar success rates at 6-month follow-up.

R137

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Effect of instrumentation technique and number of treatment visits on endodontic flare-ups: a randomised controlled trial

Aim To evaluate the effects of instrumentation technique and number of dental treatment visits on endodontic flare-ups in a randomised controlled trial.
Methodology One hundred and sixty patients who had an asymptomatic tooth with a necrotic pulp and associated apical periodontitis were included. The patients were divided randomly into four groups of 40 each. A hand (Step back) and three engine-driven (ProTaper; Dentsply Maillefer, Switzerland; Mtwo; VDW, Germany; K3; SybronEndo, USA) techniques were used for root canal instrumentation. Half of the teeth in each group were root filled at the same visit. In the other half of each group, teeth were medicated with calcium hydroxide and root filled after 1 week. The patients were recalled after 7 days and the occurrence of post-filling swelling and pain were recorded. The level of discomfort was rated as no pain, mild pain, moderate pain or severe pain. Data were analyzed using Kruskal–Wallis and Mann–Whitney *U*-tests.
Results Swelling was experienced by only four patients. Severe pain was recorded in two patients, moderate in 11 and mild in 18. Flare-ups, characterized by moderate to severe post-filling pain occurred in 8.1% of patients (7.5% after single- and 8.8% after two-visit treatment). There were no significant differences in the incidence of pain between single- and two-visit treatment ($P > 0.05$) and amongst the instrumentation techniques ($P > 0.05$).
Conclusions Instrumentation techniques used and number of treatment visits during root canal treatment of asymptomatic teeth with necrotic pulps and periapical radiolucencies had no effect on the prevalence of endodontic flare-ups.

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Post-operative pain after single-visit root canal treatment of asymptomatic teeth with different pulpal and periapical status

Aim To evaluate prospectively the incidence of postoperative pain after single-visit root canal treatment of asymptomatic teeth with vital pulps, primary apical periodontitis or requiring retreatment.
Methodology Eighty-one teeth were included in this study. All were asymptomatic with no pulpal or periapical pain pre-operatively. All treatments were comopleted in one visit by the same operator. Seventeen teeth had vital pulps (Group 1), 36 teeth were associated with primary apical periodontitis (Group 2) and, the remaining 28 teeth required retreatment due to failure of previous treatment (Group 3). A standard treatment procedure was followed for all 81 teeth. The levels of postoperative pain were categorized as none, mild, moderate or severe. Patients recorded the experienced pain at 2 and 7 days after treatments. The data were analyzed using the chi-squared test.
Results At 2 days after treatment, 10 of 17 teeth in Group 1, 24 of 36 teeth in Group 2 and nine of 28 teeth in Group 3 had no pain. The post-operative pain experience was significantly different between Group 3 and Groups 1 and 2 ($P = 0.02$). At 7 days after treatment, 14 of 17 teeth in Group 1, 30 of 36 teeth in Group 2 and 18 of 24 teeth in Group 3 had no pain. There was no statistically significant difference in the post-operative pain experience amongst three groups ($P = 0.2$) at 7 days.
Conclusions Some teeth in all groups became asymptomatic after single-visit treatment. The results of this study indicated that retreatment cases were associated with a higher incidence of post-operative pain than teeth with vital pulps and teeth with primary apical periodontitis at 2 days but not at 7 days after treatment.

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Root canal treatment protocols, materials and procedures employed by dentists in Croatia

Aim To investigate utilisation of treatment protocols and selection of materials during root canal treatment by Croatian dentists and their attitudes towards endodontic treatment.
Methodology A questionnaire with eighteen questions requested information on practice setting, usage of radiographs, rubber dam and apex locators, irrigant selection, rotary instrument use and attitudes, intracanal medicaments and root canal filling materials. The number of participants was 300.
Results The response rate was 31% ($N = 94$), the majority from dental practices in urban areas (78%). Daily root canal treatment frequency exceeded three in 75% of offices. Radiographs were taken before and after treatment in 44%, while 20% of dentists exposed radiographs only in case of complications. Rubber dam was seldom used (9% regular users). Its usage positively correlated with frequency of radiographic exposure (Spearman's rho, $P < 0.05$). Rubber dam non-users considered it to be too expensive (31%) but they also lacked training (51%). NaOCl was the most commonly used irrigant solution (90.4%) while the usage of hydrogen peroxide increased significantly with practitioner age (χ^2 test, $P < 0.05$). Apex locators were used by 66% of dentists and positively correlated with the frequency of radiographic exposure (Spearman's rho, $P < 0.05$). Hand instruments were preferred over rotary files. Problems in usage of rotary files were the price and lack of training. Usage of rotary files negatively correlated with practitioner age (Spearman's rho, $P < 0.05$). Calcium hydroxide dressing was preferred (62%), but phenol-camphor solution was still used (27%). The dominant root canal filling material used was a combination of gutta-percha and AH Plus (92%).
Conclusions Despite the majority of urban dental practices and high root canal treatment requirements, the use of radiographs to control procedures was inadequate. Rubber dam was even less utilized. Efforts should be placed on education and training in the usage of rubber dam, apex locators, rotary instruments and irrigation solution selection.