International Endodontic Journal

doi:10.1111/j.1365-2591.2009.01618.x

ABSTRACTS

Wladimir Adlivankine European Society of Endodontolgy Research Prize

RP1

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Evaluation of Nd:YAG and Er:YAG irradiation and antibacterial photodynamic therapy on *Enterococcus faecalis* biofilms

Aim To compare the antimicrobial efficacy of two high power lasers (Nd:YAG and Er:YAG) and two commercial antimicrobial photodynamic therapy (aPDT) systems with the traditional root canal irrigant on *Enterococcus faecalis* biofilms grown on dentine disks *in vitro.*

Methodology Twenty four hour *E. faecalis* biofilms were grown on dentine disks in a microtiter plate and subjected to the following treatment or control groups: aPDT using the Denfotex system, aPDT using the Helbo system, Er:YAG laser irradiation (2940 nm, 50 mJ and 100 mJ, 15 Hz, 40 s), Nd:YAG laser irradiation (1064 nm, 2W, 15 Hz, 40 s) and immersion in sodium hypochlorite (NaOCl) 0.5% for 1, 5, 10 and 30 min. Surviving bacteria were harvested and the number of CFU per disk was determined by plate count.

Results Significant differences ($P < 0.03$) in viable counts compared to untreated controls were observed for: aPDT (Helbo) (2 log10 reduction), Er:YAG irradiation using 100 mJ pulses (5 log10 reduction) and all NaOCl treatments (>6 log10 reduction). NaOCl 0.5% for 30 min effectively eliminated all bacteria. aPDT (Denfotex), Er:YAG irradiation using 50 mJ pulses and Nd:YAG treatment caused a reduction in the viable counts of <1 log10 but these results were not significantly different from the untreated controls ($P > 0.05$).

Conclusions NaOCl and Er:YAG irradiation were highly effective in eliminating *E. faecalis* grown as a biofilm. aPDT eliminated *E. faecalis* biofilm to some extent, with a difference between both systems. Nd:YAG irradiation was the least effective against *E. faecalis* biofilm in this study.

RP2

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Immunohistochemical expression of Notch signaling in the lining epithelium of periapical cysts

Aim The immunohistochemical expression of the receptors Notch 1 and Notch 2, the ligand Delta 1 and the transcription factors HES 1 and HES 5 in the epithelium of well defined periapical cysts.

Methodology Immunohistochemistry was carried out on 55 formalin fixed and paraffin-embedded, well-defined periapical cysts, with minimum inflammation, obtained from the archival tissue database of the Department of Oral Pathology and Surgery. Western Blotting was performed to evaluate the specificity of the anti-Notch antibody and the expression of Notch signaling in five fresh frozen periapical cysts. The levels of staining intensity were estimated by the performance of a semi automated image analysis system. Descriptive statistic of mean values obtained by computerized image analysis method was performed.

Results Immunostaining reaction of all Notch signaling components was observed in the cytoplasm and/or the cytoplasmatic membrane of all epithelial cells of periapical cysts. Notch 2 demonstrated the most intense staining (50.90%), followed by Notch 1 (34.55%), HES 5 (16.36%) and HES 1 (7.27%), while Delta 1 immunostaining was only moderate (36.36%) and weak (50.91%). No statistical correlation was found between the antibodies and the gender or the age of the study group.

Conclusions Notch is an evolutionarily conserved signaling mechanism that regulates cell fate decisions during development and postnatal life in organisms as diverse as worms, flies, and humans. The present observations indicate that Notch pathway is active downstream in the lining epithelium of periapical cysts, suggesting an involvement of this pathway in periapical cyst growth and expansion.

Acknowledgements The rat anti-human Notch 1 monoclonal antibody (catalog No: bTAN 20) and the rat anti-human Notch 2 monoclonal antibody (catalog No: c651.6dbhn) developed in this study was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA, USA. This study was financially supported by a grand from the University of Athens (70/4/6450).

RP3

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Survival of *Enterococcus faecalis* in root canals after conventional and experimental intracanal medication

Aim To test the susceptibility of *Enterococcus faecalis* microorganism to conventional and experimental root canal medicaments *ex vivo.*

Methodology Crowns of sixty extracted single-rooted human teeth were removed and root canals were instrumented using ProTaper instruments to size F3. After removal of smear layer with sequential usage of 17% EDTA and 5.25% NaOCl, the specimens were sterilized in distilled water and divided into six groups of ten samples in each. All samples were inoculated with the overnight inoculums of *E. faecalis* for 21 days. The following medicaments were used in test groups: calcium hydroxide paste-(Sultan Healthcare, USA) ($Ca(OH)_2$), 2% chlorhexidine gel (Consepsis Scrub, Ultradent, USA) (Chx), methyl cellulose/boric acid combination (MCBA), chitosan/boric acid combination (CBA), 2% chlorhexidine gluconate/chitosan/boric acid (ChxCBA) combination. One group was also kept infected and served as control. After 7 days of incubation, the medicaments were removed and dentine samples were collected from inner root canal walls with Gates Glidden burs of size 3, 4, and 5 into vials containing 2 mL of phosphate buffered saline, vortexed for 30 s, serially diluted to 10^{-4} and seeded on tryptic soy agar plates and incubated at 37°C for 48h before colony-forming units (CFU) were counted.

Results All the CFU values of tested medicaments were significantly lower than the control group ($P < 0.05$). Only $Ca(OH)_2$ and ChxCBA groups totally eliminated all *E. faecalis* cells (CFU = 0). *E. faecalis* microorganisms were more susceptible to Chx than the CBA and MCBA groups ($P < 0.05$).

Conclusions Chlorhexidine gluconate, chitosan and boric acid combination may effectively remove all planctonic *E. faecalis* microorganisms from the root canal and therefore should be studied further.

Epidemiology, Microbiology, Biological Sciences

R1

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An electromyographic (EMG) investigation of activity in operator upper back and neck musculature during endodontic procedures

Aim To evaluate the effect on neck muscle activity of using surgical loupes during endodontic access cavity procedures.
Methodology Work-related musculoskeletal disorders have been reported as a major problem for dentists, primarily affecting the back, shoulder and neck regions. This can result in chronic conditions, absence from work and loss of income. Magnification is primarily used in endodontics to enhance visualisation and instrumentation of root canals. However, this study considers whether surgical loupes may also contribute to improved posture of the clinician, potentially reducing muscular strain and preventing injury. Ten access cavities were prepared by one operator in plastic teeth in a phantom head model; five with the use of surgical loupes (Keeler SuperVu Galilean System 2.5× magnification, 42 cm focal distance) and alternately, five without. The subject was connected to an 8-channel wireless-datalogger EMG system (Delsys Myo IV) to record the muscle activity of the right and left shoulder and neck musculature at one, three and five-minute intervals, and subsequent statistical analysis was used to determine whether the loupes had any significant effect on the operator's muscle activity. The data was analysed using non-parametric Wilcoxon tests.
Results Standard deviations showed that there was greater variation in muscle activity in the shoulder regions compared to neck during the cavity preparations. There was a statistically significant reduction ($P < 0.05$) in activity in both shoulders (Right $P < 0.042$, Left $P < 0.043$) and right neck ($P < 0.043$) when using loupes compared to no loupes.
Conclusions The use of loupes when preparing endodontic access cavities may be of benefit to the clinician in combating musculoskeletal strain and injury, as they contribute to reduced activity in the neck and shoulder muscle regions, and could therefore reduce fatigue.

R2

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R3

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Monitoring of the clinical course of pain/discomfort in patients following non-surgical root canal treatment (NSRCT)

Aim To monitor the clinical course of the pain/discomfort experience from teeth demonstrating signs of periapical healing following non-surgical root canal treatment.
Methodology A total of 92 patients (111 teeth) were evaluated through clinical and radiographic examinations, questionnaire (Short Form of the McGill Pain Questionnaire-SFMPQ) and interview analysis at review appointments 5–14 months after root canal treatment to determine the prevalence of pain or discomfort. The course of detected pain/discomfort was monitored at a second review six months later. Pain and discomfort were classified separately based on scores on the SFMPQ. Changes in the clinical course were also assessed based on alterations in scores on the relevant questionnaires. Teeth associated with persistent pain/discomfort but exhibiting signs of complete radiographic healing were examined further using Cone Beam Computer Tomography to exclude any undetected periradicular pathosis.
Results In total, 32% (35/111) of teeth showing signs of periapical healing were associated with pain ($n = 23$) or discomfort ($n = 12$) at the first review appointment. Of the teeth with pain symptoms, the pain persisted but decreased in intensity in six teeth, changed to discomfort in five teeth and disappeared altogether in six teeth. Pain intensity increased in the remaining six. Of the teeth with discomfort symptoms, five became symptom-free and the intensity of discomfort reduced in two teeth. Symptoms remained the same or increased in another five.
Conclusions Thirty-two per cent of the teeth showing signs of periapical healing had pain/discomfort. The experience of pain/discomfort spontaneously decreased in the majority of these within six months. Monitoring and reassurance rather than intervention of these cases is therefore encouraged.

R4

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The potential association between smoking and dental periapical condition in a sample of Croatian adults

Aim To study the prevalence and extension of apical periodontitis (AP) in Croatian smoker and non-smoker patients.
Methodology Study involved 70 patients, 34 smokers and 36 non-smokers. Data collection included questionnaires and radiographic examination of participants at the Faculty of Medicine in Rijeka, Department of Endodontics and Restorative Dentistry. Patients younger than 18 years, patients having less than eight teeth, and patients who had endodontic treatment within a two year period were excluded. Two examiners interpreted panoramic radiographs with an illuminated light source in a darkened room using a magnification. The periapical region of all teeth, excluding third molars, was examined. The periapical status was evaluated by applying the periapical index system (PAI). Calibration of the two examiners was carried out using 20 panoramic radiographs, which were double-scored. Interexaminer agreement was calculated. Statistical analyses were conducted using the Cohen\'s Kappa test, *t*-test for proportions, Chi square test and one-way analysis of variance (ANOVA).
Results Apical periodontitis was found in 12% of smokers and in 9% of non-smokers (PAI ≥ 3). The differences between smoking and non-smoking groups were not statistically significant ($P = 0.062$; OR = 1.3; 95% CI = 1.0–1.6). The prevalence of AP of endodontically untreated teeth in relation to smoking and non-smoking groups was statistically significant ($P = 0.018$, OR = 1.4 (95% CI=1.1–2.1). Among treated teeth associated with AP, there was no statistically significant difference in smoker and non-smoker subjects ($P = 0.084$, OR = 0.8; 95% CI = 0.4–1.3).

Conclusions The periapical index including teeth affected by periapical pathosis did not significantly differ between smoking and nonsmoking groups. Smoking was significantly associated with a greater frequency of AP only in non root filled teeth.

R5

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Correlation between OIDP index and periapical status: a pilot study

Aim To evaluate the correlation between the score of Oral Impacts On Daily Performances (OIDP) index and endodontic status, including periapical index (PAI), determined by analysis of orthopantomograms of corresponding patients.
Methodology Digital orthopantomograms (dOPGs) (n = 61) were obtained from a private dental x-ray lab in Zagreb, Croatia. Each patient, aged from 18 to 65 years, filled a Croatian version of the OIDP questionnaire. Analysis of dOPGs was performed by two investigators using CliniView 4.1 PC diagnostic software. The following categories were determined: endodontic status, quality of endodontic treatment and PAI index. OIDP index was calculated from the questionnaire for every subject. Data were analyzed using SPSS 16.0 PC software by means of descriptive statistics and bivariate correlation analysis.
Results OIDP index score correlated positively with the number of missing teeth ($P < 0.05$) and endodontically treated teeth ($P < 0.05$). A correlation was found between OIDP index score, PAI 4 and PAI 5 score ($P < 0.05$).
Conclusions Impacts of oral conditions on daily performances are influenced by health of periapex, number of endodontically treated and missing teeth. OIDP index indicates the influence of periapical status on daily functioning of an individual.

R6

Abstract withdrawn

R7

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Influence of post-endodontic restorations on the survival rate of posterior teeth

Aim To evaluate retrospectively the influence of coronal restorations on the survival rate of endodontically treated posterior teeth.
Methodology During the years 1990–1999, 1033 root filled posterior teeth were restored with fillings, posts, crowns or bridges at the School of Dentistry in Münster, Germany. For this retrospective study, 676 of these teeth were evaluated after a mean period of 9.7 (±2.8; minimum: 5) years. The data were statistically analyzed using descriptive statistics (minimum and maximum values, standard error, and mean values). The Kaplan-Meier statistics were used to calculate the survival rate and chi-square test to determine the independance of variables. The survival rate was calculated using the log-Rank-test. A binary logistical regression was performed to determine the influence of variables on the results of the statistical evaluation.
Results In 86.2% of the cases the endodontically treated and restored teeth survived the observation period of 9.7 years. Statistically, the survivability was 13.6 (±0.2) years in total. All teeth with gold partial crowns survived without fractures (n = 24). For crowns with access cavities the survivability was 15.3 (±0.4), with crown and bridge restorations 14.0 (±0.3), with individual cores 13.9 (±0.2), with composite fillings 13.4 (±0.5), with prefabricated metal cores 12.7 (±0.6), with amalgam fillings 11.8 (±0.6), and with glass ionomer cements (GIC) 6.6 (±0.5) years, respectively. The survivability for teeth restored with GIC was significantly lower compared to all other groups ($P < 0.001$). The cavity size in teeth with fillings influenced the survival rate significantly ($P < 0.001$). The factors gender, age, jaw and kind of tooth had no significant influence on the results ($P > 0.05$).
Conclusions GIC should not be used for the permanent restoration of endodontically treated teeth. The use of cores had no significant influence on the survival rate of teeth. Crowns with access cavities showed comparable survival rates to crown restorations.
Acknowledgements Our special thanks go to PD Dr. R.J. Fischer, Institute for Medical Informatics and Biomathematics of the Westfälische Wilhelms-Universität, Münster, Germany for his help with the statistical evaluation.

R8

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Detection of mental foramen by Cone-Beam CT imaging in Taiwanese individuals

Aim To explore the position and the exit angulations of the mental foramen among a Taiwanese population using cone-beam computed tomography (CBCT).
Methodology CBCT images of 100 subjects (50 males and 50 females; mean [range] age, 39.3 [16.2 ± 2.4] years) were studied. The 3D images were reconstructed using ImplantMax software (version 3.0, Saturn, Taipei, Taiwan) for each subject. The reference planes were drawn by a horizontal line passed across bilateral centre of the mental foramen and a vertical plane intersected from the inter-proximal contact of both mandibular central incisors. The bilateral linear distances from the mental foramen to the mid-vertical line, the types of the angulated canal to the exit of mental foramen were measured. Data were determined by t test and Pearson correlation test.
Results The distance of mental foramen to the mid-vertical line was 29.38 ± 0.24 mm in the right side and 28.57 ± 0.38 mm in the left side ($P < 0.05$). Four types of the angulated canal to the exit of mental foramen; lateral exit (2.56%), anterior-upward exit (1.28%), distal-upward exit (7.69%), and distolateral-upward exit (88.46%) were found in Taiwanese individuals.
Conclusions Knowledge of the position of mental foramen and mandibular canal is essential for mandibular premolar area periapical surgery. Measurement of the mental foramen position and course of mandibular canal with 3D image of CBCT is simple and accurate for directing clinical diagnosis and treatment. Comparative studies of the anatomic landmarks between different races can be estimated non-invasively and precisely by the CBCT images data bank.
Acknowledgements The authors express thanks to Mr. Ming-Shun Lin for his excellent support of computerized tomographic techniques.

R9

Abstract withdrawn

R10

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A survey among European Dental Schools on the removal of smear layer prior to the filling of the root canal system

Aim To collect the views of the chair of the Department of Endodontics in European Dental Schools and to assess whether the removal of smear layer is taught at the undergraduate and postgraduate level.
Methodology A questionnaire was mailed electronically to 65 endodontic Departments of European Dental Schools. One question concerned the personal conviction of the department chair that the smear layer should or should not be removed. Two questions addressed whether the department taught removal of smear layer to their under- and postgraduate students. Two questions involved the recommended technique and the volume and concentration of the chemical agent used. Finally, they could indicate whether they removed smear layer only in certain situations, and if so explain their reasoning.
Results Of a total of 65 questionnaires, 47 were returned, achieving a 72.3% response rate. Thirty three respondents (70%) were convinced that smear layer should always be removed before root canal filling, whereas six (12%) used this procedure only in teeth with necrotic pulps. Nine respondents (19%) did not remove the smear layer. In 31 departments (66%) both under- and postgraduate students were taught to remove the smear layer, whereas in five departments (10.6%) this procedure was taught only to the postgraduate students. Disinfection of the root canal system was the main reason for smear layer removal. EDTA was the recommended chemical agent for smear layer removal followed by NaOCl (76%).
Conclusions European Endodontic departments favoured smear layer removal prior to root filling, mainly for aiding the disinfection of the root canal system. EDTA was the chemical agent of choice for this procedure.

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R14

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In vitro antimicrobial susceptibility of *Enterococcus faecalis* grown under nutrient-rich or nutrient-poor conditions

Aim To compare the antimicrobial susceptibility of *E. faecalis* grown on a nutritionally-poor or a nutritionally-rich medium.
Methodology *Enterococcus faecalis* was grown in defined media with increasing concentrations of horse serum (HS) to determine the threshold concentrations for 'nutrient-rich' and 'nutrient-poor' media and subsequently exposed to NaOCl, chlorhexidine and povidone-iodine. Additionally, biofilms of *E. faecalis* were generated and supplied with nutrient-rich or nutrient-poor media and their susceptibility to the antimicrobials determined. The biofilms were also assessed with culture, confocal laser scanning microscopy and fluorescence microscopy using a Live/Dead viability stain and electron microscopy.
Results Thresholds were established for nutrient-rich (10% HS) and nutrient-poor (0.1% HS). All antimicrobial agents achieved 100% bacterial kills when *E. faecalis* was grown in planktonic form. NaOCl eliminated *E. faecalis* biofilms grown under nutrient-rich conditions. Chlorhexidine achieved 66% and 0% bacterial killing after 1 minute exposure to 7 and 14-day nutrient-rich biofilms; whilst a 5 min exposure resulted in 95% and 79% reduction, respectively. There was no cultivable *E. faecalis* biofilm growth on nutrient-poor medium after 7 days; cultivable cells decreased rapidly between the first and second day of incubation but appeared viable using viability stains in conjunction with microscopy. The bacteria were cultivable again after switching from nutrient-poor to nutrient-rich medium.
Conclusions Nutrient medium had no effect on antimicrobial susceptibility of planktonically grown *E. faecalis*. NaOCl was the most effective agent tested. Biofilm cells were less susceptible to chlorhexidine than planktonic cells. *E. faecalis* biofilms grown under nutrient-poor conditions became viable but non cultivable (VBNC) after 7 days but could be resuscitated with a nutrient-rich medium.

R15

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The antimicrobial efficacy of tetracycline solutions and sodium hypochlorite on two isogenic *Enterococcus faecalis* strains

Aim To compare the antimicrobial susceptibility of isogenic *Enterococcus faecalis* strains (JH2-2 [tetracycline-sensitive] and T1 [tetracycline-resistant]) to three different irrigation solutions.
Methodology Fifty-four bovine root specimens were randomly allocated to three experimental (n = 16) and one positive-control (n = 6) group. Half of the roots in each group were inoculated with strain-JH2-2 and the remainder with strain-T1. After incubation (26 days), the specimens were irrigated with one of three test agents: (1) 2.5% sodium hypochlorite (NaOCl); (2) a mixture (3% tetracycline, 4.25% citric acid, 0.5% Tween 80); and (3) tetracycline solution (30 mg/mL). Following irrigation, dentine was filed from the superficial and deeper dentine in the canal, serially diluted, plated on 5% BHI agar plates, incubated aerobically at 37°C overnight and colony-forming units (CFU) counted. Analysis included Mann-Whitney tests and simple logistic regressions.
Results There was no significant difference between the two isogenic strains of *E. faecalis*. For both strains, the number of CFUs after irrigation with NaOCl in the superficial layer was significantly lower than after irrigation with tetracycline, whilst no significant difference was observed between NaOCl and the mixture. In the deeper layer, the CFUs after irrigation with NaOCl were significantly lower than after irrigation with either tetracycline-containing irrigant.
Conclusions The killing efficacy of NaOCl against both strains of *E. faecalis* was better than that of either tetracycline-containing irrigant in the deep dentine layer. In the superficial layer, NaOCl and the mixture performed equally well and better than tetracycline alone.

R16

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Effect of Octenidine and Chlorhexidine as intracanal medicaments on the viability of *Enterococcus faecalis*

Aim To determine the viability of *E. faecalis* in infected human root dentine in vitro after exposure to the experimental intracanal medicament Octenidine compared with Chlorhexidine.
Methodology Fifty root segments of extracted human teeth were infected with *Enterococcus faecalis* for eight weeks. Root dentine samples (rd) were collected after four and eight weeks in the control group (n = 10). For the test groups (n = 10 each), 'rd' samples were taken at weeks 4 (= baseline) and 12. One of the following medications was placed in the root canal at week 8: CH = calcium hydroxide paste; CHX-GEL = chlorhexidine gel (5.0%); CHX-GP = chlorhexidine containing gutta-percha points (active points®, Roeko, Germany); and OCT-GEL = octenidine gel (5.0%). Vital and dead bacteria were assessed by two fluorescent dyes (fluorescein diacetate/propidium iodide) to determine the portion of vital bacteria (PVB), as well as by culturing the 'rd' samples (CFU).
Results Viable as well as dead bacteria were detected in all 'rd' samples at baseline (week 4) and in the control group at week 8. OCT-GEL, CHX-GEL and CHX-GP resulted in a significantly lower PVB values at week 12 compared to CH. No bacterial growth was recorded in these 'rd' samples while CFU did not change after placement of CH. Overall OCT-GEL demonstrated the best values.
Conclusions The viability of *E. faecalis* was not affected by calcium hydroxide while chlorhexidine based dressings were more effective as intracanal medicament. Octenidine gel demonstrated the most favourable results and may be a promising alternative to chlorhexidine in the future.
Acknowledgements CAPES/MEC & CNPq/MCT/Brazil.

R17

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Ex vivo antibacterial activity of endodontic irrigant against four different microbial samples

Aim To compare the *ex vivo* antimicrobial activity of 2% Tetra-AcetylEthyleneDiamine + Sodium Perborate (TAED + P) against five different microorganisms frequently isolated from infected root canals with 5.25 % Sodium Hypochlorite (NaOCl).
Methodology To assess the antimicrobial action of the irrigant solutions, four microbial species were used: *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231). The antimicrobial efficacy of 2% TetraAcetylEthyleneDiamine + Sodium Perborate (TAED+P) (DuPont) and 5.25% sodium hypochlorite (Niclor 5 OGNA) was evaluated in presence of an organic substance (3 mg/100 mL chicken egg albumin solution) in order to simulate *in vivo* conditions. The irrigants were incubated with each microorganism and their efficacy was tested by using four different concentrations: 1 : 1, 1 : 2, 1 : 4, 1 : 8. Microbial samples were taken after 2 min and 5 min. The microbiological samples were plated to count the colony-forming units. The residual turbidity test was also performed to observe the microbial growth of *E. faecalis* samples after 12h of incubation with each irrigant solution. Data were statistically analysed by using ANOVA test at 5% significance level.
Results There was no statistical significant difference between the two irrigants after 2 and 5 minutes of incubation with each microbial sample. No residual turbidity was observed after 12h of incubation with *E. faecalis* with each irrigant solution.
Conclusions Even though all tested irrigants possessed antibacterial activity against the microbial samples tested, the time required to eliminate microbial samples did not depend on the concentration and type of irrigant used.

R18

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Efficacy of calcium hydroxide mixed with other antiseptics in elimination of *Enterococcus faecalis*

Aim To compare the efficacy of calcium hydroxide alone and mixed with 2% chlorhexidine (CHX) or camphorated paramonochlorophenol (CPMC) against *E.faecalis*.
Methodology The roots of 72 bovine anterior teeth were prepared to obtain dentinal blocks of equal size. The blocks were sterilized and ten were randomly chosen to confirm their sterility. The remaining blocks were inoculated with *E.faecalis*. Next the inoculated blocks were randomly divided into five groups, four groups of 13 and one group of ten blocks. The group of 10 blocks (group 0) was included to establish the number of CFU/mg of dentine in freshly inoculated teeth. Four groups of 13 blocks each were medicated with the following antiseptics: group 1: calcium hydroxide, group 2: calcium hydroxide + 2% CHX, group 3: calcium hydroxide + CPMC, group 4: sterile saline (control group). After medication, each specimen was sealed with sticky wax. After a one-week incubation (humidity, 37°C), the dentinal shavings from each block were collected, weighed and cultured on Petri discs containing selective agar. After a 24h incubation, the CFU/mg was estimated. The extent of viable bacteria among the groups was compared using the ANOVA Kruskal Wallis and Mann–Whitney U test ($P < 0.001$).
Results The average CFU/mg in groups 0, 1, 2, 3 and 4 were 300000.0, 5692.3, 0.7, 1.1, 90000.0, respectively. No statistically significant differences were observed between groups 2 and 3.
Conclusions Calcium hydroxide alone is not effective against *E.faecalis*. Both $Ca(OH)_2$: 2% CHX and $Ca(OH)_2$: CPMC pastes are equally effective against *E.faecalis* in this laboratory experimental model.
Acknowledgements This study was supported by a research grant from the Medical University of Lodz No 502-12-612.

R19

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Photodynamic therapy with methylene blue in root canals infected by *Enterococcus faecalis*

Aim To compare the disinfection of dentine caused using photodynamic therapy with methylene blue in two different formulations.

Methodology Twenty bovine teeth were sectioned and roots were autoclaved and incubated for two weeks with a suspension of *E. faecalis*. The specimens were randomly divided into two groups (n = 10): G1, root filled with 10 mM methylene blue dissolved in water, and then irradiated with a 660 nm diode laser with an output power of 100 mW, for 2 min; G2, root canal filled with 10 mM methylene blue dissolved in a mixture of glycerol : ethanol : water (30 : 20 : 50), and then irradiated by 660 nm diode laser with an output power of 100 mW, per 2 min. After those procedures, the dye was removed and microbial samples were collected from within the root canal. 25 μL, in triplicate, for each sample was seeded on a Petri dish with selective medium for *E.faecalis* in order to count colony-forming units (CFUs). Statistical analysis was performed (Mann Whitney test).
Results The mean CFUs were: G1 = 5.13 × 105 CFU/mL and G2 = 14.31 × 105 CFU/mL. No significant difference between the groups was detected ($P > 0.05$).
Conclusions Photodynamic therapy with methylene blue in the two formulations achieved the same degree of disinfection in root canals infected by *E. faecalis*.

R20

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Antimicrobial susceptibility of endodontic pathogens isolated from Lithuanian patients with symptomatic apical periodontitis

Aim To identify the dominating endo-pathogens, and to evaluate their susceptibility to commonly prescribed antibiotics, in patients with symptomatic apical periodontitis.
Methodology Fifty eight patients diagnosed with symptomatic apical periodontitis were examined. Of those, 47 cases had primary infections (necrotic pulp) and 11 cases had secondary infections (failed root canal treatment). The microbial samples were taken either from the root canals or, by aspiration from apical abscesses. Identification of the microorganisms was undertaken using culture methods. Susceptibility of all isolated microorganisms to antibiotics was evaluated using the E-test method. Minimal inhibitory concentrations (MIC) were determined and susceptibility was defined according to NCCLS standards. Statistical analysis was achieved using non parametric chi-square test.
Results A total of 64 cultivable isolates were recovered belonging to 25 different bacterial species, including facultative and obligate anaerobes. *E. faecalis* dominated in cases of secondary infection. Streptococci and obligate anaerobes were predominant microorganisms in cases of primary infection ($P < 0.001$). All tested microorganisms were sensitive to Penicillin G, Amoxicillin, Ampicillin, Cefotaxime. The percentage of bacteria susceptible to Clindamycin and Erythromycin was 67.2% and 58.8% respectively. Tetracycline had high level of resistance (41.2%) among all tested isolates. 55.6% of all anaerobes were resistant to metronidazole. *E. faecalis* was 100% resistant to Clindamycin and had low susceptibility rate to Tetracycline and Erythromycin.
Conclusions Penicillin and Amoxicillin are suitable for first choice antimicrobial therapy in cases of infection of endodontic origin. Clindamycin could be advised for Penicillin-allergic patients in primary endodontic treatment, however, it is not effective in cases of secondary infections. Based on the results, Tetracycline and Erythromycine should not be prescribed empirically for treating patients with symptomatic apical periodontitis.

R21

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Antimicrobial effect of Cirtus aurantifolia extract on selected endodontic pathogens

Aim To compare the antimicrobial activity of an aqueous extract of Citrus aurantifolia, a plant-derived extract, with 6% sodium hypochlorite (NaOCl) *in vitro*.
Methodology Microbial strains of *Enterococcus faecalis* (ATCC 29212), *Porphyromonas gingivalis* (ATCC 33277), *Fusobacterium nucleatum* (ATCC 25586), *Actinomyces naeslundii* (ATCC 49340), and *Candida albicans* (ATCC 10231) were used. The antimicrobial effect of test solutions was evaluated as follows: (1) paper disks were soaked with test solutions and zones of inhibition were measured using the agar diffusion test, and (2) antimicrobial activity of test solutions was also evaluated at different time intervals: 5, 10, 20, 30 minutes, and 1h. All assays were repeated five times. The data were analyzed using one-way ANOVA and repeated measures ANOVA.
Results Citrus aurantifolia extract showed smaller zones of inhibition than 6% NaOCl, but the differences were not statistically significant ($P > 0.05$). Both test solutions were able to kill all test microorganisms after a contact period of 5 min.
Conclusions Under the conditions of this study, aqueous extract of Citrus aurantifolia had a comparable antimicrobial effect to that of 6% NaOCl on selected endodontic pathogens.

R22

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A new potential antimicrobial agent for endodontic use

Aim To test the efficacy of HICA against endodontic pathogenic microbes.
Methodology HICA is a novel alpha-hydroxy acid, *D,L*-2-hydroxy-4-methyl valeric acid, the efficacy of which was tested against six bacteria and one yeast. The test concentrations of HICA-solution were 0.5%, 1%, 2% and 4%. Chlorhexidine digluconate 0.2% was used as the positive control and thioglycolate broth as the negative control. The test procedure was a modification of the method described in the CLSI standard M11-A5 for susceptibility testing of anaerobes. Colonies of reference and clinical isolates (n = 14: *Enterococcus faecalis*, Streptococcus viridans sp., *Staphylococcus aureus*, Lactobacillus sp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*) were resuspended in enriched thioglycolate broth medium (pH 5.2) and diluted to a concentration of

1x10E5 cfu/mL for test use. Test organisms were cultured with reagents in 96-well microtiter plates at 37°C for 48h in 5% CO_2. The growth was determined by a change in turbidity at 18h, 24h, 36h and 48h. At 48h 20 µl of suspension from microtitre plate wells were cultured on Brucella blood agar as a viability test. Each test was done with five replicates and repeated.

Results The growth of all bacterial and candidal isolates was inhibited by HICA in a dose dependent manner. The inhibition of both isolates of *P. aerunginosa* was time dependent. MIC90 at 48h was 2% for all bacteria and 4% for *C. albicans.* MIC100 at 48h varied from 0.5–2% for all organisms apart from lactobacilli and *C. albicans* (4%).

Conclusions Calcium hydroxide and disinfectants used in endodontics today have limited antimicrobial effect in mixed root canal infections. There is a clear need for new antimicrobial agents. HICA is a broad-spectrum bacteriocidal and fungicidal agent which may be useful in endodontics.

R23

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The synergistic effect of root canal irrigant combinations on bacterial biofilms

Aim To investigate the disruptive and killing effects of root canal irrigant combinations on single-species and microcosm bacterial biofilms of different maturity.

Methodology Single-species biofilms of *Streptococcus sanguinis* and *Enterococcus faecalis* were grown on nitro-cellulose membranes placed on agar plates. Microcosm biofilms (5, 14 days old) were grown on hydroxyapatite discs in a Constant Depth Film Fermentor. All biofilms were tested against NaOCl, Smear Clear, Corsodyl, Videne or CTAB used singly, in combination or sequentially in pairs (NaOCl with Corsodyl or Smear Clear). Outcome measures evaluated were the total number of cells recovered, the subset that was disrupted from the biofilm, and those that remained attached to the biofilm. For each measure, the numbers of live and dead bacteria were determined by using a viability stain in conjunction with fluorescent microscopy.

Results All agents and their combinations exerted some effect on cell killing and biofilm disruption but the extent varied by combination of agent and biofilm (complexity; maturity). Some combinations (Smear Clear/Videne and CTAB/Videne) formed a precipitate and were not tested further. The 14 days old biofilm was more difficult to disrupt than the 5 days biofilm. Combining irrigants did not provide a synergistic effect, but for some combinations the sequence made a difference (NaOCl/Smear Clear) to the total number of cells recovered.

Conclusions The test agents had different effects on disruption and killing dependent on the biofilm complexity and maturity. No combination showed superior disruption or killing ability than NaOCl. The sequence of irrigant used affected the total number of cells recovered but neither the combination nor the sequence affected the disruption or killing ability.

R24

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In vitro evaluation of the antimicrobial activity of nine root canal sealers: direct contact test

Aim To analyze the antimicrobial activity of root canal sealers.

Methodology Nine root canal sealers (four resin-based sealers, three zinc oxide- eugenol based (ZOE) sealers, and two calcium hydroxide based sealers) and three microbial strains (*Staphylococcus aureus, Candida albicans* and *Enterococcus faecalis*) were used. The antimicrobial activity of root canal sealers was tested by using the direct contact test based on turbidometric determination of microbial growth at three time intervals. For each set of test wells a positive control of uncoated wells containing identical size inocula were prepared, while another set of wells containing test materials but without bacterial inocula served as a negative control. Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS). Tests of differences were analyzed by *t*-tests and a value of $P \leq 0.05$ was statistically significant.

Results *Staphylococcus aureus*: All sealers showed significant differences when freshly mixed compared with its control except Endorez and Sealapex. Sealite regular and Cortisomol showed significant differences at all time intervals. Endo-REZ and Sealapex showed no significant differences at all time intervals. *Candida albicans* (90028): All sealers showed significant differences when freshly mixed. Only the 48h and the one week preparations of Sealapex showed significant differences. The 48h preparations of Topseal and AH plus showed significant differences. The ZOE based sealers showed significant differences at all time intervals. *Candida albicans* (10231): All sealers showed significant differences when freshly mixed except the two calcium hydroxide based sealers which showed no significant differences at all time intervals. AH plus and ZOE based sealers showed significant differences at all time intervals. *Enterococcus faecalis*: Topseal, AH plus, AH 26, Sealite regular and Acroseal showed significant differences when freshly mixed. The 48h and the week preparations of all root canal sealers showed no significant differences.

Conclusions Antimicrobial activity of the tested sealers depends on the time interval between mixing and testing. Most selaers exhibited antimicrobial activity when freshly mixed that was lost over time. All sealers were different from each other in their antimicrobial activity depending on the types and microbial strains.

R25

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Identification of cultivable microflora of refractory endodontic lesions

Aim To investigate the predominant cultivable microflora of refractory lesions with and without periapical abscess formation.

Methodology Refractory endodontic lesions ($n = 7$) were sampled using paper points and endodontics files to maximize microbial recovery and samples were plated onto a range of selective and non-selective media [Fastidious Anaerobic agar supplemeted with 5% [v/v] horse blood incubated anaerobically and aerobically, MacConkey agar, Veillonella agar, MTPY (for Bifidobacteria), TYC (for streptococci), Rogosa agar (for Lactobacilli) and CHROM agar (for yeasts)]. After incubation colonies were counted and random colonies were subcultured for identification. Primarily, PCR-based 16S rRNA gene sequence analysis using various combinations of universal primers [9F, 27F, 341F, 907r and 1492r] were used. To identify *Lactobacilli* spp. and *Veillonella* spp. partial sequences of *pheS* and *rpoB*, respectively, were amplified and sequenced using genus-specific primers. The identification of the organism was determined by BLAST searching the NCBI database or by interrogation of the Human Oral Microbiome Database [http://www.homd.org/].

Results All refractory lesions yielded bacteria and from the seven lesions 44 bacterial taxa and *Candida albicans* were identified. Lactobacilli were isolated from one lesion, Bifidobacteria from one and yeasts from two lesions. The lesion with lactobacilli harboured the following species: *L. paracasei, L. rhamnosus, L. salivarius* and *L. fermentum* as determined using pheS sequencing. Actinomyces

was identified in four lesions and each harboured a different species which were *A. radicidentis, A. meyeri, A. massiliensis* and 'Actinomyces strain B27SC'. Streptococci, staphylococci and propionibacteria were common isolates recovered from the majority of lesions. *Enterococcus faecalis* were isolated from one lesion. No contamination of lesion samples was identified as control cultures were sterile and *P. acnes* isolates from peri-oral skin and lesions of the same individual were different.

Conclusions The present investigation of a small number of refractory endodontic lesions confirms that the microflora of refractory endodontic lesions is polymicrobial, dominated by Gram positive fermentative organisms but with no single causative agent identifiable.

R26

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Evidence of a new archaeal phylotype in endodontic infections

Aim To explore the diversity of methane-producing *Archaea* (methanogens) in root canals containing necrotic pulp tissues.

Methodology Terminal restriction fragment length polymorphism analysis (T-RFLP) based on the mcrA gene which encodes for the methyl-coenzyme-M reductase, specific to methanogens was applied to eight endodontic samples, in which archaeal DNA was previously detected. After total DNA extraction the *mcrA* gene types were PCR amplified using a reverse primer labelled with the fluorescence dye TAMRA. Resulting PCR-amplicons were digested separately with the restriction endonucleases AluI, MspI and HpyF31. Subsequently, electrophoresis was performed on an ABI-PrismTM-310 Genetic Analyzer (Applied Biosystems, USA) and terminal restriction fragments sized using GeneMapper software (Applied Biosystems, USA). PCR amplicons of *mcrA* and 16S rRNA genes were cloned, and the identities of the sequences confirmed by the Blast sequence databases. Phylogenetic tree reconstruction was performed using the ARB software package.

Results The T-RFLP analysis and comparative sequence analysis of the *mcrA* and 16S rRNA genes revealed *M. oralis* in the majority of samples but also the existence of a second methanogenic phylotype in the mixed endodontic infections. *M. smithii* was not detected.

Conclusions *M. oralis* was the dominating methanogenic species. An additional, as yet uncultured methanogenic species in dental root canals containing necrotic pulp tissues was identified.

Acknowledgements We acknowledge Prof Kishor Gulabivala for critically reading the abstract.

R27

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Fluorescence-in-situ-hybridisation for the detection of *Treponema denticola* in teeth associated with chronic apical periodontitis

Aim To refine a protocol for the detection of *Treponema denticola* using fluorescence-in-situ-hybridisation (FISH) on known test specimens and to examine the presence and localisation of *T. denticola* in teeth associated with apical periodontitis.

Methodology Resin-embedded pure cultures of *T. denticola* were used to optimise the FISH protocol. A universal probe (EUB338), and a probe specific for *T. denticola* (TDEN) were used for bacterial detection. The established protocol was then tested on plaque samples previously identified as *T. denticola*-positive by polyclonal antibody, and resin-embedded-demineralised sections from teeth with apical periodontitis previously confirmed to include spiral morphotypes. Sections from the apical, middle and coronal third of five randomly selected teeth with apical periodontitis were observed by light microscopy (LM). Subsequently, sections from each root segment underwent FISH, using EUB338 and TDEN, and the results were assessed by confocal-laser-scanning-microscopy (CLSM). Observations were systematically documented.

Results A protocol was successfully refined and established for FISH detection of *T. denticola* using pure cultures and plaque samples. Of the experimental teeth, *T. denticola* was detected in one out of five, in the coronal section only, by this protocol. The coronal and middle sections harboured microorganisms in all of the cases, amongst which, spiral morphotypes visualised in three out of five teeth, were not labelled by TDEN. There was no evidence of bacterial presence in the apical sections of two out of five teeth.

Conclusions *Treponema denticola* was successfully detected in a human tooth with apical periodontitis using FISH in combination with CLSM.

R28

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Classification and identification of endodontic pathogens using electronic-nose technology

Aim To classify the pure microorganism cultures which has been found as endodontic pathogens and mostly anaerobe using electronic nose technology and to make a preliminary study to investigate whether an electronic nose can be used for identifying the endodontic pathogens or not.

Methodology Odour data was acquired using an electronic-nose with 32 carbon polymer sensors, from pure cultures of seven microorganisms, which are typical causes of endodontic infections. There were four odour data samples for each of the seven microorganism types. Odour data was processed using different pre-processing and dimensions reduction methods and 18 different datasets obtained. These datasets were then classified into seven groups using discriminant analysis (DA) and performance of several subtypes of DA algorithm investigated, namely linear, Mahalanobis and quadratic. In the second phase of study there were five odour data samples for each of the seven microorganism types. Randomly selected microorganism from seven microorganisms was given to the investigator blindly to identify it.

Results A quadratic approach was found successful in classifying the seven microorganism types. However in the second phase of the study the investigator was unsuccessful in identifying the selected microorganism.

Conclusions The electronic nose equipment has not been found sufficient to identify the endodontic pathogens. To make rapid diagnosis of endodontic infections use of electronic nose technology should be studied further.

Acknowledgements The authors would like to thank Dr. Hatice Ozbilge for her help during the microbiological procedures.

R29

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EM-ISH of *Fusobacterium nucleatum* in human teeth with apical periodontitis

Aim To adapt and apply a protocol for electron microscopy *in situ* hybridisation (EM-ISH) to detect the presence of *Fusobacterium nucleatum* in human teeth with apical periodontitis.
Methodology Resin-embedded pure cultures of *Fusobacterium nucleatum* and a mixed culture of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* were used to adapt an EM-ISH protocol, utilising elements from several other microscopy protocols. 90 nm ultra-thin sections were hybridised with an oligonucleotide probe specific for *F. nucleatum* (JF3), attached to digoxygenin and visualised with 10 nm gold sheep anti-digoxygenin antibody. Sections were viewed in the transmission electron microscope (TEM), images recorded and image analysis used to optimise the EM-ISH detection. The optimised protocol was applied to human teeth, using roots from three teeth with apical periodontitis, processed for TEM, from a previous study (Rojekar *et al.* 2006). Ultra-thin sections were taken from the coronal, middle and apical regions, hybridised with JF3 and examined by TEM. Image analysis was used to determine signal:noise ratios, with bacterial cell morphology providing some confirmation of correct labelling.
Results A protocol for EM-ISH was successfully adapted for the detection of *F. nucleatum* using pure and mixed cultures. *F. nucleatum* was successfully detected using EM-ISH in two out of the three teeth at the apical and middle levels. The signal:noise ratio required further optimisation for human teeth but the main elements of the protocol were established.
Conclusions A protocol for EM-ISH using the JF3 oligonucleotide probe specific for *F. nucleatum* was successfully adapted and applied to human tooth samples with apical periodontitis.

R30

Abstract withdrawn

R31

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Expression of Toll-like receptor-2 in periapical lesions of endodontic origin

Aim To demonstrate and characterise the expression of TLR2 by various cells in refractory periapical lesions of endodontic origin and to test the hypothesis that TLR2 expression would be greater in symptomatic refractory periapical lesions than asymptomatic lesions.
Methodology Immunohistochemistry was used for the localisation of TLR2 in biopsy samples from 32 teeth with failed endodontic treatment; diagnosed clinically, radiographically and histologically as either asymptomatic chronic periapical granuloma (group 1, $n = 10$), symptomatic chronic periapical granuloma (group 2, $n = 10$), periapical cyst (group 3, $n = 10$) or periapical scar tissue (group 4, $n = 2$). Five 4 μm thick sections from different levels of each sample were processed in all the groups. Positive (inflamed gingival tissue) and negative (sections without primary antibodies) controls were included in all immunohistochemistry tests. The expression of TLR2 was quantified by counting of cells expressing TLR2 in a 1 mm^2 area of each section and statistical tests were applied to determine difference in the degree of TLR2 expression among the groups.
Results TLR2 was predominated expressed by mononuclear cells in refractory inflammatory periapical lesions (groups 1, 2 and 3) while there was no expression in periapical scar tissue (group 4). Expression of TLR2 was significantly higher ($P < 0.05$) in symptomatic refractory periapical lesions (group 2) than asymptomatic lesions (group 1).
Conclusions TLR2 expression is present in various refractory periapical lesions. Periapical lesions with acute exacerbation have higher microbiological activities and this was correlated by significantly higher expression of TLR2 in these lesions.

R32

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Investigation of cytokines in response to induced apical periodontitis – a pilot study

Aim To determine if a systemic cytokine expression was evident during chronic apical periodontitis.
Methodology Dental pulps of the maxillary molars in six male rats WistarOttawaKarlsburgW (four as control) without inflammatory conditions were exposed and infected to induce chronic apical periodontitis. Under anesthesia blood samples were drawn one week preoperatively, two (acute phase; first time period) and eight weeks (chronic phase; second time period) post infection. Relative gene expression was performed to measure values of gamma interferon (IFN-γ), interleukin-1, tumor necrosis factor (TNF-α), interleukin-10 (IL-10), and transforming growth factor (TGF-β. Data were figured as mean ± standard error of mean and, for analyses, transformed by logarithm. Statistical differences between control and infection group in change over time were tested by ANOVA.
Results An up-regulation over the first time period was observed for relative expressions of IFN-γ ($P = 0.001$), TGF-β ($P = 0.002$) with a down regulation in the second time period. IL-1 was increased in the first ($P = 0.001$) as well as in the second time period ($P = 0.001$). IL-10 and TNF-α were significantly increased over the second time period ($P = 0.014$).
Conclusions Under the condition of this study the data suggest that apical periodontitis in WOKW rats is associated with increased level of systemic inflammatory markers: all tested cytokines were significantly increased whether in the first and/or second time period.

R33

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Production of immunoregulatory cytokines in periapical lesions

Aim To detect production of TGF-beta and IL-10 by inflammatory cells in culture isolated from human periapical lesions.
Methodology The study included 31 samples of periapical lesions (symptomatic lesions: $n = 13$; asymptomatic lesions: $n = 15$). Based on the predominance of T cells or B cells the lesions were divided into T-type ($n = 15$) and B-type ($n = 12$), respectively. Cytokines were measured by specific ELISA kits, whereas pheno-

typic characteristics of infiltrating lymphocytes were determined by immunocytochemistry.
Results The levels of both cytokines were detectable in all samples and did not significantly differ between symptomatic versus asymptomatic lesions. In contrast to IL-10, inflammatory cells from B-type lesions produced significantly higher levels of TGF-beta, than T-type lesions. In spite of these differences, there were positive correlations between the levels of IL-10 and TGF-beta in asymptomatic and B-type lesions.
Conclusions Results suggest that immunoregulatory mechanisms are more operative in chronic asymptomatic lesions with the predominance of humoral immune response and are supportive of the hypothesis that such processes are characteristic of an advanced stage in the development and healing of periapical lesions.

R34

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Cryopreserved dental pulp stem progenitor cells from miniature pigs: a tool for regenerative endodontics

Aim The isolation, preservation and characterization of mesenchymal Dental Pulp Stem Progenitor cells (DPSC) in miniature pigs.
Methodology All animal and Stem Cell experiments were approved by the National Institute of Animal Care and the Ethic Committee of the Dental School of Aristotle University. Immature permanent incisor teeth and unerupted premolars at the early root forming stage of three, 6–8 month-old miniature pigs were extracted and a number of teeth were evaluated histologically. Mesenchymal Stem Progenitor Cells were isolated from Dental Pulp and Apical Papilla tissues. Pulp cell cultures, were performed via the enzyme digestion method. Cells were cryopreserved at -196°C. Flow cytometric analysis before and after the cryopreservation procedure was performed. The expression of cell surface antigen markers for mesenchymal stem cells STRO-1, CD-90, CD-105, and CD-146 was examined.
Results 9–15 $\times$ 10^6 cells were obtained from each tooth. Porcine pulp Stem Cells were positive for CD-90 (strong expression), CD-105 and CD-146 (slight expression) and negative for STRO-1. Similar results were obtained before and after the cryopreservation procedure. Immunohistochemical analysis showed the specific distribution pattern of these cells both in pulp and apical papilla tissues.
Conclusions The different potential of these cells needs to be further explored. Cryopreservation of pulp derived Stem Progenitor Cell population provides a useful tool to approach Stem-Cell based regeneration of the Dentine–Pulp Complex.

R35

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Cell membrane polarity of mature human odontoblasts

Aim To investigate the odontoblast plasma membrane polarity and the expression of tight junction (TJ) proteins in native mature human odontoblasts as well as odontoblasts cultured with and without TGF-β.
Methodology Influenza virus (A/WSN) hemaglutinin (HA) and vesiculostomatitis virus (VSV) G-protein budding model in mature human odontoblast organ culture was used to observe cell polarity. TJ proteins ZO-1, claudin (CL)-1, -4, -10 and -11 were examined with immunohistochemical staining (IHC). The effect of TGF-β1 on TJ protein gene expression in culture was studied with DNA microarray.
Results Confocal microscopy immunofluorescence and TEM demonstrated that odontoblast apical surface was towards pulp and basolateral plasma membrane was restricted to the dentinal tubuli, the polarization border being located at the odontoblast-predentine junction. In IHC stainings, strong ZO-1 expression was seen in odontoblast cell layer, with weaker staining for claudins (CL-1 = CL-11 > CL-10), with CL-4 being negative. In microarray analysis, TGF-β1 showed a tendency to reinstate the gene expression of selected TJ genes, in which the expression was absent in control cultures.
Conclusions Odontoblast polarization is based on histological appearance as columnar cell with polarized cytoplasmic organelles. However, little is known about the odontoblast plasma membrane organization. Several cells (e.g. epithelial cells, osteoblasts) that perform vectorial functions have asymmetric (polarized) distribution of plasma membrane molecules, tight junctions (TJs) being responsible for formation and maintenance of plasma membrane polarization. Mature human odontoblasts demonstrate clear plasma membrane polarity. ZO-1/claudin expression patterns indicate that odontoblast TJs may be weak, allowing control of odontoblast cell layer permeability. TGF β1-induced preservation of TJ gene expression profile indicates that TGF-β may control odontoblast cell layer integrity by controlling TJ protein expression.
Acknowledgements The study was supported by the Academy of Finland, the Finnish Dental Society Apollonia, and KEVO grants from the Oulu University Hospital.

R36

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Gene expression of alkaline phosphatase (ALPL) is not sufficient to characterise 'pulp derived cells' as 'odontoblast-like cells'

Aim To compare the gene expression profiles of odontoblasts with pulp derived cell cultures obtained from the same developing teeth.
Methodology Total RNA was isolated from the odontoblasts of three caries free impacted wisdom teeth of the same male donor (15 years old). Additionally, total RNA from the third passage of pulp derived cell cultures obtained from the same teeth and cultivated with DMEM supplemented with 8%(v/v) foetal calf serum and 1%(v/v) penicillin–streptomycin-solution (Invitrogen, Germany) in cell culture flasks (Corning, The Netherlands) at 37°C with 5% CO2. Quantitative RT-PCR of 84 genes relevant to biomineralisation was performed on an iCycler (BioRad, Germany) utilising RT2 Profiler PCR Arrays (SABioscience, USA). Relative gene expression levels were calculated according to the delta-delta Ct method. Normalisation was achieved by geometric averaging the data of three housekeeping genes.
Results There were significant differences in the gene expression between odontoblasts and the pulp derived cell cultures, especially in genes relevant to dentinogenesis, e.g. dentine sialophosphoprotein (DSPP) was upregulated in odontoblasts 380 000 fold, ameloblastin (AMBN) 91 000 fold, dentine matrix protein 1 (DMP1) 19 000 fold and amelogenin (AMELY) 4600 fold (all $P < 0.0001$; t-test). In contrast, alkaline phosphatase (ALPL) was

expressed in both groups, but the 1.3 fold 'upregulation' in odontoplasts was not significant ($P = 0.3325$; t-test).
Conclusions Alkaline phosphatase (ALPL) is not a suitable biomineralisation marker in dentinogenesis and its expression is not an appropriate proof of 'odontoblast-like cells'. Furthermore, an uncritical use of unauthenticated pulp derived cell cultures must be avoided in order to obtain valid results.

R37

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Proliferation and RNA amount of human pulp derived cells (hPDCs) and human bone marrow stem cells (hBMSCs)

Aim To compare the proliferation and RNA amount of primary hPDCs and hBMCSs on two different support materials.
Methodology Group 1: HPDCs were derived from two caries free impacted third molar teeth from a female donor, aged 21 years. The pulp tissue was cut in pieces of 1 mm^3 and pre-cultivated up to transfer phase (T) 2. Group 2: HBMSCs were derived from bone marrow of two female donors 18 and 19 years old. The cells were expanded up to T3. Both cells types were pre-cultivated and fed with DMEM supplemented with 8% (v/v) foetal calf serum and 1% (v/v) penicillin-streptomycin-solution (Invitrogen, Germany). In T3 respectively T4 they were seeded on 13 mm diameter glass sheets (Group 1: $n = 288$, Group 2: $n = 288$) (Menzel, Germany) and on mineralised collagen tapes (Group 1: $n = 288$, Group 2: $n = 288$). After three and after six weeks of cultivation, the cells were removed with 0.05% (v/v) Trypsin EDTA (Invitrogen, Germany). Analysis was performed by cell counting (Casy, Schärfe, Germany), and after RNA extraction (RNeasy, Qiagen, Germany) by determination of the RNA amount/1Mio cells (GeneQuant, Pharmacia, Germany).
Results Proliferation of hPDCs was slightly higher on both support materials than proliferation of hBMSCs on average at each time of assessment. RNA amount normalised to 1Mio cells was similar for both cell types. On glass the proliferation and the RNA amount was up to 10-fold higher than on tapes, in spite of normalising the RNA amount to 1Mio cells.
Conclusions The use of different support materials has a strong impact on cell proliferation and on their RNA-transcription. This must be taken in consideration before planning analysis of proliferation and gene expression in cells.
Acknowledgements Supported by the German Research Foundation (RE 1562/1, 1-3), priority programme SPP 1117.

R38

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Comparison of the biological effects of calcium and silicate cement and Mineral Trioxide Aggregate on MG63 cells

Aim To compare bone cell (MG63) biocompatibility and bone marker expression after calcium and silicate base cement (CS) and mineral trioxide aggregate (MTA) treatment.
Methodology MTA was mixed according to the manufactuers instructions (liquid / powder ratio of 0.3 mL/g); Calcium Silicate was also mixed with liquid / powder ratio of 0.3 mL/g The specimens (2 mm diameter × 1 mm height) were stored in an incubator at 100% humidity and 37°C for 1 day of hydration, then sterilized on ultraviolet light for 1h and placed in 96-well micro plate and cultured with cells. X-ray diffraction (XRD) was employed to identify material surface structure, and the tetrazolium bromide colorimetric assay (MTT) was used to evaluate cell viability. The relative mitogen activation protein kinase expression was compared with Western blot, and bone marker expression was evaluated with reverse transcriptase polymerization chain reaction (RT-PCR).
Results CS and MTA had similar chemical structures and biocompatibility with MG63 cells. CS and MTA cements allowed good MG63 cell proliferation when analysed using high phosphor extracellular signal-regulated kinase (p-ERK) expression levels. CS and MTA cements showed the evident type I collagen (Col), osteocalcin (OC), alkaline phosphatase (ALP), Bone sialoprotein (BSP) and osteopontin (OPN) expression.
Conclusions Both MTA and CS cements are biocompatible and appear to have osetoconduction effects on bone cells.

R39

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Genotoxicity of resin-based root canal sealers

Aim To evaluate the *in vitro* genotoxicity and cytotoxicity of two different resin-based root canal sealers.
Methodology The sealers tested were: Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA) and RealSeal (SybronEndo, USA). Each component of the material (Epiphany Primer, Epiphany Thinning Resin, Epiphany Sealant, RealSeal Primer, RealSeal Thinning Resin and RealSeal Root Canal Sealant), components in permutual combinations and all components mixed together were tested on human peripheral blood lymphocytes using the comet assay and ethidium bromide/acridine orange viability staining. Simultaneously, untreated negative control cultures were analysed in the same manner. DNA damage was evaluated following 4h treatment, and after 24h of repair period in the absence of any material's component.
Results After 4h treatment, except thinning resin, each tested component and different combinations of components induced a significant increase in DNA migration ability ($P < 0.05$). After 24h, combination of primer, thinning resin and sealant of both tested materials caused cell death inducing intense apoptosis. After the repair period, cells exposed to Epiphany Sealant and RealSeal Root Canal Sealant, both in polymerized and nonpolymerized form, exhibited a level of DNA damage that was similar to the control.
Conclusions Primer and thinning resin of both resin-based root canal sealers and their combinations caused substantial DNA damage mediated by their cytotoxicity, rather than genotoxicity.

R40

Abstract withdrawn

R41

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In vivo periapical tissue response from coronal leakage following root filling using different sealers and techniques

Aim To evaluate the periapical inflammatory responses resulting from coronal leakage in dogs' teeth root filled with one or other of three resin based sealers, AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany), RealSeal (SybronEndo Corp., Orange, CA, USA) and RealSeal SE (SybronEndo Corp., Orange, CA, USA); and two obturation techniques, lateral condensation (LC) and warm vertical condensation associated with the injection technique (CW).
Methodology Premolars, central and lateral incisors from five dogs received root canal treatment employing NiTi instruments and Endo PTC cream with 1% sodium hypochlorite. Working length was established at a distance of 1.0–1.5 mm short of the radiographic apex and root canals were filled according to their groups as follows: GI - AH Plus/lateral condensation, GII - AH Plus/ thermoplastic, GIII - RealSeal/lateral condensation, GIV - RealSeal/ thermoplastic, GV - RealSeal SE/lateral condensation, GVI - RealSeal SE/ thermoplastic, a positive control group and a negative control group. The coronal access was left unsealed, exposing the root filling, coronally, to the oral environment for a period of seventy-five days. Dogs were euthanized by anaesthetic overdose. Teeth were surgically removed and histologically processed with 6 μm serial sections and haematoxylin and eosin (HE) staining. Photomicrographs were obtained at various magnifications, digitalized and analyzed with respect to tissue response by three independent evaluators. Kruskal-Wallis and comparative Dunn test were used at =0.05.
Results The thermoplastic technique were associated with better periapical conditions than the lateral condensation technique ($P = 0.0055$). The condensation technique resulted in more intense inflammatory reactions when RealSeal SE was used ($P = 0.002$). RealSeal SE was less effective against leakage, thus producing greater levels of chronic inflammation irrespective of the obturation technique ($P = 0.0088$). No difference was observed between sealers ($P > 005$).
Conclusions The association of continuous wave of condensation and injection techniques showed better sealing capacity that resulted in fewer levels of periapical response. AH Plus and RealSeal presented similar behaviour.

Diagnosis, Root canal preparation, Surgery

R42

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Comparison of two systems for radiographic assessment of apical periodontitis

Aim To study and compare the performance of the Strindberg system and the Periapical Index (PAI).
Methodology Apical periodontitis is primarily assessed by evaluation of periapical radiographs. Two different systems have been used both in clinical trials and in epidemiological studies. The Strindberg system, based on case series, and PAI, based on a comparison histology and radiography. Four observers, two experienced and two inexperienced, evaluated the periapical conditions of 97 teeth using periapical radiographs. Each observer evaluated a tooth twice using PAI and twice using Strindberg's system. The observers were mutually blinded and the first evaluation was unavailable when the second evaluation was made. In the Strindberg system three scores were used: healthy (1), unsure (2), and diseased (3). PAI was considered both on the five-point scale and dichotomized as healthy (1,2) or diseased (3,4,5). Kappa statistics were used to describe intra- and inter-observer agreement. The proportion of negative/positive assessments by one system among teeth with a negative/positive assessment by the other system was used for comparison.
Results Intra-observer variation for Strindberg's system had an average kappa value of 0.70, inter-observer variation had an average kappa value of 0.49. For PAI the corresponding average kappa values were 0.60 and 0.27. When PAI was dichotomized (12/345), average kappa values were 0.76 and 0.56. On average 89% of the teeth with Strindberg score 1 was evaluated as healthy by PAI and on average 78% of teeth with PAI 1 or 2 were given Strindberg score 1. On average 83% of the teeth with Strindberg score 3 was evaluated as diseased by PAI and on average 86% of teeth evaluated as diseased by PAI had Strindberg score 3. In all situations the individual observers varied considerably.
Conclusions Intra- and inter-observer variation between the two systems are comparable. To prefer one system before the other should reflect the type of study and evaluation needed.

R43

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Use of ultrasound real-time examination and colour-doppler to evaluate initial response to root canal treatment

Aim To assess the possibility of monitoring the initial response to root canal treatment in periapical lesions, by using the echographic examination with the application of the colour-power-doppler to assess the changes in the vascularization around and within the lesion in response to the treatment.
Methodology Twelve patients aged between 18 and 70 and presenting with apical periodontitis diagnosed clinically and radiographically were selected. Patients were scheduled for a two visits root canal treatment: during the first appointment the canal/s were cleaned, shaped and medicated with calcium hydroxide; in the second appointment, one week apart, the canal/s were obturated. Besides routine radiographs, echographic examinations and CPD of each case were carried out as follows: (1) before treatment; (2) one week after instrumentation, disinfection, and application of intermediate medication; (3) four weeks after completion of treatment. All the exams were evaluated by two independent observers after obtaining a K value of 8.5. The results were reported in a chart.
Results All lesions presented a vascular supply before treatment. In nine cases a progressive reduction of the vascularization within and around the lesions was documented during the one week and the four weeks controls with the echography and the CPD. In two cases there was an increase of vascularization at the one week post treatment control and a decrease at four weeks. In one case the vascularization remained stable during both the controls.
Conclusions It appears that the use of echographic examination with CPD is suitable to evaluate the responses of the tissues to the administration of a specific treatment at different time intervals.

R44

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The ability of radiographs to determine root canal curvature

Aim To evaluate the ability of mesial and buccal radiographs to determine canal curvature.
Methodology Micro Computed Tomography (MCT) acquisitions with a voxel size of 38 μm were used to select 60 molar root canals having a curvature of more than 15°. The selected roots were fully developed and contained no S-shaped canals. The angle of each root canal was determined by tracing a line in the centre of the root canal. The angle of curvature was measured using the commonly used Schneider method. Subsequently, root canals were conventionally radiographed from the buccal and mesial aspects and the canal curvature on the radiographs was also measured using the Schneider method. The maximum curvature from both aspects (mesial or buccal) was recorded for each root canal and compared with that determined by the MCT.
Results In 48% of the canals the curvature in the MCT was more than that of the conventional radiographs. The difference was 5–10° in 23% of the canals [99% CI: 15;36] and 10–20° in 25% of the canals [99% CI: 16;37]. In the remaining 52% of the canals the angle of curvature on the radiographs was similar to that determined by MCT (±4°).
Conclusions Radiographs of the mesial and buccal aspects of the root are not adequate to determine the curvature of the root canals.

R45

Abstract withdrawn

R46

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Micro computed tomographic study of the mesiobuccal root canal morphology of the maxillary first molar

Aim To observe the mesiobuccal root canal morphology of the maxillary first molar using micro computed tomography.
Methodology Twenty mesiobuccal roots were resected at the furcation level from randomly selected extracted human maxillary first molar teeth. The roots were cleaned to remove adherent hard and soft tissues and disinfected. Each root was then scanned using a Sky Scan micro CT scanner (Sky Scan 1172 X-ray microtomograph, Antwerp, Belgium) at a slice thickness of 11.6 μm. From the shadow images, cross sectional images of the root were reconstructed (NRecon Version 1.4.0; SkyScan). A three dimensional image was made using image processing software (Amira 4.1.2 software, Mercury Computer Systems, Berlin, Germany). The images were analysed to record the number and configuration of the canals, the presence of accessory canals, connections between the canals, and number of canal orifices and foramina.
Results The majority of roots had complex root canal systems with a second mesio-buccal canal (MB2) present in 90% (n = 18) of the roots examined. The MB2 was a completely independent canal in only 10% (n = 2) of the roots. Inter-canal connections were observed in 55% (n = 11) of the roots. In the teeth examined, a single apical foramen was found in 15% (n = 3), two foramina were present in 2, 20% (n = 4) and three or more foramina were present in 65% (n = 11). In 50% (n = 10) of the teeth there were two orifices at the furcation level, 40% had one orifice and 10% had 3 orifices. Accessory canals were present in 85% of the roots. Only 60% of root canals could be classified using Weine's classification.
Conclusions Micro computed tomography offers an ability to examine root canal anatomy in fine detail and confirms that the morphology of the mesiobuccal root of the maxillary first molar teeth is complex and that present morphology classifications do not fully reflect the complexity.

R47

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Influence of surfactant/thickener on NaOCl-induced removal of applied collagen from canal walls during ultrasonic irrigation

Aim To test the efficacy of syringe or ultrasonic irrigation in the removal of an applied collagen layer from root canal walls using sodium hypochlorite with or without surfactant or thickener.
Methodology One hundred and eighty human, single-rooted extracted teeth were prepared to apical size 40 with an 0.08 taper. They were longitudinally split, into halves, their root canals were coated with a standard layer of stained Type 1 collagen, and then reassembled in a silicone matrix. They were then randomly divided into two groups: (1) static irrigation (n = 90); and (2) ultrasonic irrigation (n = 90). These were randomly subdivided into: (a) 2.5% sodium hypochlorite (n = 30); (b) 2.5% sodium hypochlorite with 0.2% surfactant (n=30); and (c) 2.5% sodium hypochlorite with 'thickener' (n = 30). Digital images were taken before and after standard irrigation protocols. The percentage of canal surface area of the apical 5 mm of root coated with remaining stained collagen, was quantified (ipWin4®). The data were analysed using paired t-tests and non-parametric K-sample tests.
Results Ultrasonic irrigation resulted in significantly ($P < 0.001$) less remaining stained collagen compared with static irrigation, regardless of irrigant used. The differences were: 22% (9%, 35%) for NaOCl alone; 50% (38%, 63%) for NaOCl with surfactant; and 39% (26%, 52%) for NaOCl with thickener. The addition of surfactant or thickener had no significant effect on static irrigation but significantly ($P < 0.001$) improved the efficacy of ultrasonic irrigation by 21% (8%, 34%) or 17% (4%, 30%), respectively.
Conclusions The addition of a surfactant or a thickener to sodium hypochlorite irrigant enhanced the efficacy of collagen removal only when ultrasonically activated.

R48

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Evaluation of smear layer removal after use of a canal brush: a SEM study

Aim To evaluate smear layer removal after use of a new canal brush (Coltene/Whaledent GmbH+Co. KG, Germany).
Methodology Thirty anterior teeth were randomly divided into three equal groups. Group I was instrumented with ProTaper rotary instruments (Dentsply Maillefer, Switzerland); following use of each instrument root canals were irrigated with 1 mL of 5.25% NaOCl and 1 mL of 5% EDTA. Group II was instrumented with ProTaper instruments. Following the use of each instrument, canals were irrigated with 1 mL of 5.25% NaOCl and 1 mL of 5% EDTA. At the end of instrumentation, canals were flushed with 5% EDTA, then the canal brush was used for 20 s with a final irrigation of 5.25% NaOCl. Group III was instrumented with the same instrument sequence and irrigated with only 5.25% NaOCl. Before the final irrigation, the canal brush was used for 20 s for each root canal. At the end of instrumentation, all teeth were sectioned buccolingually and examined by SEM at 1500× magnification in the apical, middle and coronal portions of the canals. Analyses of SEM images were performed by two independent evaluators in a blind manner using a 4-point scoring system. The scores were then compared and if a difference was found, the two evaluators jointly examined the sample with a different score, if they could not reach an agreement a third evaluator helped with the scoring.
Results There was no significant difference between group I and group II in the coronal and middle thirds. Group III showed the highest average scores in all three thirds of the canals. There was no significant difference between the scores in the apical part of three groups.
Conclusions Iirrigating with brushing was not significantly better than irrigating alone in removing the smear layer on the canal walls.

R49

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Efficiency of cleaning procedures to remove chlorhexidine-calcium hydroxide paste in root canal extensions

Aim To assess cleaning efficiency of five different cleaning techniques to remove artificially placed calcium hydroxide/chlorhexidine paste from simulated apical grooves and depressions within straight human root canals.
Methodology Seventy five straight human single-rooted teeth were instrumented to size 80 taper .02 (Mity Roto, Loser, Germany)

and assigned to five experimental groups (n = 15). The teeth were split longitudinally and grooves and depressions were prepared according to Lee *et al.* (2004) and packed with a 50 : 50 mixture of calcium hydroxide paste (Calxyl, Oco, Germany) and aqueous chlorhexidine (2%) under an operating microscope (Zeiss OPMI Pico, Zeiss, Germany). Teeth were re-assembled and additional paste and ActivPoints size 70 (Coltène/Whaledent, Germany) were placed. After one week medication was removed and five irrigation methods were performed (n = 15): group1: citric acid (40%) and NaOCl (5%) using syringe irrigation (Endoneedle Buquet, Vedefar, Belgium), group 2: citric acid and NaOCl, activated by CanalBrush (Coltène/Whaledent) using a sonic toothbrush (Sonicare, Philips, Germany), group 3: CanalBrush (Coltène/Whaledent) with permanent rotation, group 4: citric acid and NaOCl activated by CanalBrush (Coltène/Whaledent) used with the Endo-Eze AET handpiece (Ultradent, USA), group 5: citric acid and NaOCl ultrasonically activated by a NiTi tip (VDW, Germany) using Piezon Master 600 (EMS, Switzerland). Images of specimens were taken using a lightmicroscope (Zeiss Stemi SV6). Lateral extensions were evaluated for remaining dentinal debris using a 4-step scoring system (Lee *et al.* 2004).

Results All cleaning procedures left residues of medication in lateral extensions. No significant differences occurred between groups (Kruskal-Wallis test: $P > 0.05$). The highest amount of calcium hydroxide/chlorhexidine paste remained in grooves and depressions when canals were irrigated without activation. Coronal depressions were significantly cleaner than middle and apical depressions (Friedman test: $P = 0.04$). Sonicare/CanalBrush showed slightly higher cleaning efficiency compared with the other cleaning procedures.

Conclusions Within straight and wide root canals all tested cleaning methods were similar in terms of cleaning efficiency.

R50

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Efficiency of different irrigation systems in oval root canals: manual irrigation, ultrasonic irrigation and RinsEndoTM

Aim To compare the cleaning efficiency of irrigation with syringe, ultrasonics, and RinsEndoTM in oval root canals.

Methodology Fifty five extracted premolars with a single oval root canal (ratio of long to short canal diameter >2× and <4×) were embedded into a muffle system and separated into three 3 mm high segments. These segments were prepared in the muffle with the FlexMaster NiTi-System to size 40, .02 taper. After each instrument the root canal was rinsed with 5 mL 1% NaOCl manually (syringe tip ISO 30), with ultrasonic irrigation, or with RinsEndoTM. 10 mL EDTA and 10 mL 1% NaOCl were used for final irrigation. The three root segments were split longitudinally into two u-shaped halves, which were evaluated under a scanning electron microscope (200× and 1000× magnification) by a blinded endodontist for remaining debris and smear layer using a classification developed by Hülsmann *et al.* (1997). The influences of technique and localization on either score were studied by SAS (Version 9.1, SAS Institute) with a nonparametric test for longitudinal data (Brunner *et al.* 2002). The significance level was set to alpha =5% for all tests.

Results Ultrasonic irrigation removed debris significantly better from the root canal than all other techniques. RinsEndoTM showed significantly better results than manual irrigation. Looking at the smear layer RinsEndoTM achieved significantly better results than ultrasonic or manual irrigation, whereas ultrasonic irrigation was significantly better than manual irrigation. There were no significant differences for the removal of debris ($P = 0.1938$) and smear layer ($P = 0.2194$) in the different parts of the root canal (coronal, middle, apical). There were no interactions between irrigation technique and localisation ($P = 0.3372$).

Conclusions Irrigation with RinsEndoTM and ultrasonic devices cleaned oval root canals significantly better than manual irrigation, independent of localisation in the root canal.

R51

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Cleaning efficiency and canal straightening of curved canals instrumented with rotary NiTi instruments

Aim To assess cleaning efficiency and canal straightening after instrumentation of curved root canals with an amount of curvature between 25 and 50 degrees using different NiTi rotary instruments.

Methodology Forty severely curved root canals of human molars were selected for the study and randomly assigned to one of the following four groups (n = 10): group 1: BioRace; group 2: FlexMaster; group 3: Mtwo; group 4: ProFile. Following standard access root canals were explored and digital radiographs were taken to assess pre-operative canal curvature. Root canals were instrumented to size 40 .04 taper according to the manufacturers' instructions and instrumentation time was recorded. The root canals were only rinsed with distilled water in order to exclude an interference of irrigants on cleaning efficiency of the instruments. Radiographs were taken to assess amount of canal straightening. Following instrumentation the roots were dried and longitudinally cracked and specimens were prepared for SEM analysis. Each root half was photographed in the apical, middle, and coronal section of the root canal. SEM photos were scored for debris, smear layer and root canal surface according to Hülsmann *et al.* (1997). Statistics were computed using SPSS Win 14.0 (Kruskal-Wallis test, ANOVA, and Friedman test).

Results All instruments produced substantial amounts of smear layer. Debridement was effective even with water irrigation. Debris and smear layer scores decreased from coronal to apical (Friedman test: $P < 0.001$). Kruskal-Wallis test revealed significant differences for root canal surface in the middle canal levels with smoother canal surfaces after instrumentation with ProFile ($P = 0.015$). BioRace (0.11 degrees) and ProFile (0.61 degrees) instruments tended to straighten canals less when compared with Mtwo (1.78 degrees) and FlexMaster instruments (1.63 degrees) (ANOVA and Student-Newman-Keuls test: $P < 0.001$).

Conclusions In severely curved root canals NiTi rotary instruments sufficiently debrided root canals and produced consistent canal shapes with slight to moderate canal straightening.

R52

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Efficiency of a new dynamic root canal irrigation device in curved canals

Aim To investigate the efficiency of a new dynamic root canal irrigation device in curved root canals, and to compare it with conventional rinsing protocols.

Methodology After preparation of three moderately and three severely curved root canals, the roots were split longitudinally into

two halves. Three standardized holes were cut in one canal wall 2, 4 and 6 mm apart from the apex to simulate uninstrumented canal extensions. Canal walls and depressions were covered with artificially produced debris made of dentine and sodium hypochlorite (1%). The two root halves were reassembled and each root canal was irrigated according to four protocols: Group 1, syringe hand irrigation; Group 2, ultrasonic irrigation; Group 3, RinsEndo 1 introducing the needle in the cervical portion of the canal; and Group 4, RinsEndo 2 placing the tip of the needle at the canal orifice. Standardized images of the canal walls were taken, and the amount of debris left in the canal and in the depressions was microscopically evaluated by four independent dentists using a score range from 'A' (no debris left) to 'E'.

Results RinsEndo 1 most frequently obtained score A (12/24 = 50%) compared to RinsEndo 2 (7/24 = 28%) and ultrasonic irrigation (4/24 = 17%). RinsEndo 1 more effectively removed debris from the depressions than the other methods. Irrigation by hand syringe left either debris on the canal walls or in the depressions.

Conclusions RinsEndo is a powerful device to effectively remove debris from curved root canals. Ultrasonic irrigation is less effective, whereas hand irrigation is insufficient to clean the canals.

R53

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Effect of size and taper of the apical preparation, root canal curvature and diameter of needle tip on the insertion depth of irrigation needles

Aim To investigate the effect of size and taper of the apical preparation, root canal curvature and diameter of needle tip on the insertion depth of irrigation needles into root canals.

Methodology One hundred and four root canals from extracted human teeth were selected. They were divided into four groups according to the degree of canal curvature (0–5°; 6°–15°; 16°–25°; >25°). Root canals were enlarged apically to size 25, .06 taper. Both a conventional 25G and a 30G irrigation needle with a lateral opening were consecutively inserted until binding. The remaining distance (d) between the needle tip and the working length (WL) was recorded and related to the length of root canal. Next, root canals were further enlarged apically to size 40, .04 taper and the procedure of inserting the two different irrigation needle to determine 'd' was repeated. Mean values and 95%-confidence intervals served as basis for the statistical analysis of the four groups.

Results In curved canals (>6°), the needle did not reach WL in any case. 'd' decreased with decreasing root canal curvature and increasing apical preparation size regardless of the needle diameter. Using a 25 G needle resulted in 'd' values ranging from 2.2 mm to 5.0 mm, representing approximately 22% to 50% of the canals length. In contrast, the 30G needle could be introduced nearly to WL even in moderately curved canals (<26°), when the apical preparation size was size 40, .04 taper.

Conclusions When enlargement to an adequate apical width is assured, only a 30G irrigation needle allows delivering of the irrigant to the most apical region of a curved root canal, in contrast to a smaller apical preparation with a wider taper.

R54

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Comparative study of the demineralizing effects of several endodontic irrigants

Aim To compare the Ca^{++} removal capability of 17% EDTA (Sigma-Aldrich Co, St Louis, MO), 0.1% octenidine dihydrochloride (Octenisept, Schülke & Mayr, Norderstedt, Germany) and super oxidized water (SOW, Medilox, O&M Medikal, Turkey) from root canal dentine in 5, 10 and 15 minutes intervals.

Methodology Fifteen extracted human mandibular premolars were decoronated and roots were adjusted to 10 mm in length. The roots were bisected longitudinally and randomly distributed to each group (n = 10). All surfaces were isolated with nail varnish except the root canals. The samples were immersed in the test solutions for 5, 10 and 15 minutes and the extent of Ca^{++} released into the solutions was measured by flame photometry. Analysis of data was performed using Kruskal-Wallis and Friedman tests.

Results For all time intervals the extent of Ca^{++} removal from root dentine were significantly different between the test groups (Kruskal-Wallis, $P < 0.05$). 17% EDTA removed significantly more Ca^{++} than 0.1% octenidine dihydrochloride which was more than SOW within time (Friedman, $P < 0.05$). The extent of removal of Ca^{++} by 17% EDTA statistically ranked as follows; 15 min > 5 min = 10min.

Conclusions 17% EDTA showed maximum demineralizing effect on the root canal dentine at 5, 10 and 15 minutes intervals.

R55

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Qualitative and quantitative analysis of anolyte and catholyte solutions after electrochemical activation procedures

Aim To evaluate the main products of electrochemical activation that have been proposed as alternative root canal irrigants, for an observation period of 14 days, in standard storage conditions.

Methodology A STEL-10H-120-01(226) device (LET, Moscow, Russia) was used for the harvesting of acid anolyte, cathodic anolyte and alkaline catholyte solutions. Sterile saline (0.9% w/v NaCl) and an aqueous solution of tap water mixed with NaCl 2.5 g/L were the two initial salt solutions. An alkaline sodium hypochlorite solution (NaOCl) 2.5% w/v was chosen as a control group. Concentrations of available hypochlorite (ClO-), hydrogen peroxide (H_2O_2) and sodium hydroxide (NaOH) were estimated by iodometric titration, titration with a standard solution of potassium permanganate and acid-base titration, respectively. Volumetric and colorimetric alterations were recorded, visually, by two independent experienced observers, during observation periods of 0h, 24h, 48h, 7 days and 14 days. All measurements were repeated three times. Additionally, pH and oxidation-reduction potential values were determined. All solutions were stored in dark, in closed non-opaque polypropylene containers at room temperature.

Results After electrochemical activation, the available concentrations of ClO-, H_2O_2, NaOH molecules depended upon the strength of the initial salt solution. Cathodic anolyte solutions presented higher

values of available ClO- molecules compared to acid anolyte solutions, at all observation periods. 2.5% w/v NaOCl presented the highest values of available ClO- molecules overall solutions, at all observation periods. Available H_2O_2 was detected in very low concentrations. Oxidation-reduction potential and pH values remained stable during all observation periods.
Conclusions Anolyte and catholyte solutions exist in a metastable phase for 7 days and act as available sources of the same species that provide to sodium hypochlorite antimicrobial and solubilizing properties. Future studies should be conducted for the development of enhanced electrochemically activated solutions, in order to provide a more sufficient source of oxidants and detergent agents.

R56

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The effect of surface modifiers on dentine softening properties of EDTA, NaOCl and Chlorhexidine Gluconate

Aim To evaluate the effects of root canal irrigants with or without surface tension modifying agents on the microhardness of root canal dentine.
Methodology Fourty root halves were prepared by longitudinal dissection of the distal roots of 20 freshly extracted mandibular human third molar teeth. The specimens were embedded in autopolymerizing acrylic resin and the dentine surfaces were exposed by grinding and polishing. After obtaining flat exposed root canal dentine, microhardness values of untreated samples were recorded by a Vicker's tester (Zwick). The root halves were randomly assigned into five groups comprised of eight samples each and were treated with one of the irrigants: 17% EDTA (disodium ethylenedinitrilotetraacetate, Vista Dental WI, USA), 2% chlorhexidine gluconate (Klorhex, Drogsan, Turkey), 2% chlorhexidine gluconate with surface modifiers (CHX-Plus, Vista Dental, USA), 6% NaOCl (ACE, Procter & Gamble, Turkey) & 6% NaOCl with surface modifiers (Chlor-XTRA, Vista Dental) for 5 minutes. Following surface treatments, microhardness values of dentine were recorded at close proximity to the initial indentation areas. Control and test samples data were analyzed statistically by Wilcoxon Signed Ranks test ($P < 0.05$). Comparison of the experimental groups were performed by Kruskal-Wallis ($P < 0, 05$) and Manny–Whitney U test with Bonferroni adjustment ($P = 0.005$).
Results EDTA caused a significant decrease in the microhardness of root dentine ($P < 0.05$). No significant change in terms of microhardness were observed following NaOCl or chlorhexidine application with or without surfactant.
Conclusions NaOCl and Chlorhexidine gluconate solutions with or without surface modifiers did not cause a significant softening effect on root canal dentine.

R57

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Evaluation of decalcifing potency of two chelating agents in root canal preparations

Aim To compare the decalcifing potency of two chelating agents during root canal preparations.
Methodology Thirty extracted canines were divided in three groups for use of RC- Prep materials. Group A RC-Prep (Primer) made in USA, group B RC- Prep (RC-CTF) made in Iran, and group C as control. Root canals were prepared using standard method followed by irrigation with 5% sodium hypochloride. The teeth were then divided into two sections (mesial and distal) by a special cutting device. The amount of calcium on polished sections was measured using elemental analysis (electron beam probe system). The mean value data were analysed using one-way Analysis of Variance (ANOVA).
Results The mean values for the measured calcium were group A: 28713 ± 1129, group B: 39441 ± 680, and group C: 54458 ± 2024. Statistical analysis showed that the mean value for group A was significantly different from other groups. There was also significant difference in the mean values for group B and C. The results indicate that the decalcifying potency for RC-Prep (Primer, USA) is significantly higher (PO.001) than that of RC-Prep (RC-CTF).
Conclusions RC-Prep (Primer) decalcifying potency is significantly higher than RC-Prep (RC-CTF) during root canal preparation.

R58

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A systematic method to accurately register temperatures in root canals *in vivo*

Aim To elaborate a systematic method to accurately register temperatures in root canals *in vivo*.
Methodology A type T thermocouple microprobe: Copper-Constantan (IT-23, physitemp instruments inc, USA) was inserted into a needle (2G3 × 1″, 06 × 25 mm Nr 16, Terumo, USA) and fixed with self-curing acrylic (Palavit, Kulzer, Germany) on an endo plugger (Maillefer). Root canals were prepared with rotary NiTi instruments (Mtwo VDW, Germany) to a diameter of 0.30 mm, 6% taper. Subsequently the measuring device was inserted into the root canal in agreement with the patient. As a test NaOCl (1%) at room temperature was injected into the root canal and temperature was recorded. Temperature was recorded with a microprobe thermometer (BAT 12 Physitemp, USA).These measures were repeated three times in four different teeth in three different patients.
Results In the clinical situation of a prepared tooth, the measuring device could easily be inserted to 4 mm from the root apex. The different results obtained were reproducible and reliable. The use of the temperature measuring device was not a barrier to the endodontic treatment. Temperature of the irrigation solution evolved from 20°C to 36°C in less than 1 min. Temperature stabilised between 36.2°C and 36.8°C.
Conclusions This method was reliable to gather information about the temperature of irrigants inside root canals. Since it is said that NaOCl is more effective at higher temperatures, it could be useful to verify the clinical importance of NaOCl preheating protocols.

R59

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Efficacy of EDTA and NaOCl solutions on dentinal tubule erosion depending on time and concentration

Aim To determine the efficacy of EDTA depending on irrigation time and concentration of NaOCl on dentinal erosion.
Methodology Twenty-one extracted single-rooted human teeth were instrumented with step-back preparation and 1.25% NaOCl.

After removing the crowns and the apical two-third, the roots were split longitudinally and divided into six groups for subjecting to final irrigation as follows: group A and B were irrigated with 17% EDTA (5 mL) for 1 min, followed by 1.25% NaOCl (5 mL) for 5 min or 5% NaOCl (5 mL) for 5 min; group C and D, 17% EDTA (5 mL) for 5 min, followed by 1.25% NaOCl (5 mL) for 5 min or 5% NaOCl (5 mL) for 5 min; group E and F, 17% EDTA (5 mL) for 1 min or for 5 min. Photomicrographs of dentinal walls were produced using a scanning electron microscope (×2500) at cervical third. The dentinal tubule diameter were calculated by image analysis toolkit and data were statistically analyzed using Kruskal-Wallis and Dunn tests ($P < 0.05$).

Results When the root canals were irrigated with EDTA alone for 1 min (2.28 ± 0.12 μm) or 5 min (2.27 ± 0.17 μm), there was no significant differences. However, dentinal tubule diameters were measured to be significantly increased to 2.66 ± 0.21 μm, 2.57 ± 0.40 μm, 3.06 ± 0.40 μm and 2.91 ± 0.37 μm in groups A,B,C and D respectively ($P < 0.05$).

Conclusions The using of NaOCl as final irrigation increased dentinal erosion depending on the irrigation time of EDTA solution.

R60

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Temperature decrease of preheated NaOCl in root canals, an *in vivo* study

Aim To measure the rate of temperature decrease of preheated NaOCl after injection in root canals *in vivo*.

Methodology After obtaining consent from the patients, root canals of twelve teeth were prepared to size 0.06 taper. Syringes containing 3 mL of 1% NaOCl were preheated in a water bath using a baby bottle warmer (Avent, Philips, UK) and a cup. The heated NaOCl was injected into the root canals and the temperature was measured (IT-23 microprobe and Bat 12 Microprobe Thermometer Physitemp Instruments Inc, USA). Time was recorded from the end of injection until the temperature decreased below 37°C.

Results The mean temperature in the cup was 73.3°C. In the syringe the mean temperature was 68.3°C. The mean time to obtain stabilisation of the temperature below 37°C was 44 s.

Conclusions Previous *ex vivo* studies reported that preheated NaOCl had an increased effectiveness in terms of antimicrobial properties and tissue dissolution ability. This cannot be extrapoled to the clinical situation since in this study, temperature decrease was rapid.

R61

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Efficacy of ultrasonic versus laser activated irrigation to remove artificially placed dentine debris plugs

Aim To assess the efficacy of laser activated irrigation (LAI) with Er:YAG and Er,Cr:YSGG as compared to passive ultrasonic irrigation (PUI) at the irrigation time proposed for LAI (4× 5 s) and at the irrigation time as proposed by van der Sluis *et al.* (2007) for the intermittent flush technique (3× 20 s).

Methodology A split root model with an artificial root canal wall groove according to Lee *et al.* (2004) was used–root canals were prepared to an apical size 40 with ProFiles .06 taper. Five groups of fifteen straight canine root canals were evaluated i.e. Group 1: hand irrigation during 20 s with 2.5% NaOCl (CI); Group 2: PUI during 4× 5 s with the size 20 Irrisafe (Satelec Acteon group, Merignac, France) (PUI 1); Group 3: PUI during 3× 20 s with the Irrisafe (PUI 2); Group 4: LAI with the Er,Cr:YSGG laser and Z2 (200 μm) Endolase tip (Biolase, San Clemente, USA) at 75 mJ during 4× 5 s (LAI 1); Group 5: LAI with the Er:YAG laser (HoYa Versawave, Cortaboeuf, France) and a 200 μm endodontic fibre at 75 mJ during 4× 5 s (LAI 2). Before and after irrigation images from the groove were made. The quantity of dentine debris in the groove after the experimental protocols was evaluated using a scoring system.

Results Statistically significant differences ($P < 0.05$) were found between CI and all other groups, between PUI 1 and the other groups.

Conclusions Laser activated irrigation with erbium lasers (Er:YAG or Er,Cr:YSGG) during 20 s (4× 5 s) are as efficient as passive ultrasonic irrigation with the intermittent flush technique (3× 20 s).

R62

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Apically extruded debris using hand-held or rotary ProTaper instruments compared to conventional K-Files

Aim To compare the quantity of debris extruded apically between hand-held and rotary ProTaper instruments during chemomechanical preparation. A step-back technique, with push-pull motions using K-files was used as a control

Methodology Forty-five single-rooted human teeth were selected and divided into three groups of fifteen canals. Two groups were instrumented with either hand-held or rotary ProTaper instruments according to the manufacturer's instructions. The third group was instrumented with K-files using the step-back technique. Every effort was made to standardize the amount of irrigant used for each canal. Apically-extruded debris and irrigant was collected in pre-weighed vials. The mean weight of the dry extruded debris for each group was analysed statistically using the non-parametric Kruskal-Wallis ANOVA on the Ranks test, and the all pair-wise Student-Newman-Keuls comparison test.

Results All instrumentation techniques produced extruded debris and irrigant. The mean dry debris weight for the rotary ProTaper group was 0.97 mg (range: 0.1–3.1 mg), for the hand-held ProTaper group 1.41 mg (range: 0.1–3.7 mg) and for the control group 2.21 mg (range: 0.6–6.2 mg).The mean amount of extruded debris with the step-back technique was significantly higher than the two ProTaper systems ($P < 0.05$). No significant difference was observed between the hand-held and rotary ProTaper system, although the former showed a tendency for greater debris extrusion.

Conclusions The ProTaper instruments, regardless of their mode of use (rotary or hand-held) resulted in less debris extrusion compared to the conventional filing with K-files. No significant difference was observed between the two ProTaper systems.

R63

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Effects of ultrasonic and sonic systems on pulp tissue removal from simulated accessory canals and transportation of the curved main canal

Aim To compare ultrasonic and sonic systems used for intracanal sodium hypochlorite activation regarding their necrotic pulp tissue removal from simulated accessory canals and transportation of the curved main canal.
Methodology Transparent epoxy resin models (N = 50) were used. The simulated main canal had the dimensions of a size-D spreader and a 20° curvature according to Schneider. The two simulated accessory canals had a diameter of 0.2 mm and were placed at a 45° angle from the main canal in the apical third. They were filled with minced bovine pulp tissue immediately before the experiments. Sodium hypochlorite (2.5% NaOCl) was activated four times for 30 s with intermittent replenishing of the NaOCl. For the activation, the following systems were used (N = 10 models, each): ultrasonic hand-piece (EMS) with (i) straight stainless steel files (Endosonore), (ii) pre-bent stainless steel files, or (iii) nickel-titanium tips (ESI), and (iv) a sonic system (EndoActivator) with plastic tips. All tips were size 15. Passive placement of the irrigant in 10 models served as negative controls. Digital photographs were analysed for tissue removal and maximum canal transportation individually by two blinded observers. Data were compared between groups using one-way ANOVA/Bonferroni, alpha =0.05.
Results All ultrasonic tips under investigation caused the NaOCl solution to dissolve statistically similar amounts of tissue in the simulated accessory canals, whilst the sonic system showed no effect on tissue removal ($P \geq 0.05$ compared to negative controls). Canal transportation was significantly higher ($P < 0.05$) with straight stainless steel files than with nickel-titanium tips, whilst no transportation was caused by the sonic system.
Conclusions Pre-bent stainless steel files or nickel-titanium tips caused considerable tissue dissolution in simulated accessory canals and, compared to straight stainless steel files, limited transportation of the main canal. The sonic system under investigation showed no effects on the investigated outcomes.

R64

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Accuracy of apical root canal gauging assessed on μCT scans

Aim To assess the accuracy of apical root canal gauging by K-files after a crown-down procedure in maxillary molars using micro-computed tomography (μCT).
Methodology Twelve maxillary molars with fully developed roots and four separate root canals were selected based on initial μCT scans. Canals were accessed and then pre-flared with ProFile .04 instruments using a crown-down preparation to three quarters of estimated working length. Working length was electronically determined using a size 06 K-file. Progressively larger files were inserted passively to working length. The first binding file was termed the initial apical file (IAF). Subsequently, the apical third of the molars was scanned with the IAF in situ and then without. Cross-sectional areas of the instrument and the canal and the largest and smallest root canal diameters were measured 1 mm from the instrument tip. In addition, volumes of the apical 2 mm of the instrument and the canal were calculated. Values were grouped according to canal type (mesiobuccal, mesiopalatal, distobuccal, and palatal). Data were compared using one-way ANOVA/Bonferroni, alpha =0.05.
Results IAF ranged from size 8 to 30 and was lowest in mesiopalatal and highest in palatal canals. The mean canal area filled by the instruments 1 mm from the tip was below 40% in all canal types ($P > 0.05$ for overall comparison). The mean ratio (±SD) between largest and smallest canal diameter at that level was 3.0 ± 2.6 for mesiobuccal, 3.3 ± 2.6 for mesiopalatal, 4.1 ± 3.5 for distobuccal, and 1.6 ± 0.4 for palatal canals, indicating an irregularly shaped cross-section. The mean volume filled in the apical 2 mm was 39 ± 10%, 47 ± 20%, 38 ± 11% and 38 ± 16% for mesiobuccal, mesiopalatal, distobuccal and palatal canals, respectively ($P > 0.05$).
Conclusions Two- and three-dimensional analysis of μCT scans revealed that apical gauging cannot predict canal dimensions in maxillary molars, because canal shapes are irregular.

R65

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Efficacy of rotary Ni-Ti ProTaper instruments during root canal preparation of extracted teeth

Aim To evaluate the efficacy of nickel titanium ProTaper instruments to prepare the entire circumference of root canals in the apical, middle and coronal sections of different groups of teeth.
Methodology One hundred sixty five extracted teeth of various types were divided into eleven groups ($n = 15$). All root canals were prepared using ProTaper instruments to size F3 at working length. A modification of the Bramante muffle mould was used to examine the root canal before and after instrumentation at a level of 3, 6 and 9 mm from the apex. Images of the root cross-section before and after instrumentation were superimposed and digitally subtracted.
Results Canals in which the entire circumference was prepared in all sections were only found in mesial root canals of mandibular molars. Uninstrumented areas in all sections were found in most groups of teeth, except maxillary lateral incisors, in the buccal and palatal canals of first maxillary premolars, in the mesial and distal root canals of maxillary molars, and mesial root canals of mandibular molars.
Conclusions The preparation technique did not allow preparation of the entire canal circumference in most groups of teeth.
Acknowledgements We wish to acknowledge Mr. V. Karagiannis, Research Associate of School of Dentistry, for his assistance with the statistical analysis.

R66

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Comparison of root canal preparation using Alpha and Mtwo NiTi instruments

Aim To compare various parameters of root canal preparation using Alpha (Gebr, Brasseler GmbH, Lemgo, Germany) and Mtwo (VDW, Munich, Germany) rotary nickel-titanium instruments.
Methodology Fifty extracted mandibular molars with mesial root canal curvatures between 20° and 40° were embedded in a muffle system. All mesial root canals were prepared using either Mtwo instruments to size 40 in a single-length-technique or Alpha instruments to size 35 in a crown-down-sequence as recommended by the manufacturer. In both groups preparation was performed with Endo-IT-professional-motor (VDW, Munich, Germany), root

canals were irrigated with 2 mL NaOCl (3%) after each file. The following parameters were evaluated: straightening of root canal curvature, postoperative root canal diameters, cleaning ability, safety issues, and working time. Statistical analysis was performed using Mann-Whitney's U-test and Wilcoxon's test.

Results Both NiTi systems maintained curvature well; the mean degree of straightening was less than 1° (Alpha: mb 0.5°, mL 0.5°; Mtwo: mb 0.5°, mL 0.7°).The difference was not statistically significant. 85% (Alpha) and 87% (Mtwo) of the postoperative cross-sections were acceptable showing round and oval diameters, differences were not significant. Neither of the systems completely eliminated debris and smear layer, the results did not differ significantly between the systems. Concerning safety issues seven procedural incidents occurred with Alpha instruments (four fractures, three working length losses). Mtwo preparation resulted in five fractures and two working length losses. Mean working and irrigation time was slightly shorter for Alpha (mb 192 s, mL 130 s) than for Mtwo (mb 206 s, mL 147 s).

Conclusions Alpha and Mtwo Ni-Ti instruments respected original root canal curvature well. Concerning debris and smear layer none of the systems showed a completely satisfying cleaning ability. Sufficient experience in using the instruments should be present to reduce procedural errors to a minimum.

R67

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The evaluation of two rotary instrumentation techniques under 'operator related variables' standardized conditions

Aim To evaluate the enlargement characteristics of two Ni-Ti rotary instrument systems that use two different preparation techniques, in simulated root canals under 'operator related variables' standardized conditions.

Methodology Forty simulated canals in resin blocks were used. The pre-and postoperative images of the blocks were taken with a digital camera and a special appliance in which distance and angle between camera and specimen were fixed. Simulated canals were divided into two groups for Mtwo (VDW, Germany) to use with Single Length Technique and for ProTaper (Dentsply Maileffer, Switzerland) instrument systems with Crown-Down Technique. All preparations were made with a special computer-controlled device used in a previously published article to standardize the 'operator related variables'. The pre-and post-operative images of the canals were superimposed by using an image analyze program. Superimposed composite photographic images of canals were measured at 11 levels from the apical tip. The distances between the pre-and postoperative outer lines of the simulated root canals were measured at both the inner and outer sides of the curve. The amount of removed material and the symmetry of the preparation were evaluated. Statistical analyses were done with Mann-Whitney Test.

Results Significantly more material was removed by the Mtwo at nine levels, ProTaper at two ($P < 0.05$), and no significant difference was determined at 11 levels. The preparations made with Mtwo was more symmetrical at four levels and ProTaper at four ($P < 0.05$) and no significant difference was determined at three levels.

Conclusions Mtwo removed significantly more material than ProTaper at different levels of the curved root canals under controlled conditions of operator-related variables.

R68

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Efficacy of different preparation systems in enlarging the apical size of curved root canals: a micro computed tomography study

Aim To evaluate the efficacy of rotary and manual systems in increasing the apical preparation size from 30 to 40 in curved canals.

Methodology Thirty root canals with a minimum curvature of 20° were selected using preoperative Micro Computed Tomography (MCT) acquisitions with a voxel size of 38 μm. The maximum initial apical size was 20. Another two MCT scans were performed after preparation of the root canals to size 30 and 40. The preparation was performed using three techniques: NiTi Hand-Files, Mtwo (VDW, Munich, Germany) and ProTaper (Maillefer, Ballaigue, Switzerland). Preparation of the canals was performed by experienced operators and in a dental mannequin to simulate the clinical conditions. At a level of 1 mm short of the working length, the percentage of canal original-circumference touched by the instruments (PC) was measured from the post preparation MCT scans. The mean PC for each preparation system was calculated. Additionally, a pair-wise (for each canal) analysis for the preparation sizes 30 and 40 was performed.

Results After preparation of the canals to size 40, the mean PC for NiTi-Hand-Files (63%[95%CI: 55;73]) was statistically significantly higher than Mtwo (55%[95%CI: 43;59]) and ProTaper (53%[95%CI: 41;58]). The increase in PC after canal preparation from size 30 to 40 was higher in NiTi-Hand-Files (10%[95%CI: 5;15]) than in Mtwo 4%[95%CI: 1;5] and ProTaper 3%[95%CI: 1;5]).

Conclusions In curved root canals, NiTi-Hand-Files were more effective than Mtwo and ProTaper in enlarging the canal from size 30 to 40.

R69

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Shaping ability of hand instrumentation compared to ProTaper mechanical instrumentation in simulated curved root canals performed by a novice and experienced operator

Aim To compare the shaping ability of hand and mechanical instrumentation (K-Flexofiles vs. ProTaper, Dentsply/Maillefer, Switzerland) in severely curved simulated canals performed by a novice operator (student). To assess differences in the ProTaper preparation shape between a novice and experienced (endodontist) operator.

Methodology Thirty simulated canals with curvature of 40° were prepared using two different preparation methods: group A (novice) and C (experienced) with the ProTaper instrument set (apical preparation to F3) according to the recommendations of the manufacturer; group B with a crown-down step-back preparation with Gates Glidden and K-(Flexo)files (MAF size 30, step-back to size 50). Pre and post-operative pictures, recorded using a digital camera, were superimposed. Measurements were carried out at five different points:

canal orifice (O); half-way to the orifice in the straight section (HO); beginning of the curve (BC); apex of the curve (AC); endpoint (EP).
Results Outer and inner curve widths, total canal width and transportation of the central axis, were significantly lower in group A compared to group B at all points ($P < 0.05$), except for the outer curve width at HO, the inner curve width at BC, AC and EP, and transportation at O; Mean transportation and aberrations in group C were less than group A, but no statistical significant differences were found between both groups at any of the measuring points.
Conclusions Mechanical preparation with ProTaper resulted in a more uniform and centered preparation shape with less aberrations in comparison with hand instrumentation. ProTaper instrumentation performed by a novice and experienced operator showed no significant shaping differences.

R70

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Determination of the optimal apical preparation size in premolars

Aim To investigate the cross sections of the apical part of the root canal of premolars and subsequent recommendations for their optimal apical preparation size.
Methodology One hundred and forty root canals from extracted human premolars (86 maxillary, 54 mandibular) were selected. After preflaring, electronic working length (WL) was measured to identify where the apical preparation size (APS) is to be established. Subsequently, non-tapered, non-cutting special instruments (SI) were used to reach WL. The diameter of the largest special instrument width that reached the WL was defined as DSI. The roots were sectioned apically and the diameter of a potential rotary instrument was determined for each section, allowing a complete circumferential cutting of the root canal wall. The estimation of APS was related to DSI.
Results Enlarging single-rooted premolars to an individual APS of DSI +0.3 mm (6 file sizes), complete preparations of root canal walls resulted most apically in 72%, whereas an enlarged preparation of DSI +0.5 mm (10 file sizes) reached 84%. In two-rooted premolars, an APS of DSI +0.3 mm completely processed the root canal walls in only 55% (buccal canals) and 79% (palatal canals). Theoretically, when shaping these premolars to DSI +0.5 mm, complete apical preparations of root canal walls resulted in 81% in buccal and 96% in palatal canals, respectively.
Conclusions APS should be at least six sizes larger than the first apical file that binds apically. Commonly, it implies shaping of premolars to ISO 50.

R71

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Accuracy of three different apex locators considering different meter readings: an *ex vivo* study

Aim To compare the accuracy of three different apex locators (EAL) considering two different meter readings.
Methodology EALs used in this study were ES-02 and ES-03 (Artronic, Zagreb, Croatia) and ProPex (Dentsply Maillefer, Ballaigues, Switzerland). Root-canal lengths of 46 extracted human one-rooted teeth were determined visually with a size 15 file (actual length-AL). The teeth and labial connector were embedded in alginate and two sets of measurements were performed for each meter readings: '0.5 bar' and 'Apex' reading (0.0). The differences between the measured and control values were statistically tested by using the analysis of the direction of discrepancies, non-parametric Friedman, and Wilcoxon tests, as a post hoc.
Results The mean difference between AL and measured values at meter reading 'Apex' was 0.10 mm with Propex, 0.31 mm with ES-02 and 0.71 mm with ES-03. At meter reading '0.5 bar' that difference was 0.28 mm with ProPex, 0.41 mm with ES-02 and 0.87 mm with ES-03. Wilcoxon test showed that at meter reading 'Apex' ProPex is more accurate than ES-02 ($Z = 4.23$; $df = 2$; $P < 0.001$) and ES-03 ($Z = 5.08$; $df = 2$; $P < 0.001$). ES-02 is more accurate than ES-03 ($Z = 3.78$; $df = 2$; $P < 0.001$). At meter reading '0.5 bar' ES-02 and ProPex are equally accurate ($Z = 1.82$; $df = 2$; $P > 0.05$), and ES-03 is less accurate than other two AELs ($Z = 5.02$; $df = 2$; $P < 0.001$). Generally, Wilcoxon test showed that mean difference between meter readings 'Apex' and the actual length (M = 0.37 mm) were lower than the mean difference between the measurement '0.5 bar' and the actual length (M = 0.52 mm) ($Z = 3.94$; $P < 0.001$).
Conclusions ProPex was the more accurate EAL at meter reading 'Apex', compared with ES-02 and lastly ES-03. At meter reading '0.5 bar' ES-02 and ProPex were equally accurate, whereas ES-03 was the least accurate.

R72

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An *in vivo* comparison between two electronic apex locators: Dentaport ZX and Raypex 5

Aim To compare *in vivo* two different electronic apex locators: Dentaport Zx (J. Morita Corporation, Tokyo, Japan) and Raypex 5 (VDW, Munich, Germany).
Methodology Twenty single-rooted permanent teeth scheduled for extraction because of periodontal disease were selected from six adult patients (ranging from 56 to 67 years old) and were divided in two groups. Before the extraction, an access cavity was opened and the crown was cut to establish a stable reference point for all measurements. In 10 teeth the working length was determined with Dentaport Zx (group A) using the K-file with the largest diameter that reached the apex and the file was then fixed with a dual-curable flow resin composite. The procedure was repeated in the other 10 teeth using Raypex 5 as electronic apex locator (group B). The teeth were then extracted and rendered transparent. The distance between the tip of the file and the foramen was calculated on digital photographs in a mesio-distal view for each tooth. Positive values indicated measurements exceeding the foramen, negative values indicated measurements short of the foramen, 0 indicates the file flush to the apex. Means and standard deviations were calculated and data were analysed statistically using the *t*-test ($P < 0.05$).
Results The mean distance between the tip of the file and the foramen was 0.078 ± 0.388 mm for the Raypex 5 and -0.273 ± 0.401 for the Dentaport Zx. The t-test showed no significant difference between the two electronic apex locators ($P > 0.05$).
Conclusions This study confirms the accuracy of Raypex 5 and Dentaport Zx in working length determination. The differences between the two apex locators were not statistically significant.

R73

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Accuracy of three different apex locators: A clinical study

Aim To compare the accuracy of three different electronic apex locators (EALs) in patients treated for three different endodontic diagnoses.
Methodology Research was performed on 418 endodontic patients, aged 20–58. Initially, each tooth was diagnosed for pulpitis (P), being necrotic without (N) or necrotic with (AP) periapical pathosis, respectively. Working length (WL) was randomly determined with three EALs: ES-02 and ES-03 (Artonic, Zagreb, Croatia) and ProPex (Maillefer, Ballaigues, Switzerland). A total of 794 roots was analysed (245 roots P, 323 roots N, 126 roots AP). Following root filling the distance between the radiological apex and the end of the root-filling was assessed radiographically. Those roots where the fillings ended within 1.5 mm from the radiological apex were deemed as having an accurate WL. The data was statistically analyzed and non-parametric Kruskal-Wallis and Mann-Whitney were used as a post hoc test.
Results Kruskal-Wallis tests showed that all three EALs were, regardless of make or technical principle, equally accurate in all three diagnoses. They showed no differences in roots with P ($\chi2 = 2.18$; df = 2; $P > 0.05$), neither in roots with N ($\chi2 = 0.63$; df = 2; $P > 0.05$), nor in roots with AP ($\chi2 = 3.62$; df = 2; $P > 0.05$). However, diagnosis-related, Mann-Whitney tests showed that inaccurate WLs were significantly more frequent in roots with AP than in roots with N ($Z = 6.47$; $P < 0.05$) and roots with P ($Z = 8.60$; $P < 0.05$); inaccurate WLs were more frequent in roots with N than in roots with P ($Z = 3.30$; $P < 0.05$), for all three EALs.
Conclusions Regardless of make or technical principle, all three EALs are equally accurate; however, in relation to different diagnoses, they uniformly demonstrated the same degree of discrepancy.

R74

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Efficacy of the walking bleach technique on root filled teeth

Aim To evaluate the bleaching efficiency of the 'walking bleach' technique on root filled teeth in regard to the cause of tooth discoloration.
Methodology Bleaching of 40 discoloured root filled teeth was conducted. Based on the patient's dental history and clinical examination, discoloured teeth were allocated in four groups in respect to the cause of discoloration: trauma, pulp necrosis, endo-sealer and unknown. Walking bleach technique was performed by applying sodium perborate and 30% hydrogen peroxide mixture in a ratio of 2g of powder to 1 mL of liquid, changed weekly. Aesthetic results were evaluated before and after the treatment using the colour comparison with the standardized colour-coded key (Vita, Vita Zahnfabrik). Duration of the treatment and number of shades before and after bleaching were recorded. For statistical purposes, a numerical value was assigned to each tab (each tooth sample was assigned a corresponding number). Bleaching was considered to be successful when the tooth was of the same colour or lighter than adjacent teeth. Statistical analysis of variance (ANOVA) was performed to determine if a significant difference ($P < 0.05$) existed among the groups.
Results The shortest treatment time was observed in the trauma group (15.7 days). The longest treatment time was observed in the endo-sealer group (42.5 days). The greatest number of shades before and after bleaching was observed in the group caused by pulpal necrosis (9.7). The least number of shades was found in the endo-sealer group (4.5).
Conclusions Teeth with internal discoloration caused by root filling materials had poorer prognosis regarding bleaching success compared with teeth discoloured by trauma or pulp necrosis.

R75

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Effect of soft tissue massage on efficacy of incisive and mental nerve block

Aim To assess the effect of soft tissue massage on the efficacy of incisive and mental nerve block.
Methodology Thirty eight adult volunteers received incisive nerve blocks of 2.2 mL 2% lidocaine with 1 : 80 000 epinephrine over 60s on two separate occasions at least one week apart. At one visit the soft tissue at the injection site was massaged with a finger for 60s (active treatment). At the other visit the mandibular premolar teeth were massaged (control treatment). Order of treatments was randomised. An electric pulp tester was used to measure pulpal anaesthesia in mandibular first molar, first or second premolar and lateral incisor teeth at base-line and every two minutes for the first 10 min then every 5 min for 45 min following the injection. Pulpal anaesthesia was defined as no response to the maximal stimulation of 80 on the pulp tester on two consecutive tests. The assessor of pulpal anaesthesia was blinded to the type of massage used.
Results Over the 45 min of the trial the anaesthetic success following soft tissue and tooth massages was 52.6% and 42.1% respectively in lateral incisors; 89.5% and 86.8% respectively in premolars; 50.0% and 42.1% respectively in first molars. These differences were not significant ($\chi2 = 0.844$, 0.126 and 0.477 respectively). When the data for all teeth were combined it was noted that pulpal anaesthesia was more successful in the first ten minutes after injection following soft tissue massage compared to tooth massage ($\chi2 = 4.057$; $P = 0.044$).
Conclusions Digital soft tissue massage at the injection site had no significant influence on the efficacy of pulpal anaesthesia after incisive nerve block over a 45 min period. In the first ten minutes after injection soft tissue massage provided better pulpal anaesthesia compared to massage of the teeth.

R76

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Use of Corega® cream and OraSeal® Caulking to seal dental rubber dam, a clinical evaluation

Aim To investigate the sealing ability of Corega® cream (Stafford Miller, Ireland), also known as Poligrip Denture Adhesive in USA

instead of OraSeal® Caulking (Ultradent Products Inc. USA) when using dental rubber dam during an endodontic treatment.
Methodology A study was conducted with 40 patients who needed root canal treatment of a mandibular first molar. After placement of the rubber dam (Hygenic®, Coltène/Whaledent, USA) two groups of 20 patients were formed. In the first group Corega® cream was applied using a 3 mL syringe with White Mac® tip (Ultradent Products Inc., USA). After application, the Corega® cream was caulked with a cotton wad, submerged in a Hibitane® (SSL Healthcare, Belgium) solution. The same procedure was conducted with the second group using OraSeal® Caulking. During and at the end of the endodontic treatment the detection of saliva contamination was visually investigated by microscope (Optomic®, Spain). Patients were asked if a bad taste, due to the use of 3% sodium hypochloride, was observed.
Results The first group obtained a 85% tissue isolation, no bad taste was reported. The second group obtained a 80% tissue isolation with one bad taste noted.
Conclusions Although Corega® cream is not specifically designed to seal rubber dam, but is a denture adhesive, mainly composed of methylvinylether/maleic acid, sodium-calcium mixed partial salt, cellulose gum and paraffinum liquidum, it has a sealing ability as good as OraSeal® Caulking. A deficient seal was easily restored for both products.

R77

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Pulpal temperature rise induced by different light-activated bleaching techniques

Aim To evaluate the intrapulpal temperature rise induced by different light-activated bleaching techniques.
Methodology The coronal root portions of 108 extracted sound human mandibular insisors were sectioned approximately 2 mm below the cementoenamel junction. The root cavities of the crowns were enlarged to facilitate the correct placing of the thermocouple sensor in the pulp chamber. Teeth were divided into three equal groups as either two different bleaching agents containing heat-enhacing colorant was applied to the labial surface or no bleaching agent was applied. Light sources used were a conventional halogen (20 s), a light-emitting diode (LED) unit (20 s) and a diode laser (20 s). 12 specimens were used for each system and bleaching-agent or no bleaching agent combination. Intrapulpal temperature changes were recorded every 5 s during the procedure. The differences between the initial and the highest temperature readings for each specimen were taken and calculated temperature changes were averaged to determine the mean value in tempertaure rise. The values of tempertaure rise were compared using two way analysis of variance (ANOVA) at a preset α of 0.05.
Results Temperature rise varied significantly depending on light source ($P = 0.002$). The diode laser induced significantly higher temperature increases (23.10°C) than the other light sources (halogen and LED). There was no statistically significant difference between the temperature rise values of halogen (3.56°C) and LED (3.06°C) units ($P = 0.891$). There were no statistically significant difference between no bleaching agents and between the bleaching agents ($P = 0.347$).
Conclusions Within the limitations of this *ex vivo* study light activated bleaching with diode laser caused a higher intrapulpal temperature rise when compared to LED and halogen light sources.
Acknowledgements This research was supported by Gazi University, Scientific Projects Funding.

R78

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Surface roughness of resected root-ends using recommended burs; an operating microscope study

Aim To compare root ends resected using high-speed or low-speed surgical burs followed by a finishing bur.
Methodology Twenty canals in single rooted extracted teeth were prepared using ProTaper instruments to size F3. Canals were filled with white mineral trioxide aggregate (MTA) and orifices sealed with grey Cavit and glass ionomer cement. Radiographs verified filling quality. Ten teeth were resected using a low-speed tungsten carbide surgical bur (H33L, Komet, Brasseler, Germany) and ten with a high-speed tungsten carbide surgical bur (H162, Komet). Five specimens from each group were polished using an ultra-fine 30-fluted composite finishing bur (H135UF, Komet) (group 1: low-speed, no finishing; group 2: low-speed, finishing; group 3: high-speed, no finishing; group 4: high-speed, finishing). Root ends were examined by 11 examiners (three endodontists and eight post-graduate students) using an operating microscope at ×25. Roughness of dentine and MTA were scored on a scale of 0–3 from smooth to very rough.
Results Low-speed burs resulted in greater roughness of dentine than high-speed. The finishing bur greatly reduced surface roughness in group two and to a lesser extent in group four. The bur type had little influence on the roughness of the cut MTA, which in all cases was smoother than dentine.
Conclusions Use of high-speed surgical burs followed by a finishing bur produced the smoothest dentine and MTA surfaces on resected root ends.
Acknowledgements We wish to thank Komet and Dentsply for their assistance with the supply of instruments for this research.

R79

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Root-end cavity disinfection with chlorhexidine and laser

Aim To investigate the effect of chlorhexidine and an ER,CR:YSGG laser (Waterlase, BIOLASE Technology, St Clemente, CA, USA), respectively on *Enterococcus faecalis* within the root-end cavity and the adjacent dentinal tubules.
Methodology Thirty-nine extracted single rooted premolars were decoronated and prepared using a crown-down technique. Three millimetres of each apex was resected using diamond coated crown burs (Dentsply Maillefer, Ballaigues, Switzerland) and a root-end cavity was prepared in each root using ultrasonic retro-tips (Dentsply). Cementum was removed with 3M ESPE Sof-Lex Contouring & Polishing Discs (3M, St. Paul, Minnesota, USA) and the smear layer was removed with 17% EDTAC (Dentalife Pty Ltd, Croydon, VIC, Australia) and the roots were divided into three groups. Roots were placed in Brain Heart Infusion (Difco Laboratories, Detroit, MI, USA) and autoclaved. Group 1 received no bacterial killing. Group 2 was infected and had chlorhexidine irrigation of the root-end cavity and remained in contact with the chlorhexidine for a further 5 min and group 3 was infected and cavities treated with laser for 20 s at 1.5W. The roots were stained using LIVE/DEAD stain (Baclight Bacterial Viability Test, Invitro-

gen, Eugene, Oregon, USA), sectioned and these were examined under a confocal laser scanning microscopy (Axiovert 200, Carl Zeiss Ltd., Jena, Germany).
Results Laser-treated specimens featured a defined kill zone, while those treated with chlorhexidine produced a more diffuse pattern of kill.
Conclusions Chlorhexidine and the laser were able to kill bacteria within the dentinal tubules of the root-end cavity under these experimental conditions.
Acknowledgements We wish to thank Dentsply for providing instruments and materials for this research, Biolase for the laser and laser tips and Mr Andrew McNaughton for his guidance with confocal microscopy.

R80

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Infection at the root apex: a confocal microscope study of bacterial viability

Aim To assess using LIVE/DEAD stain (Baclight Bacterial Viability Test, Invitrogen, Eugene, Oregon, USA) and confocal microscopy whether *Enterococcus faecalis* is able to penetrate into the dentinal tubules in the apical 3 mm of root canals.
Methodology Twenty one extracted single-rooted intact premolars were decoronated and prepared using a crown-down technique. Cementum was removed semi-circumferentially using 3M™ ESPE™ Sof-Lex™ Contouring & Polishing Discs (3M, St. Paul, Minnesota, USA) and the smear layer of some roots was removed by treatment with 17% EDTAC (Dentalife Pty Ltd, Croydon, VIC, Australia). Roots were placed in Brain Heart Infusion (Difco Laboratories, Detroit, MI, USA) and autoclaved. Preparations were inoculated with *E. faecalis* V583 and incubated for ten days with medium replenishment every second day. Roots were stained, sectioned and examined under a confocal laser scanning microscopy (Axiovert 200, Carl Zeiss Ltd., Jena, Germany). Positive and negative controls were also examined.
Results *E. faecalis* invaded the entire length of dentinal tubules in the apical 3 mm of the root under the conditions of this experiment irrespective of the presence of smear layer and/or cementum.
Conclusions (1) Confocal microscopy and LIVE/DEAD stain is an excellent method to observe bacteria in situ within dentinal tubules. (2) The presence of smear layer and/or cementum did not prevent *E. faecalis* from invading the dentinal tubules in the apical 3 mm of the root.
Acknowledgements We wish to thank Dentsply for providing instruments and materials for this research and Mr Andrew McNaughton for his guidance with confocal microscopy.

R81

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Effectiveness of MTA root-end fillings following cavity lasing

Aim To examine the effect of Er,Cr:YSGG laser irradiation (Waterlase, BIOLASE Technology, Inc., San Clemente, CA, USA) on the seal of root-end cavities filled with MTA (ProRoot MTA, Dentsply Tulsa Dental, OK, USA).
Methodology Thirty palatal roots of maxillary teeth were resected and prepared using crown down technique with ProFiles (Dentsply) and filled with gutta-percha and sealer. Three millimetres of their apices were resected and root-end cavities prepared using ultrasonic retro-tips. The teeth were randomly divided into 3 groups of 10. Group 1 was root-end filled with grey MTA. Group 2 was treated with laser for 20 s at 1.5 W onto the root-end cavity and filled with MTA. Group 3 was treated with 17% EDTAC (Dentalife, VIC, Australia) for 5 min, treated with laser and filled as above. The teeth received three coats of nail varnish over the external surfaces except for their apical regions and were immersed in 0.2% methylene blue dye for 72h. The roots were split longitudinally. The maximum linear leakage was measured using a Nikon Measurescope (Nippon Kogaku, Tokyo, Japan) and the results analyzed using linear regression.
Results There was no statistical difference in leakage between Groups 1 and 2. Less leakage was found between Group 3 and the other two groups ($P = 0.007$).
Conclusions Used in conjunction with EDTAC the laser improved the apical seal of the MTA root-end fillings.
Acknowledgements This project was supported by a Dentsply Research Grant to A Patel. The company supplied instruments and materials for the work. We thank Associate Professor Peter Herbison for statistical advice.

R82

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Scanning electron microscopy study of root-end cavities following laser irradiation

Aim To observe morphological changes in root-end cavities after Er,Cr:YSGG laser treatment.
Methodology Thirty palatal roots of maxillary first molars were resected and prepared using a crown-down technique with ProFiles (Dentsply) and filled with gutta-percha and sealer. Three millimetres of the apices were resected and root-end cavities prepared using ultrasonic retro-tips. The teeth were randomly divided into three groups of 10. Group 1 (control) was split into two longitudinally. Side A was treated with 17% EDTAC (Dentalife, Victoria, Australia) for 5 min while side B had no intervention. Group 2 was treated with the laser (Waterlase, BIOLASE Technology, Inc., San Clemente, CA, USA) for 20 s at 1.5 W onto the root-end cavity. Group 3 was treated with 17% EDTAC for 5 min and treated with laser as above. Groups 2 and 3 were longitudinally split and the specimens examined using a scanning electron microscope (SEM). An image was taken at the cervical, middle and apical part of each cavity. The dentine was graded for debris and smear layer by a blinded operator and results analyzed using ordinal logistic regression.
Results There was no statistical difference amongst the different cavity levels within the three groups, or between groups 1 and 2. Group 3 (EDTAC + laser) was significantly different ($P < 0.05$) when compared to groups 1 and 2.
Conclusions The Er,Cr:YSGG laser in conjunction with EDTAC improved the removal of smear layer and debris.
Acknowledgements This project was supported by a Dentsply Research Grant. The company is thanked for supplying instruments and materials. We wish to thank Associate Professor Peter Herbison for statistical advice and Professor Mike Swain for comments on imaging.

Canal Filling, Materials Science

R83

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Forces applied during lateral condensation technique depending on different investigators and tapering of the root canal

Aim Practitioners with different levels of expertise were advised to fill root canals of varying taper. The forces applied during root filling with the lateral condensation technique were recorded.

Methodology Three groups of practitioners were chosen for this study: students, dentists, and endodontic specialists. Each of the three groups consisted of 12 test persons. Each person had to fill four prefabricated plastic root canals with tapers of 0.02, 0.04, 0.06 and apical size 30 (ProTaper F3) in the same order. The vertical forces, applied by the test persons during the filling procedure were measured by a force sensor. With an appropriate software tool these results were further analyzed.

Results The following results were obtained: 1. The 36 test persons applied mean maximum loads of 1.10–2.12 kg Experts used the largest loads with 2.12 kg, while students used 1.48 kg and practitioners 1.10 kg. Two. Average values of the applied loads ranged between 0.51 and 0.92 kg: the experts' average load was 0.92 kg, students used 0.65 kg, and practitioners 0.51 kg. In both groups of measurements the experts used larger loads during condensation procedures.

Conclusions A correlation of the practitioners level of expertise and the applied forces during the filling procedure was observed. In the literature, forces necessary to provoke vertical root fractures are described as being at minimum of approximately 7.2 kg (Lindauer, Holcomb). Although the loads applied during root filling vary among the test persons, none approached the value for provoking vertical fractures.

R84

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The effect of varying the elastic modulus on the stress induced during root canal obturation

Aim To illustrate the effect of the varying elastic modulus of root dentine, gutta-percha (GP) filling material, and the periodontal ligament (PDL) on the stresses induced during root canal filling using 3-D Finite Element tooth root models.

Methodology A model of a human maxillary central incisor root with supporting periodontal ligament and bone was constructed. After root canal preparation and embedding in epoxy resin, the root was serially sectioned at 0.5 mm intervals. The sections were imaged, magnified and digitized. The finite element software (MSC Nastran for Windows V 4.5) was used to import the digitized images. Construction curves for the modeling of gutta-percha, periodontal ligament and bone were carried out. Hexahedral elements built-up the solid 3-D finite element mesh. Appropriate constraints and standardized loading were applied. Analysis was based on linear, elastic, homogenous, and isotropic material behaviour under static loading. Changing the elastic modulus of model materials was carried out as one material at a time. The remaining materials values were kept as the original model.

Results Using warm GP with a low modulus induced higher dentinal stresses than cold GP having a higher modulus. The low dentine modulus induced lower stresses than the higher modulus values. The removal of smear layer using demineralizing agents and concomitant production of a low modulus demineralized dentine layer might aid in the reduction of stresses induced during obturation. The presence of opaque and translucent dentine with two different moduli did not affect the induced stresses. The presence of the PDL had a cushion effect in reducing the stresses induced in bone; however, the induced dentinal stresses were higher compared with the model with missing PDL.

Conclusions Utilizing the proper values of material properties in tooth modelling by Finite Element Analysis is important in order to predict more accurately the stress induced during root canal filling.

R85

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Sensitivity of dental films and high-definition volume-computerized tomography (fpVCT) in detecting remaining root filling material

Aim To compare dental films and volume-computerized tomography in detecting remaining root filling material.

Methodology Thirty-two root canals in extracted human molars were prepared and obturated. After root filling material was removed, the teeth were split longitudinally and the root halves were embedded in transparent epoxy resin with two reference elements. A conventional radiograph (Dentus M2, AGFA-Gevaert, Morstel, Belgium; Heliodent MD, Sirona Bensheim, Germany) and fpVCT scans (General Electric, New York, USA) were taken and digitized. Reconstruction software developed virtual pictures, which were superimposed using Adobe Photoshop. Microscopic investigation of the root halves served as control. Again photographs were taken and digitized. Detectability and extension of remaining filling material were evaluated. Statistical evaluation was performed using the Chi-square test.

Results Using the fpVCT as many remnants of root canal filling material were detected as radiographically with no significant difference between both techniques ($P = 0.18$). The sensitivity was 78% for the dental film and 70% for the fpVCT, respectively. The extension of the remaining filling material was significantly different for both techniques ($P = 0.001$). In only 42% of fpVCT pictures the dimension of remaining filling material was the same as in the control. Frequently the dimension was larger than in the control (27%) or smaller (20%). In dental films the dimension of the remaining filling material was the same as in the control (64%) or smaller (29%), and only infrequently larger (7%).

Conclusions The detectability of remaining root filling material with fpVCT after retreatment is not significantly superior than with dental films. fpVCT shows good results in 3-dimensional diagnostics, but has a high radiographic exposure rate and should only be used in difficult cases for 3-dimensional diagnostics.

R86

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Step-wise assessment of root canal filling and retreatment using high-resolution micro-computed tomography

Aim To assess relative volumes of sealer and gutta-percha in root canals of maxillary first premolars after root canal filling and sequential enlargement of the canal during retreatment.
Methodology Fifteen human maxillary premolars with one canal per root were used. Canals were prepared using ProFile instruments to size 35, .04 and subsequently filled with gutta-percha and AH-Plus sealer using the cold lateral compaction technique. Canals were then retreated using a size 35, .04 taper followed by a size 40, .04 taper and finally a size 45, .04 taper ProFile to working length. A sodium hypochlorite solution (1% NaOCl) was used as the sole irrigant. Teeth were scanned using high-resolution micro-computed tomography (μCT) initially and after each treatment step. Using previously defined and validated threshold levels, empty root canal volumes, root dentine, sealer and gutta-percha could be differentiated from and correlated to each other. Data were averaged between the two roots per tooth. Appropriate parametric and non-parametric tests were used to statistically compare the data, alpha = 0.05.
Results The root filling took 86.6 ± 4.7% of the prepared canal volume. The difference between prepared and filled canal volume was highly significant (paired *t*-test, $P < 0.001$). Removal of the root filling using the last ProFile used for canal preparation left a median of 2.7% (IQR = 4.1%) of the root filling volume in the canal. This volume was not significantly reduced by the subsequent enlargement steps (Kruskal-Wallis, $P = 0.344$). The median percentage of gutta-percha in the root filling before retreatment was 90.1% (IQR = 7.3%). The relative amounts of gutta-percha and sealer did not change during the retreatment with the three instruments (Kruskal-Wallis, $P = 0.944$).
Conclusions Under the conditions of the current study, a substantial part of the prepared root canal volume could not be filled. Retreatment of the filled root canal by the last rotary instrument used for canal preparation removed over 95% of the root filling volume with no further reduction by larger instruments. The gutta-percha to sealer ratio was not altered.

R87

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Technical quality of root fillings completed by undergraduate students using rotary versus hand preparation techniques

Aim To evaluate and compare technical quality of root fillings performed by undergraduate students in Riga Stradins University using rotary and hand preparation techniques.
Methodology All root filled teeth completed by 4th year undergraduate students during the period 2006–2008 using rotary instrumentation technique were included in the study. Teeth with readable postoperative radiographs that had received primary treatment were examined radiographically for quality. Comparison with previous evaluations during the periods 1996–1999 and 2003–2004 where hand preparation technique were used was carried out. A 'satisfactory' root filling was categorized when the filling completely obturated the root canal system and was within 0–2 mm of the radiographic apex, with no iatrogenic problems. The two examiners were calibrated for radiographic quality of root fillings. Intraexaminer agreement was measured by Cohen's kappa (k) values. The radiographs were read by one operator. The Fisher's exact test was used to determine difference between technical quality using rotary and hand preparation techniques ($P < 0.05$).
Results Cohen's kappa score for the intraexaminer agreement was k = 0.8. A total 192 root canals when rotary instrumentation was used were included in the study. Overall 50% (96) of the treated canals were categorized as satisfactory in terms of radiographic quality of fillings but 50% (95) cases the treatment was unsatisfactory, i.e. incomplete filling with visible iatrogenic problems. Results from previous evaluation revealed satisfactory result in 57% (550) cases but unsatisfactory in 43% (418) cases. Fisher's test demonstrated that the difference in obtained results was not statistically significant ($P = 0.0796$).
Conclusions Results did not show sufficient evidence that rotary instruments provided better technical quality of root fillings performed by undergraduate students in Riga Stradins University.

R88

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Low-vacuum scanning electron microscopy for micromorphological evaluation of adhesively bonded root canal dentine

Aim To characterize the micromorphology of the adhesive interface between root canal dentine (RCD)/adhesive-sealer (AS)/core-material (CM) using a new imaging method: low-vacuum scanning electron microscopy (LVSEM). Influence of chemo-mechanical modification of RCD and influence of root-canal geometry upon adhesive interface micromorphology to RCD were investigated on native specimens without demineralization/deproteinization.
Methodology Root canals in single-rooted teeth were instrumented and subjected to irrigation with (1) 5% NaOCl/10% citric acid and (2) 5% NaOCl/10% citric acid/5% NaOCl. An adhesive interface was created by bonding three adhesive obturation systems (Real-Seal-SybronEndo; EndoREZ-Ultradent; HybridRootSeal-Morita) and respective core materials (1) to root canal halves (open system) or (2) by obturating the chemo-mechanically instrumented canals with the respective materials (closed system). Micromorphology of the RCD/AS- and AS/CM-interfaces was characterized in cervical, middle and apical root-canal thirds using new LVSEM imaging on moist, polished, native specimens with reduced SEM specimen preparation procedures and reduced artefact formation. Chemical characterization was performed using energy-dispersive x-ray analysis.
Results LVSEM enabled micromorphological characterization of the AS/RCD interdiffusion zone with RCD and obturation material (CM/AS) in their natural spatial arrangement. Hybrid-layer, resin-tags and lateral-resin-tags are identified in RCD as in coronal dentine, but micromorphology of the interaction zone depended upon irrigation-protocol and localization within the root-canal: smear layer removal exposed a collagen-rich, hybrid-layer-like zone between RCD/AS, absent when NaOCl was used as final irrigant after smear layer removal despite intimate adaptation of the AS to RCD. Resin-tags predominated in cervical and middle root canal thirds. The formation of a monoblock could not be confirmed with either obturation system.
Conclusions With adhesive obturation materials, the irrigation protocol influences the micromorphology and thereby the quality of the adhesive interface to RCD.

R89

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Evaluation of the quality of root canal filling performed with thermoplasticised and cold gutta-percha techniques

Aim To compare the quality of root fillings obtained with the single-cone technique, Thermafil and the continuous wave of condensation.
Methodology Sixty extracted single-rooted teeth were treated with ProTaper Universal instruments (Dentsply-Maillefer, Switzerland) and then randomly divided into three subgroups ($n = 20$) depending on the filling technique used: group A, single-cone technique; group B, Thermafil; group C, continuous wave of condensation. In each group the obturation was performed using ProTaper Universal gutta-percha points and obturators corresponding to the size of the last instrument used and AH-Plus sealer (Dentsply DeTrey GmbH, Konstanz, Germany). Thirty teeth, ten for each group, were sectioned horizontally at 2, 5, 7 and 10 mm from the apex; the percentage of root-canal space occupied by gutta-percha and sealer, voids and the gutta-percha/sealer ratio were calculated. Thirty teeth, ten for each technique, were cleared and photographed under a stereomicroscope to evaluate the quality of the root filling. Data were analysed by Kruskal-Wallis test to determine statistical differences; the significance was determined at the 95% confidence level.
Results The percentage of gutta-percha/sealer filled area at 2 and 5 mm from the apex was significantly higher ($P < 0.05$) in group B and C than in group A (A: 91.25%, B: 100%, C: 99.78% at 2 mm; A: 96.70%, B and C: 100% at 5 mm). In group B, six of 10 samples had a small contact between the carrier and canal wall. Thermafil produced the highest gutta-percha/sealer ratio at all levels. The cleared samples showed more accessory canals filled in the Thermafil and continuous wave of condensation groups.
Conclusions All the techniques analysed showed good results. Thermafil and the continuous wave of condensation technique had less sealer, fewer voids and good penetration into lateral canals.

R90

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Percentage of gutta-percha filled area in root canals instrumented and obturated with different techniques

Aim To evaluate the efficacy of different obturation techniques in root canals instrumented either by hand or rotary instruments with regard to the percentage of gutta-percha filled area (PGFA).
Methodology One hundred and sixty extracted mandibular premolars with single, straight root canals were studied. Root canals were prepared to an apical size of 30 by hand in a modified crown-down technique or using ProTaper (Dentsply Maillefer, Switzerland) and HEROShaper (Micro-Mega, France) systems. Teeth were divided into eight groups ($n = 20$) according to the following instrumentation and obturation techniques: G1: Hand-files, lateral condensation (LC), G2: Hand-files, Thermafil (Dentsply Tulsa, USA), G3: ProTaper, LC, G4: ProTaper, single-cone (ProTaper Universal, Dentsply Maillefer, Switzerland), G5: ProTaper, ProTaper-Obturator (Dentsply Tulsa, USA), G6: HEROShaper, LC, G7: HEROShaper, single-cone (MM-GP points, Micro-Mega, France), G8: HEROShaper, HEROfill (Micro-Mega, France). AH26 (Dentsply DeTrey, Germany) dyed with carbon black powder was the sealer used in all teeth. Horizontal sections were cut at 1, 3, 5, 7, 9, 11 and 13 mm from the apical foramen. A total of 1120 sections obtained were digitally photographed under a stereomicroscope set at 48× magnification. The cross-sectional area of the canal and the gutta-percha was measured by digital image analysis and the PGFA was calculated for each section. The data were statistically analyzed using one-way ANOVA, Tukey HDS and student's *t*-tests ($P < 0.05$).
Results The mean of the PGFA in Thermafil, ProTaper-Obturator and HEROfill groups was significantly higher than the other groups. In G3 and G4, PGFA showed no significant difference in apical segments whereas PGFA was significantly higher at middle and coronal segments in G3. In G6 and G7, PGFA showed no significant difference in apical and middle segments whereas PGFA was significantly higher at coronal segments in G6.
Conclusions The coated carrier gutta-percha systems revealed significantly higher PGFA among all groups.

R91

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The effect of obturation technique on sealer penetration in dentinal tubules

Aim To evaluate the pattern of sealer penetration at different levels of the root in association with different obturation techniques.
Methodology Ten maxillary double rooted premolars were collected after extraction. The root canals were prepared according to the crown down/step back technique. Throughout instrumentation, the canals were copiously irrigated with 2.5% sodium hypochlorite and with a final rinse of 17% EDTA (Pulpdent Corporation, Watertown, MA, USA). A dye (Sudan Black B, Polis-Service, Belgium) was administered by paper points into both root canals of the same teeth. To differentiate sealer cement from the surrounding dentine, AH26 was coloured by the addition of a small amount of Sudan Black B, which was added until completely dissolved within the sealer cement. One root canal was obturated by a cold lateral condensation technique. The second root canal of each tooth was obturated with a hybrid condensation technique (cold lateral condensation of the apical 3 mm followed by condensation with gutta condensers (Dentsply Maillefer, Switzerland). All teeth were imbedded in polymethylmetacrylat (Dentaurum, Ispringen, Germany) and sectioned at 1, 4, 7 and 10 mm from the apex. Macro images were acquired from both sides of sections (Nikon D300 with 120 mm Medical Nikor macro lens). The amount and extent of ink penetration were measured and compared.
Results The extent of penetration apically was less than coronally for both cold and hybrid condensation. These results are statistically significant (ANOVA test: $P < 0.0001$). Hybrid condensation resulted in no more extensive sealer penetration than cold lateral condensation. Differences between two methods were not statistically significant for all sections ($P = 0.74$).
Conclusions Sealer penetration is less apically then coronally. Hybrid condensation presented no more widespread sealer penetration around the contour of the prepared root canal, sealing ability is expected to be the same for both techniques.

R92

Abstract withdrawn

R93

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Pull-out resistance of GuttaFlow used with gutta-percha cones of different surface structure

Aim To assess pull-out resistance of gutta-percha cones with four different surface designs placed with or without a primer.
Methodology Eighty straight single-rooted teeth were instrumented to size 55,.02 taper, decoronated to a remaining root canal length of 8 mm and embedded in epoxy-resin (Biresin, Sika, The Netherlands). The teeth were randomly assigned to one of the four following groups (n = 20) according to the surface structure of experimental gutta-percha cones size 50,.02 taper adjusted to fit at working length with tug-back (Coltène/Whaledent, Germany): group 1: coarse; group 2: fine; group 3: three retention rings; group 4: smooth. Each group was further divided into subgroup A: no primer, and subgroup B: primer. GuttaFlow (Coltène/Whaledent) was used as sealer in all groups. After a storage of seven days at 37°C and 100% humidity, the gutta-percha cones were pulled out of the root canal using a universal testing machine (Zwick, Germany; cross-head speed: 1 mm/min). Statistics were computed with SPSS Win 14.0 (SPSS Corp., USA) using Kolmogorov-Smirnov test, two-way ANOVA, and post hoc tests.
Results Bond strength was (MPa/SD): group 1A: 0.09/0.03; group 1B: 0.17/0.02; group 2A: 0.10/0.04; group 2B: 0.19/0.03; group 3A: 0.06/0.04; group 3B: 0.16/0.05; group 4A: 0.08/0.04; group 4B: 0.18/0.03. Two-way ANOVA revealed significant differences for cone type (P = 0.026) and of primer (P < 0.001). Cones with the 'fine' surface showed significantly higher retention than the '3 rings' version (Bonferroni-Holm test: P = 0.017).
Conclusions Resistance of gutta-percha cones against extrusive forces within root canals obturated with GuttaFlow can be mainly improved by using a primer, additionally by using gutta-percha cones with a finely-roughened surface structure.

R94

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Bonding epoxy based sealers to different root dentine areas with or without the smear layer

Aim To evaluate the bonding of two epoxy-based sealers to the apical, middle, and coronal part of root canal dentine with or without smear layer.
Methodology Eighteen human maxillary central incisors were sectioned below the cemento-enamel junction, split longitudinally in a bucco-lingual direction, divided into three parts: coronal, middle and apical (n = 108). Group A- the smear layer was removed by rinsing the specimens for 3 min with 17% EDTA, followed by 2.5% NaOCl. Group B were rinsed with NaOCl only. Three subgroups were assigned: RealSeal (SybronEndo, CA, USA), AH Plus (DENTSPLY Maillefer, CH), and Z-250 (3M ESPE, USA) as a control. The materials were applied using jig with plastic mould (Ultradent Products Inc. USA), thereafter samples were placed in 37°C (100% humidity) for one week. Shear bond testing was performed with universal testing machine (Lloyd Instruments, UK).
Results The mean shear bond strength values were 1.5 MPa (SD 1.2) for RealSeal and 0.9 MPa (1.1) for AH Plus without EDTA, and 2.2 (1.3) and 1.3 (1.0) MPa, respectively with EDTA. Both materials showed higher bond strength in the apical part, mean values from 1.1 MPa to 2.6 MPa (depending on the pretreatment and material), compared to 0.9 and 2.3 MPa in the middle and coronal parts. None of these differences were statistically significant. The composite resin showed significantly higher bond values (ANOVA, P < 0.05) for root dentine than the epoxy based sealers with and without EDTA, 7.0 (4.6) and 4.6 MPa (2.6), respectively.
Conclusions Removal of the smear layer from the root canal dentine wall increased the bonding ability of epoxy based root canal sealers and allowed better seal for the apical part of the root.

R95

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Sealing ability of MTA and portland cement as apical plugs in an immature apex model

Aim To evaluate and compare the sealing ability of mineral trioxide aggregate (ProRoot® MTA, Dentsply Tulsa, USA) and Portland cement (PC) when used as apical plugs at either of two thicknesses (3 mm and 5 mm) in an immature apex model with either of two sizes of the apical foramen [Small (S = 90), Large (L = 120)].
Methodology Ninety-four, single-canalled teeth were used, where the canals were prepared in a way to create divergent canal walls apically. Orthograde apical plug placement was performed, leaving the rest of the canal empty. Eight experimental groups (n = 10): MTA3S, MTA3L, MTA5S, MTA5L, PC3S, PC3L, PC5S, PC5L, and two control groups (n = 7): positive and negative. The teeth were coated with varnish except over the apex; the negative control teeth had apices also coated. The sealing ability was assessed by the dye extraction method using 2% buffered methylene blue (pH = 7). Optical densities (OD) were measured using a microplate spectrophotometer at a wavelength of 550 nm.
Results Every experimental group was significantly different from each of the positive and negative controls (P < 0.05). The PC3S group was significantly different from each of the four MTA groups (P < 0.05); however, it was not significantly different from the other PC groups (P > 0.05). GLM Univariate test showed that the type of the material had a significant effect on the OD measurements (P < 0.05), but the thickness of the material and the size of the immature apex did not have a significant effect (P > 0.05).
Conclusions MTA could provide a better seal than PC as a short apical plug in cases of immature apices with smaller apical foramen size.

R96

Abstract withdrawn

R97

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Apical leakage of retreated root canals obturated by gutta percha or Resilon/RealSeal

Aim To compare apical dye penetration of retreated root canals obturated with gutta percha and Zinc Oxide Eugenol (GP-ZOE) or Resilon-RealSeal.
Methodology Forty extracted human teeth with single canal teeth were decoronated, accessed and instrumented by K-file to apical size 40. The prepared roots were randomly divided into three experimental groups of 12 roots each in addition to a positive and a negative control groups of two teeth each. In group I, roots were re-enlarged to apical size 50 and obturated with GP-ZOE. In group II and III, they were firstly obturated with GP-ZOE, which was later removed and re-enlarged to apical file size 50. GP-ZOE or Resilon-RealSeal were used for obturation in group II and III respectively. The apical leakage was assessed using Indian ink penetration for two days. All teeth were cleared and the maximum dye penetration measured and averaged. Data was analysed by one way ANOVA and LSD test.
Results The retreated root canals obturated with GP- ZOE (GR II) displayed the highest mean dye penetration. The retreated root canal obturated with Resilon-RealSeal (Gr III) had significantly less dye leakage than the retreated root canal obturated with GP- ZOE(GR II) ($P < 0.05$) but not for the root canal obturated with GP-ZOE (GR I).
Conclusions The use of Resilon-RealSeal in retreatment could improve apical sealability.

R98

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Apical leakage and sealer extrusion of root fillings using five different sealer placement techniques

Aim To assess apical sealing efficiency and extrusion of four root canal sealers placed by five different sealer placement techniques.
Methodology Two hundred straight single-rooted teeth were selected and randomly assigned to one of the 20 following groups ($n = 10$), Root canals were instrumented to size 45, .04 taper and irrigated according to the manufacturers' instructions. Canals were obturated with the following sealers: group 1, AH Plus DeTrey Dentsply, Germany); group 2, GuttaFlow (Coltène/Whaledent, Germany); group 3, Maxcem (Kerr-Hawe, Switzerland); group 4, RelyX Unicem (3M-Espe, Germany). Groups were further divided into five subgroups according to the sealer placement technique: A, CanalBrush (Coltène/Whaledent); B: cannula; C: lentulo spiral; D: paper point; E: sonic tip. Coronal leakage was assessed in a standardised dye penetration test (3 min/30 G). Linear dye penetration was investigated microscopically. Statistical tests were performed using SPSS Win 14.0 using Kolmogorov-Smirnov test, two-way ANOVA, repeated *t*-tests, and post hoc tests.
Results Linear dye penetration (mm)/apical sealer extrusion (mg): group1A: 3.0/0.9; group1B: 1.7/4.0; group1C: 2.0/0.5; group1D: 2.8/0.6; group1E: 2.8/0.5; group2A: 2.3/0.9; group2B: 2.8/2.8; group2C: 2.8/0.7; group2D: 3.1/0.8; group2E: 1.1/1.1; group3A: 2.8/0.9; group3B: 1.8/0.7; group3C: 3.5/1.3; group3D: 3.5/1.5; group3E: 3.5/1.7; group4A: 5.0/0.7; group4B: 3.1/0.4; group4C: 2.8/1.3; group4D: 3.8/0.4; group4E: 4.8/0.2. Two-way ANOVA revealed significant differences for linear dye penetration and sealer extrusion on sealer, placement technique, and their combination ($P < 0.05$). Best sealing efficiency was coupled with highest sealer extrusion for AH Plus and RelyX Unicem but not within the GuttaFlow group. The best seal for Maxcem was coupled with the least sealer extrusion in that group. RelyX Unicem revealed less apically extruded material than the other sealers except when placed with a lentulo spiral.
Conclusions The extent of linear dye penetration and amount of extruded sealer did not display uniform results for each sealer. Therefore, the placement technique should be chosen individually for each sealer.

R99

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Comparison of apical sealing of two canal sealers between immediate versus delayed post-space preparation

Aim To compare the effect of immeadiate versus delayed post space preparation on the apical seal using AHPlus and EndoREZ sealers.
Methodology Forty four single rooted teeth were prepared using a step-back technique and obturated with gutta-percha/AHPlus (Dentsply, Germany) and gutta-percha/EndoREZ (Ultradent, USA) by the lateral compaction technique. Four teeth were used for positive and negative control groups. The teeth were divided randomly into six groups; and the post space were prepared either immeadiately after filling or after the obturated teeth were stored in saline at 37°C after a week using hot pluggers, or using gates glidden drills leaving 5 mm root canal filling in the apical part. Leakage was determined by a computerized fluid-filtration device.
Results Immediate post space preparation of the gutta-percha/AHPlus group had a mean leakage value of 4.97 $\mu L/min.10^{-4}$; and the delayed post space preparation had a value of 4.94 $\mu L/min.10^{-4}$. Immediate post space preparation of gutta-percha/EndoREZ group had a mean leakage value of 4.97 $\mu L/min.10^{-4}$; whereas the delayed post space preparation group had a mean leakage value of 5.57 $\mu L/min.10^{-4}$. Positive control group value was 398.16 $\mu L/min.10^{-4}$ and there was no fluid filtration in negative control group.
Conclusions Gutta-percha/AHPlus delayed group achieved better sealing than the other groups. Delayed post space preparation in gutta-percha/EndoREZ group showed the higher percentage of leakage.

R100

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Evaluation of apical sealing ability of different obturation materials

Aim To evaluate and compare apical sealing of root canals obturated using the cold lateral condensation technique with different materials.
Methodology The root canals of 70 single-rooted human mandibular premolars were prepared according to the crown-down and step-back technique. Each canal was rinsed with 3% NaOCl (Parcan, Septodont, France) during and after preparation. The teeth were randomly divided into three groups of 20 samples each, and the remaining 15 teeth served as controls: seven as positive and eight as negative controls. Teeth were obturated using cold lateral conden-

sation technique and different materials: RealSeal system (Resilon, SybronEndo, USA), gutta-percha with AH Plus (Dentsply Maillefer, Germany) and gutta-percha with Acroseal sealer (Septodont, France). Teeth were immersed in 1% methylene blue for 72h After that period, the roots were splitted longitudinally, and dye penetration was measured using a stereomicroscope (Zeiss, Axioskop 40).
Results The seven positive control specimens had total dye penetration of the root canal system, whereas the eight negative control teeth had no dye penetration into the roots. The dye penetration has occurred in all experimental groups. RealSeal group exhibited the least mean apical leakage value (0.25 ± 0.23 mm) among the teeth filled with gutta-percha using the sealer, AH Plus (0.28 ± 0.21 mm) and Acroseal (1.08 ± 0.49 mm). According to the Student t test, Acroseal showed significantly more leakage ($P < 0.001$) than RealSeal and AH Plus materials. Microleakage of RealSeal system was not significantly different ($P > 0.05$) from that of AH Plus sealer with gutta-percha.
Conclusions Cold lateral condensation of RealSeal and gutta-percha with AH Plus sealer had the least apical dye penetration and provided the best seal.

R101

Abstract withdrawn

R102

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Effect of endodontic sealers on the sealing ability of adhesively cemented posts

Aim To evaluate the effect of Gutta Flow, AH plus and Real Seal sealers on the sealing ability of ParaPost luted with a resin cement ParaCem.
Methodology Thirty-six canals of maxillary incisors were prepared chemo-mechanically and filled with gutta-percha and three different sealers: Gutta Flow in group 1, AH plus in group 2, and Real Seal in group 3 – using a single cone technique. After that the teeth were kept moist in a humidor at 370 for 48h to allow the sealer to set. All root canals were enlarged with a size 2, 3, 4, 5 and 6 Gates-Glidden drills mounted in low-speed handpiece. The working length of the drills was established with silicone stoppers. All root canals were flushed with sterile water and dried with paper points. In teeth of all groups the ParaCem Universal DC was applied according to the manufacturer instruction and the size 5.5 ParaPosts Fiber Lux were cemented. The specimens were further stored in a 2% methylene blue for 72h at 37°C and cross sectioned in regular (1 mm) intervals beginning from the coronal ends. Depth of linear penetration was measured under a stereo microscope at 20× magnification. The maximum leakage value was limited to 9.0°mm. The extent of leakage among the groups was compared using the non-parametric Kruskal Wallis analysis of variance and Mann-Whitney U test ($P < 0.05$).
Results Median leakage [mm] in groups 1, 2 and 3 were 4.0, 5.0 and 2.0, respectively. All groups revealed significantly different distribution of leakage penetration ($P < 0.05$).
Conclusions Group 3 (Real Seal) had better sealing ability than the other groups. The worst results were observed in group 2 (AH Plus).
Acknowledgements This study was supported by a research grant from the Medical University of Lodz N° 503–2044–2.

R103

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Coronal leakage of post-space-prepared root canals obturated by three different root canal filling techniques

Aim To assess the coronal seal produced by six sealers used with three different obturation techniques after post-space preparation.
Methodology One hundred and eighty straight human single-rooted teeth were selected. The root canals were instrumented with FlexMaster (VDW, Germany to size 40 then with ProFile (Dentsply Maillefer, Switzerland) to size 45,.04 taper according to the manufacturers' instructions. The teeth were randomly assigned to six groups ($n = 30$): group 1: AH Plus (DeTrey Dentsply, Germany); group 2: low-viscous impression silicone (experimental); group 3: FibreFill (Pentron, USA); group 4: GuttaFlow (Coltène/Whaledent, Germany); group 5: Maxcem (Kerr-Hawe, Switzerland); group6: Pulp Canal Sealer (Sybron, USA). Groups were further divided into three subgroups ($n = 10$) according to the obturation technique used: A: lateral condensation; B: sectional root filling technique; C: single-cone technique. Seven days later post space cavities were prepared to a remaining length of the root canal fillings of 5 mm using the ER post preparation system (Komet, Germany). Coronal leakage was assessed in a standardised dye penetration test (3 min/30 G). Linear dye penetration was investigated microscopically. Statistical tests were performed using SPSS Win 14.0 using Kolmogorov-Smirnov test, two-way ANOVA, and post hoc tests.
Results Linear dye penetration: means (SD) in mm: group 1A: 4.6 (2.1); group 1B: 3.1 (0.7); group 1C: 2.9 (1.7); group 2A: 3.7 (1.4); group 2B: 2.5 (1.0); group 2C, 2.5 (1.1); group 3A: 4.1 (1.4); group 3B: 4.9 (1.3); group 3C: 4.9 (1.4); group 4A: 1.2 (0.4); group 4B: 1.4 (1.0); group 4C: 1.8 (1.2); group 5A: 4.5 (1.3); group 5B: 3.2 (2.2); group 5C: 4.7 (1.7); group 6A: 5.3 (1.0); group 6B: 2.5 (1.4); group 6C: 5.4 (1.1). Linear dye penetration was significantly affected by sealer type, obturation technique, and their combinations (two-way ANOVA, $P < 0.001$).
Conclusions Root fillings using the sectional obturation technique tended to leak less than other techniques. GuttaFlow allowed less leakage to occur compared with the other sealers.

R104

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Effect of six obturation techniques on filling of lateral canals

Aim To evaluate the filling of artificially prepared lateral canals using six different gutta-percha obturation techniques.
Methodology Sixty extracted human single-rooted teeth were used. Root canals were cleaned and shaped by using a step-back technique. Six lateral canals were prepared in the coronal, middle and apical thirds in each root using size 15 engine driven reamer. The teeth were randomly divided into six groups of 10 and obturated by lateral condensation, Microseal (Tycom,USA), JS Quick-Fill (JS Dental Mfg. Inc., USA), Softcore (CMS-Dental Aps., Denmark), System B (SybronEndo, USA) with Obtura II (Obtura Corp.,USA), and Thermafil (Tulsa Dental, USA) techniques using Kerr Pulp Canal sealer (Kerr Manufacturing Co, USA). Radiographs of the obturated canals were taken mesiodistally and scored according to the extension of filling of lateral canals.

Results Statistically more lateral canals were obturated by Thermafil and System B/Obtura than the other techniques ($P < 0.05$). However, no significant difference was found between Thermafil and System B/Obtura techniques ($P > 0.05$). Also, statistically more lateral canals were filled in middle thirds of the canal than in the apical and coronal thirds in all techniques.
Conclusions Thermafil and System B/Obtura techniques were superior to other techniques in filling lateral canals.

R105

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An evaluation of a combined bacterial leakage technique by centrifugation and fluorescein

Aim To evaluate a bacterial coronal leakage technique by centrifugation combined with dye penetration with fluorescein.
Methodology Forty-two extracted human teeth with straight, single root canals all cut to the same length were prepared until the master apical file was 45. The teeth were divided randomly into five experimental groups and two control groups (six teeth each). In the experimental groups, roots were obturated with size 6 gutta-percha points, lateral condensation, System B (SybronEndo, USA) with Obtura II (Obtura, USA), Thermafill (Dentsply, USA) and Epiphany System (Pentron, USA). The negative controls were obturated with lateral condensation and covered completely with two layers of nail varnish and six teeth were prepared but not obturated to serve as positive controls. AH Plus (Dentsply, USA) was used as sealer. The teeth were glued into a polypropylene tube (coronal chamber) which was placed in a plastic tube containing 1000 μL of physiological water. An inoculum (500 μL) of *Pseudomonas aeruginosa* containing fluorescein was placed in each coronal chamber. The specimens were then centrifuged (179*g* for 5 min). The number of cfu/mL in the lower chamber was then determined. The teeth were then sectioned to allow visualization of dye penetration that was measured from coronal to apical in mm.
Results There was a significant correlation between dye penetration and bacterial leakage. The teeth in each group except the negative controls showed partial dye penetration and bacterial leakage in varying amounts. There was no significant difference in leakage between the lateral condensation, System B – Obtura II, Thermafill and Epiphany filling techniques.
Conclusions Centrifugation of a bacterial suspension in combination with a fluorescein dye can be used as a reliable technique to test leakage in root canal fillings *ex vivo*.

R106

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Sealing ability of MTA, GIC and composite resin when repairing large furcal perforations

Aim To evaluate the sealing ability of different repair materials and the pathway of bacterial contamination after closure of large pulp chamber floor perforations.
Methodology Perforations were made in the furcation area of extracted human molars and sealed with either mineral trioxide aggregate (MTA) (tooth-coloured (Group1) or grey (Group 2)), glass ionomer cement (Group 3) or resin composite (Group 4). The bacterial leakage method was used with *Enterococcus faecalis* as microbial tracer. The time of leakage (in days) was recorded for each specimen. Statistical analysis of the bacterial leakage experiment was performed using the survival analysis and pairwise comparison of the groups. A *P*-value less than 0.05 was considered statistically significant. After a study period of 50 days, tooth specimens were prepared and inspected for the presence of bacteria by a scanning electron microscope (SEM).
Results After 30 days leakage was detected in 29%, 20%, 60%, and 86% of the samples in groups 1, 2, 3, and 4, respectively. The percentage of leaking samples were similar in groups 1–3 ($P > 0.05$) while it was significantly higher in Group 4 than in the other sealer groups and the negative control group ($P < 0.05$). SEM inspection revealed the presence of bacteria in all leaking specimen. Bacteria were observed along the filling-dentine interface as well as in dentinal tubules at some distance from the filling.
Conclusions The resin composite material leaked significantly more than MTAs and glass ionomer cement when used to repair furcation perforations. Bacteria could penetrate into dentine even distant from the perforation filling.
Acknowledgements The authors would like to thank Ms. I. Nedzelskiene (Kaunas University of Medicine, Department of Dental and Oral Pathology, Kaunas, Lithuania) for statistical and I.-S. Dragland (NIOM-Nordic Institute of Dental Materials, Haslum, Norway) for laboratory assistance.

R107

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Different ways to improve the secondary protective seal over root fillings: a preliminary study

Aim To improve the adhesive seal over root fillings and to compare with conventional cements.
Methodology Root canals of 80 molars were instrumented to size 60, .02 taper and filled with AH plus (DeTrey Dentsply, Germany) and gutta percha and divided into eight groups (each $n = 10$). After removal of residual sealer with an alcohol moistured foam pellet, etch-and rinse with 37% H3PO4 for 10 s and application of Syntac (Ivoclar Vivadent, Liechtenstein) was performed for groups 1–5. In group 1 two thin layers of Tetric flow (Ivoclar Vivadent) (~2 mm) were applied (reference group), in group 2, prior to applying the two layers, all canal orifices were sealed separately with small amounts of Tetric flow, in group 3 a combination of Tetric flow and Tetric ceram was used instead of Tetric flow alone, in group 4, the access cavity was additionally cleaned with CleanPro-Powder (3M ESPE, Germany), used in an AirFlow Handpiece (KaVo, Germany), in group 5, Grandio flow (Voco, Germany) was used (lower shrinkage). In group 6 Coroseal (Ivoclar Vivadent) was applied. In group 7, the access cavity was closed with a 2 mm layer of Harvard Cement (Richter&Hoffmann, Germany), in group 8 with a 2 mm-layer of Ionofil-molar (Voco). Linear dye penetration was measured (centrifugation 3 min at 30 G) within methylene blue 5%. Statistical tests were performed using SPSSR Win 14.0 (Kolmogorov-Smirnov-test, ANOVA with SNK post hoc-test; alpha=0.05).
Results Linear dye penetration (mm) (means/SD) (letters indicate homogeneous subgroups regarding ANOVA/SNK): group1 (0.60/0.52) (a,b), group2 (0.84/0.60) (a,b), group3 (0.66/0.55) (a,b),

group4 (0.48/0.41) (a), group5 (1.65/1.60) (b,c), group6 (0.35/0.37) (a), group7 (3.30/1.37) (d), group8 (2.40/1.08) (c). Significant differences were apparent (ANOVA, $P < 0.001$).
Conclusions The following methods may offer good potential: CleanPro Powder, Coroseal. Grandio flow, exhibiting low shrinkage but high shrinkage stress, and conventional cements cannot be recommended.

R108

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One year effect of chlorhexidine on bonding of fibre-reinforced composite root canal post to dentine

Aim To determine the long term effect of chlorhexidine on attachment of fibre-reinforced composite root canal posts to root canals.
Methodology Forty human extracted third molar root canals were prepared for post cementation with each post systems own burs. Four commercially available FRC posts: Glassix (H.Nordin sa, Chailly-Montreaux, Switzerland), D.T.Light-Post (Bisco Inc., Schaumburg, IL, USA), Unicore (Ultradent, Salt Lake City, UT, USA), everStickPOST (Stick Tech Ltd., Turku, Finland) with three cements: Duo-link (Bisco, Inc., Schaumburg, IL, USA), PermaFlo DC (Ultradent) and RelyX Unicem (3M ESPE, Seefeld, Germany), were used. After etching, except with self-etching RelyX Unicem, the post spaces were irrigated either with 2% chlorhexidine (CHX) or physiological saline for 60s. The roots ($n = 5$ per group) were cut into 2 mm thick dentine discs. The discs were stored in artificial saliva for one year. The bond strength was measured with push-out method, and the failure mode was evaluated with a stereomicroscope. Kruskal-Wallis Test with Mann Whitney Test was used to analyse the differences in bond strength.
Results Higher long-term bond strength was observed with CHX in all groups, the improvement varying between 6% and 94%. However, the difference was statistically significant only with D.T. Light-Post/Duo-Link (94%; $P < 0.05$). With CHX, pure adhesive-to-dentine failures decreased between 14% and 45% and mixed fractures with partial cohesive dentine fracture increased between 7% and 25% in different groups, when compared to respective controls.
Conclusions Previous studies have shown chlorhexidine to significantly improve the longevity of resin-dentine bond with composite fillings. With the posts, the improvement of bond strength seems to be more limited, but together with marked reduction in purely adhesive-to-dentine failures and increase in cohesive dentine failures they indicate that chlorhexidine may improve long-term post adhesion to root canal dentine. 2% chlorhexidine can be recommended as final irrigant before post cementation.
Acknowledgements The study was supported by the Academy of Finland and the Finnish Dental Society Apollonia.

R109

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Long term solubility of Portland-type cements with and without serum contamination

Aim To compare the water-solubility of MTAs, Portland Cements (PCs) and Diestone over 84 days after mixing with the recommended mixing solution and with a mixture of recommended solution and serum.
Methodology Samples ($n = 5$) of grey and white MTAs (Angelus and ProRoot), PCs (Lafarge) and dental Diestone (BPB, Newark, UK) were mixed to standard consistency with the recommended solution or 50:50 (v/v) recommended solution:defibrinated horse serum and packed into 10 mm × 1 mm ring moulds. After curing for 24h at 37°C/100% humidity, specimens were immersed in 7 mL distilled water and stored at 37°C. Specimens were moved to new 7 mL aliquots of water at 2, 7, 14, 28, 42, 56 and 84 days, and material solubility measured by the residue method. Cumulative solubility was recorded over time. Differences in solubility of the materials, changes over time with and without serum contamination and influence of serum contamination were compared using two-way ANOVA, one-way ANOVA and Tukey's post hoc tests ($P < 0.05$).
Results Cumulative solubility over 84 days for materials mixed with recommended solution was: PC White (9.37%) < MTA Angelus Grey (9.59%) < PC Grey (9.75%) < MTA Angelus White (9.80%) < MTA ProRoot White (11.94%) < MTA ProRoot Grey (16.48%) < MTA BIO (37.99%) < Diestone (47.13%). Serum contamination significantly increased the solubility of MTA Angelus (Grey 10.49%) and (White 11.47%), MTA ProRoot (Grey 19.70%) and (White 15.19%) and Grey PC (10.26%) ($P < 0.05$), but significantly decreased the solubility of MTA BIO (29.72%) ($P < 0.05$), and had no significant effect on White PC ($P = 0.63$).
Conclusions All materials, with and without serum contamination dissolved progressively over 84 days. Heavy serum contamination significantly increased the solubility of most materials. Although MTAs are hydraulic cements, caution may be needed in sites where they are likely to become heavily contaminated with tissue fluids.

R110

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Radiopacity of resin-based root canal sealers

Aim To compare the radiopacities of eight resin-based root canal sealer and a zinc oxide eugenol based sealer according to ISO specification 6876/2001.
Methodology Nine root canal sealers: Epiphany (Pentron, USA), EndoRez (Ultradent, USA), Roekoseal (Colténe-Whaledent, Switzerland), GuttaFlow (Coltene-Whaledent, Switzerland), Diaket (3M/ESPE, Germany), AH Plus (Dentsply, Germany), AH 26 (Dentsply, Germany), FibreFill (Pentron, USA), and Kerr Pulp Canal sealer (Kerr Manufacturing Co, USA) were evaluated. Ten acrylic plates were prepared containing nine wells with a 5 mm diameter and 1 mm thickness and an aluminum stepwedge. The wells were filled with nine different freshly mixed root canal sealers and radiographed using an indirect digital technique. Mean grey values were determined by digital anaysis and converted into equivalent mm Aluminum thickness for each sealer. The data were evaluated statistically using one-way ANOVA and Tukey post hoc tests ($P = 0.05$).
Results The opacity of the samples ranged from 3.22 mmAl to 8.92 mmAl. AHPlus, Epiphany, Kerr Pulp Canal sealer and Roekoseal had higher radiopacity values than the other resin based sealers ($P < 0.05$). All materials had radiopacities above the international standard for root canal sealers (>3 mmAl).
Conclusions Although the materials tested had different radiopacities, all sealers presented radiopacity values above the values required by ISO 6876/2001 standards.

R111

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Confocal microscopy study of marginal adaptation of total-etching and self-etching bonding systems in endodontically treated teeth

Aim To analyze marginal adaptation of total-etching and self-etching bonding systems to enamel and dentine walls, using a confocal laser microscopy with teeth connected to a permanent intrapulpal pressure device.
Methodology Forty teeth were selected, distributed in eight groups of five teeth with two cavities in each tooth. They were filled with composite resin using two self-etching bonding agent: Xeno V and AdheSe One (groups A-E and B-F respectively). The total-etching bonding agents, Excite and XP Bond were used in groups C-G and D-H respectively. Groups A, B, C, D were connected to a perfussion device. The percentage of cavity perimeter without bonding was measured through a confocal laser microscopy image. Variance analysis was used for the data analysis and the Tukey test for paired comparisons with a significance level of 95% ($P < 0.05$).
Results The percentage of cavity perimeter without bonding was as follows: A: 16%; B: 22%; C: 9%; D: 10%; E: 0%F: 2%, G: 0%; H: 0%. There were significant differences among groups with and without pulpal pressure.
Conclusions The marginal adaptation of the dental bonding systems analyzed was significantly better in the groups where intrapulpal pressure was not applied. The self-etching bonding systems studied showed better adaptation to dentine. Total-etching agents had better marginal adaptation to enamel.

R112

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Mechanical properties and dimensional characterisation of Roeko CanalBrush™

Aim To evaluate the mechanical properties and dimensional characterisation of Roeko CanalBrush™ (Coltène/Whaledent, Germany).
Methodology Ten new CanalBrushes were examined to determine the length of the working part, number of lateral brushes, length of lateral brushes, distance between brushes, tip length, and diameter at each millimetre from the tip. Cyclic fatigue testing was performed to evaluate resistance to fracture of the polypropylene microbrushes ($n = 20$) in an artificial root canal for 5 and 10 min. A total of 30 CanalBrushes were used in a tooth with a straight circular root canal, in a tooth with a straight oval root canal and in a tooth with a curved root canal for 1, 3 and 5 min at 300 rpm. The working part of each instrument after each period of use was examined on digital images to search for signs of deformation or fracture of the lateral brushes. Data were analysed by Kruskal-Wallis test to determine any statistical difference; the significance was determined at the 95% confidence level.
Results No instruments fractured during cyclic fatigue tests and no lateral brushes fractured after use. Wear of the lateral brushes increased with time of use ($P < 0.05$). All the instruments were identical ($P > 0.05$): mean length of the working part was 17.1 mm, the tip was 1 mm in length, lateral brushes were 30 for each side and were 0.4 and 0.5 mm in length with a distance between each brush of 0.4 mm. Diameters at each millimetre from the tip were regular among the instruments, with mean values varying from 0.2 mm at the tip to 0.6 mm at the end of the working part.
Conclusions Dimensions of Roeko CanalBrushes were standardised well. In this laboratory setting the instruments were used safely without risk of fracture in curved canals or separation of the lateral brushes.

R113

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Surface porosity of hand-mixed, syringe-mixed and encapsulated set endodontic sealers

Aim To investigate the surface porosity of hand-mixed, syringe-mixed and encapsulated set endodontic sealers using image analysis.
Methodology Eight different root canal sealers (AD Seal/Meta Biomed; AH Plus/Dentsply; MM Seal/Micromega; AH Plus Jet/Dentsply; EndoREZ/Ultradent; Epiphany/Pentron; GuttaFlow/Coltène; RealSeal/Sybron Endo) were prepared in accordance with the manufacturer's recommendations and dispensed into plastic rings ($n = 10$/group). Following set of the specimens, scanning electron photomicrographs of the sealer surfaces were taken at standard magnification. The area fraction and the minimum-maximum diameters of surface pores were calculated on the micrographs using ImageJ open-source image analysis software. Statistical comparisons were made between the sealers with respect to the area fractions and number of pores (Kruskal-Wallis test, $P < 0.05$).
Results The endodontic sealers differed significantly in both the percentage of area fraction and pore count ($P < 0.001$). The surface porosity of the methacrylate-based sealers Epiphany and RealSeal were significantly lower than those of resin- and silicone-based sealers. With the exception of AD Seal, Hand-mixed sealers displayed significantly higher surface porosity than those of syringe-mixed ones ($P < 0.001$). The encapsulated sealer GuttaFlow exhibited the highest surface area fraction of pores.
Conclusions The mixing method may influence the surface porosity of set endodontic sealers.

R114

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Cyclic fatigue resistance of nickel-titanium rotary instruments with different cross-sectional configurations and manufacturing methods

Aim To compare cyclic fatigue resistance of five nickel-titanium (NiTi) rotary files with different cross-sectional configurations and manufacturing methods (twisted vs. electropolished vs. grinding) using a simulated fatigue device.
Methodology Five brands of NiTi rotary instruments with size 25, .06 taper were tested: Twisted File ($n = 20$, SybronEndo, USA), ProFile ($n = 20$, Dentsply, USA), RaCe ($n = 20$, FKG Dentaire, Switzerland), K3 ($n = 20$, SybronEndo, USA), and Mtwo ($n = 20$, VDW, Germany). Fatigue tests were performed with a specific cyclic fatigue device that evaluated the time to failure of rotary instruments inside an artificial canal. The artificial canal was made of stainless steel with a 60 degree angle, and 5 mm radius of curvature. A continuous, 6 mm axial oscillating motion was

applied at 0.5 cycles per second to simulate a clinical pecking motion. The time until fracture occurred was measured. In addition, three instruments from each brand were embedded in composite resin, sectioned horizontally at 3 mm from the tip and observed with a scanning electron microscope (SEM); the cross sectional area was calculated in square centimetres (Paint.NET; dotPDN LLC). All data were analyzed statistically. Fractographic analysis of fractured surfaces was performed to determine the mode of fracture using a scanning electron microscope (SEM).

Results Cross-sectional area did not have a significant effect on the time of instrument fracture. The Twisted File was significantly more resistant to cyclic fatigue than all the other groups ($P < 0.01$). ProFile, Mtwo were significantly more resistant than K3 ($P < 0.05$) There was no significant difference between ProFile, Mtwo and RaCe and between RaCe and K3. SEM revealed brittle fractures as the predominant initial failure mode. The brittle fracture region demonstrated fatigue striations and a large number of secondary cracks. These fractures typically led to a central region of catastrophic ductile failure, which was characterized by microvoid formation and dimpling.

Conclusions The new manufacturing process (twisted method) appeared to improve the fatigue resistance of NiTi rotary instruments. Cross-sectional area was not an influencing factor.

Acknowledgements This study was supported by 2008 SNUDH research fund.

R115

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Cyclic fatigue of Ni-Ti rotary instruments in simulated apical abrupt curvature

Aim To evaluate the cyclic fatigue resistance of five NiTi rotary instruments in an abrupt apical curvature.

Methodology Cyclic fatigue testing was performed in tapered artificial canals constructed on the dimension of the instruments with a 2 mm radius of curvature and an angle of curvature of 90 degrees. The centre of the simulated curvature was 2.5 mm from the tip of the instrument. Ten FlexMaster (VDW, Munich, Germany) tip size 25, .06 taper, ten Mtwo (Sweden & Martina, Italy) tip size 25, .06 taper, ten ProFile tip size 25, .06 taper from Dentsply-Maillefer (Ballaigues, Switzerland), ten ProFile tip size 25, .06 taper from Dentsply-Tulsa (Tulsa, USA) and ten F2 ProTaper Universal instruments (Dentsply-Maillefer, Switzerland) were rotated until fracture occurred and the number of cycles to failure (NCF) recorded. Data were analysed by one-way ANOVA and Tukey HSD test to determine any statistical difference among groups; the significance was determined at the 95% confidence level.

Results Mtwo had the highest fatigue resistance (NCF 124) ($P < 0.05$); there was no statistical difference ($P > 0.05$) between ProFile from the two different brands despite ProFile from Maillefer having a higher fatigue life (NCF 75) compared to ProFile from Tulsa (NCF 66). No difference ($P > 0.05$) was registered between FlexMaster (NCF 53), and ProFile from Tulsa; ProTaper F2 showed a statistically ($P < 0.05$) lower fatigue life respect to the other instruments tested (NCF 29).

Conclusions Life-span registered for the instruments tested in an apical abrupt curvature was: Mtwo > ProFile from Maillefer > ProFile from Tulsa > FlexMaster > ProTaper.

R116

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Effect of rotational speeds on the cyclic fatigue of a nickel-titanium rotary endodontic instrument

Aim To evaluate the effect of different rotational speeds on the cyclic fatigue of ProTaper Universal (Dentsply Maillefer, Switzerland) nickel-titanium rotary endodontic instruments.

Methodology A total of 150 (30 sets) ProTaper instruments were used. Each set consisted of five instruments S1, S2, F1, F2 and F3. The sets were equally divided into three groups A, B and C according to speed of rotation (A: 160 rpm, B: 250 rpm and C: 350 rpm). Each set was used to prepare eight curved mesial canals of mandibular first molars clinically. Then they were further used to prepare the mesial canals of extracted mandibular first molars until instrument separation or deformation occurred; this was considered to be failure. One set of instruments from each speed were excluded from preparation of extracted teeth and subjected to evaluation of surface characteristics by Scanning Electron Microscope (SEM). The number of canals prepared by each instruments till failure was recorded and analyzed statistically.

Results Shaping instruments had higher mean numbers of prepared canals than finishing instruments in all speed groups. All instruments prepared larger number of canals at the lower speed values. But there was no statistical difference between speed groups in case of F1 and F2 instruments. In case of F3 instrument slow speed (160 rpm) had significantly higher mean number of canals when compared with 250 and 350 rpm. Eleven instruments were deformed; ten S1 and only one S2 instrument. SEM evaluation demonstrated that instruments used at higher speed values showed higher incidence of surface defects.

Conclusions Lower speed increased the clinical life of ProTaper NI-TI instruments. All used instruments survived for the clinical preparation of eight canals at speeds of 160 to 350 without any signs of deformation or separation. Shaping instruments had a longer clinical life than finishing instruments. Permanent deformation occurred only in the shaping instruments with higher incidence in the S1 instrument.

R117

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Deformation and surface texture of ProTaper and Mtwo NiTi rotary instruments in severely curved canals

Aim To evaluate deformation and changes on the surface texture of ProTaper and Mtwo nickel-titanium rotary instruments following their use in severely curved root canals of extracted human teeth.

Methodology A total of 30 root canals of mandibular molars were selected and divided into two equal groups, each was balanced in terms of curvature. The curvatures of the mesial canals ranged between 40° and 90° (mean 64.3°) according to Schneider's method. Two different designs of nickel-titanium rotary instruments were used; ProTaper® (Dentsply Maillefer, Ballaigues, Switzerland) and Mtwo® (VDW, Munich, Germany). Each set of instruments was changed after the third use. Pre- and post-instrumentation images of size 25 instruments and fractured instruments were taken by using a scanning electron microscope (SEM).

Results Fourteen instruments were fractured during the preparation. The fractured eight ProTaper (40%) and 6 Mtwo (30%) instruments were analysed by SEM. More fractures were observed in the ProTaper group. Most of the fractures occurred in S1 (ProTaper) and size 10, .04 taper (Mtwo) instruments. Distortion of spirals and surface wear were identified in both groups.
Conclusions Both instrument systems showed deformation and fracture during the preparation of severely curved root canals of extracted human teeth.

R118

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Fracture resistance of root filled treated premolars with one remaining cavity wall restored using different techniques and materials

Aim To compare the fracture resistance of root filled treated maxillary premolars with one remaining cavity wall restored using two types of custom-made fibre-reinforced composite post materials compared with titanium posts and direct composite restorations.
Methodology Forty-eight maxillary premolars with one remaining buccal cavity wall were root filled treated. The teeth were randomly assigned to four groups of 12 specimens each. In the first group, polyethylene woven fibre (Ribbond); in the second group, custom-made glass fibre-reinforced composite posts (everStick); and in the third group, titanium posts (Filpost) were applied to the post spaces. The resin composite crown was then built up. In the fourth group (control), no post-space preparations were made; however, standardized cavities (3.5 mm depth and 1.5 mm diameter) in the palatal canal entrances were made and the cavities were filled with resin composite. The resin composite crown was built up similarly to the other crowns. The restored teeth were placed into a universal testing machine at a loading angle of 300, and the load was applied with a stainless steel ball (diameter 4 mm) at a crosshead speed of 1 mm/min until the fracture occurred. The data were statistically analyzed using one-way analysis of variance (ANOVA) ($P = 0.05$).
Results There was no significant difference in fracture resistance between the different materials ($P > 0.05$).
Conclusions The presence and type of post did not influence experimental fracture loads in single-rooted maxillary premolar teeth with one cavity wall remaining.

R119

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Effects of sodium hypochlorite on gutta percha and resilon cones; an atomic force microscopy study

Aim To evaluate the effects of sodium hypochlorite on gutta percha and resilon cones by AFM (atomic force microscopy).
Methodology Six standardized size 30 gutta percha (Sure Endo, Korea) and resilon cones (Penthron Technologies) were used for this study. An untreated gutta-percha and resilon cone were used as controls. Cones were immersed in NaOCl for different time periods; 1, 5, 10, 20, 30 min. Gutta-percha and resilon cones were cut 3 mm from their tip and attached to metal base with double sided tape for AFM evaluation. The analyses in AFM were performed on 15 different regions located between 1 and 2 mm from the tip. Scanned areas were uniform squares 5 μm × 5 μm. Tapping mode imaging (TMI) profiles were obtained for the first time. Three dimensional images of gutta-percha and resilon cones were recorded and the root mean square (RMS) which is a statistical measure of the magnitude of a varying quantity, and depth analysis was chosen to investigate the structure of gutta percha and resilon cones. All RMS and depth values were evaluated statistically by Kruskal-Wallis and one way ANOVA tests according to Shapiro Wilk test.
Results The RMS values of gutta-percha cones were increased for all time periods when compared with the control sample but there were no statistically significant differences. The depth analysis values were decreased for 1, 5, 10 and 20 min but no statistically significant differences were detected ($P > 0.05$). Both RMS and the depth analysis values for resilon cones were decreased when compared with the control sample and statistically significant values were obtained ($P \leq 0.05$).
Conclusions NaOCl solution used for disinfection of gutta percha and Resilon cones cause physical alterations on Resilon cones.

R120

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Bond strength of luting cements to root canal dentine using a pull-out test

Aim To assess the bond strength of adhesive and non-adhesive luting cements to root canal dentine.
Methodology Root canals of 80 single-rooted teeth were instrumented to size 60,.02 taper, decoronated to a remaining root canal length of 8 mm, and embedded in epoxy-resin. Teeth were divided into eight groups ($n = 10$) according to different luting cements: group 1: Embrace (Pulpdent, USA), group 2: zinc phosphate cement (Harvard, Richter & Hoffmann, Germany), group 3: Ketac-Cem (3M-Espe, Germany), group 4: Maxcem (KerrHawe, Switzerland), group 5: Multicore Flow (Ivoclar-Vivadent, Liechtenstein), group 6: Panavia F 2.0 (Kuraray, Japan), group 7: Paracem (Coltène/Whaledent, Switzerland), group 8: RelyX Unicem (3M Espe). Spreader blanks size 55, .02 taper were adjusted at working length with a slight tug fit, silica-coated, and inserted into the root canal following placement of the luting cements. After 10 days storage (37°C/100% humidity) the spreaders were pulled out of the root canals using a universal testing machine (Zwick, Germany; crosshead speed: 1 mm/min). Fracture modes were investigated microscopically. Statistics were computed with SPSS Win 14.0 using Kolmogorov-Smirnov test, one-way ANOVA, and *t*-tests.
Results Fracture modes were predominantly cohesive or adhesive at the cement-dentine interface. Bond strength was (MPa/SD): group 1: 12.1/3.8; group 2: 7.0/2.2; group 3: 5.5/1.6; group 4: 5.2/2.0; group 5: 16.8/2.3; group 6: 12.0/6.6; group 7: 18.9/4.6; group 8: 17.7/3.6. The multi-step adhesive luting systems Paracem and Multicore Flow as well as the single-step system RelyX Unicem revealed the highest bond strength to the root canal wall. The weakest bond strength was obtained with the self-adhesive Maxcem and the two non-adhesive luting cements Ketac-Cem and Harvard. Significant differences in terms of bond strength were present between the tested groups (one-way ANOVA: $P < 0.05$).
Conclusions Luting cements showed different pull-out bond strength to dentine depending on the type of cement used. Adhesive luting improved significantly shear bond strength.

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An evaluation of the bond strength of Epiphany sealer to dentine treated with different irrigants

Aim To test the impact of different irrigation regimens on the bond strength of a resin-based sealer (self-etch Epiphany) to dentine and to investigate possible reactions between the irrigants used.
Methodology The occlusal 2 mm of seventy sound extracted third molars were sectioned to obtain flat dentine surfaces. The teeth were randomly assigned into seven groups ($n = 10$) according to the irrigation regimen each dentine surface received. Group I: deionized water, Group II: 5% NaOCl, Group III: 5% NaOCl followed by 10% sodium ascorbate, Group IV: 5% NaOCl then BioPure MTAD (Dentsply/Tulsa, Tulsa, OK), for 5 min, Group V: NaOCl then 10% sodium ascorbate followed by a 5 min treatment with MTAD, Group VI: 5% NaOCl followed by 2% chlorhexidine for 5 minutes, Group VII:. 5% NaOCl, then 10% sodium ascorbate followed by 2% chlorhexidine. After conditioning the dentine surfaces, they were dried with paper points. Epiphany was injected to a thickness of 1 mm inside a stainless steel tube placed on the dentine surface then the sealer was light-cured for 40s. Specimens were prepared for shear bond strength assessment. Loading was performed on a universal testing machine at a speed of 0.5 mm/min. ANOVA and Tukey multiple comparisons were used to compare the results among the experimental groups.
Results The mean shear bond strength obtained for Group I to VII was 0.3 MPa, 0.3 MPa, 0.9 MPa, 0.5 MPa, 1.1 MPa, 0.5 MPa, 1.0 MPa respectively. There was a statistically significant difference between group III and groups I, II and IV ($P < 0.05$).
Conclusions Of the tested irrigants only Sodium Ascorbate was capable of abolishing the adverse effect of NaOCl on the bond strength of Epiphany to dentine whereas MTAD and Chlorhexidine were not capable of achieving a similar effect. In addition MTAD had no effect on the bond strength of Epiphany to dentine.
Acknowledgements This research was supported by Grant number 103/2008 from JUST.

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Effects of different curing units and luting agents on push-out bond strength of translucent posts

Aim To evaluate the effects of different curing units and luting agents on push-out bond strength of translucent fibre posts.
Methodology Thirty extracted incisor teeth with single root canals were sectioned at the cementoenamel junction and the canals were root filled. A post space was then prepared. The smear layer was removed using ultrasonic agitation associated with 17% EDTA and 5.25% NaOCl irrigating solutions. The roots were divided into two groups according to two dual-curing luting systems. FRC Postec Plus fibre posts (Ivoclar/Vivadent, Liechtenstein) were luted with self-etch system (Panavia F2.0, Kuraray, Japan) or self adhesive system (MaxCem, Kerrhawe, Switzerland). The roots were divided three subgroups of five each. Light activation of luting agents was completed by a quartz-tungsten-halogen (Blue Swan, Dentanet, Turkey) in groups 1 and 4, a blue light-emitting diode (Blue Swan, Dentanet, Turkey) in groups 2 and 5 or a plasma arc (Remecure, Remedent, Belgium) in groups 3 and 6. After 24h storage in 100% humidity at 37°C, the specimens were sectioned transversally into 1 ± 0.05 mm thick discs. The adhesion between the post and the root canal walls was assessed using the push-out test. The data were analyzed using three-way ANOVA using luting system, curing units and root region (apical and coronal) as factors.
Results Micro push-out bond strengths were significantly affected by the type of luting agent ($P < 0.0005$). Significant differences on bond strength were observed between the coronal and apical section ($P < 0.0005$). Curing of luting agents with different light sources did not affect the bond strength of fibre posts ($P > 0.05$).
Conclusions Self adhesive systems showed higher bond strength than self-etch system. Use of different curing units had no effect on retention of translucent fibre posts.

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Microtensile bond strength of dual and chemical cured cements used in various glass fibre post

Aim To asses the micro-tensile bond strengths of two different adhesive cements and two different glass fibre posts to dentine in the apical, middle and cervical thirds of the root canal.
Methodology Forty extracted human single rooted mandibular premolar teeth were used. Post spaces were prepared using a Reforpost drill (Angelus, Brazil) (size 2, 1.3 mm) to a depth of 10 mm. The specimens were divided into four groups (A1, A2, B1, B2) of 10 teeth each. Group A1: Clearfil Esthetic Cement (Kuraray, Japan) + Everstick (Stich Tech Ltd, Finland) Group A2: C&B Metabond (Parkell, USA) + Everstick Group B1: Clearfil Esthetic Cement + Reforpost (AngelusBrazil) Group B2: C&B Metabond + Reforpost. The roots were sectioned perpendicular to the root axis into 1 mm ± 0.3 mm thick slabs (Mecatome T201A, France). Each slab was trimmed with a diamond bur to expose the resin cement on buccal and lingual sides and microtensile bond strength of each slice was determined (Micro Tensile Tester T-61010 K, US). The fracture surfaces were observed by a stereomicroscope (SZ-PT Olympus, Japan) at a magnification of ×30 to determine the failure modes. The bond strength values were evaluated using one way ANOVA and Tukey multiple comparison intervals.
Results Group A2 had the highest bond strength ($P < 0.05$). Cervical canal dentine was associated with the highest bond strength ($P < 0.05$). Adhesive failure between cement and dentine of the group A1, A2, B1, B2 were 70%, 76.6%, 76.6%, 43.3% respectively. Most of the post cohesive failure were seen in group A2 and B2 (n: 5).
Conclusions The bond strength of post systems was effected by type of post, type of adhesive cement and root canal dentine.

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Ex vivo comparison of the Push-Out Bond Strength of a new Endodontic Obturation System (Smartseal) and various gutta-percha obturation techniques

Aim To compare the adhesion between root dentine and the components of two obturation systems: Smartseal and gutta percha/AH 26, by using the push-out bond strength test.
Methodology A total of 40 extracted human single-rooted teeth were used. The root canals were instrumented using the Protaper rotary system and the canal spaces were filled as follows: Group 1, Smartseal sealer and a 0.06 taper Smartpoint calibrated to apical tip size 30; Group 2, Smartseal sealer and an F3 SmartpointPT; Group 3, AH 26 sealer and a single F3 Protaper gutta percha cone; and Group 4, AH 26 sealer and 0.02 taper gutta-percha using the cold lateral condensation technique. Two horizontal sections (coronal and apical) were removed from each root sample and the bond strength was measured using the push-out test. Data were analyzed using one-way analysis of variance (ANOVA).
Results No significant differences ($P > 0.01$) were found between the mean bond strengths of the various groups at either the coronal or apical cross-sectional level.
Conclusions There was no difference in adhesion between the Smartseal obturation system and gutta-percha/AH 26 applied using either the single cone or lateral condensation technique.

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