Abstracts of Research

To be presented at the 2014 Annual Session of the American Association of Endodontists
April 30 – May 3, Gaylord National Resort and Convention Center, National Harbor, Md.

Abstracts appear as they were submitted by the presenters. The letters in the upper left corner represent the type of presentation: OR for Oral Research Presentation, PR for Poster Research Presentation.

Please refer to the schedule below to determine when the abstracts will be presented.

Schedule of Presentations

Oral Research Presentations

Resident Sessions

**Wednesday, April 30**
Endodontic Outcomes, Genetics
Chesapeake 3
10:30 a.m. – 12:15 p.m.
Abstracts 1 – 7

Irrigation
Chesapeake 3
1:30 – 3 p.m.
Abstracts 15 – 20

Instrumentation
Chesapeake 9
1:30 – 3 p.m.
Abstracts 21 – 26

Microbiology
Chesapeake 3
3:30 – 5 p.m.
Abstracts 27 – 32

Endodontic Materials
Chesapeake 9
3:30 – 5 p.m.
Abstracts 33 – 38

**Thursday, May 1**
Clinical Endodontics
Chesapeake 3
8 – 9:30 a.m.
Abstracts 39 – 44

Oral Biology
Chesapeake 3
10 – 11:30 a.m.
Abstracts 51 – 56

Oral Research Presentations, continued

Neuroscience and Local Anesthesia
Chesapeake 3
1:30 – 3 p.m.
Abstracts 63 – 68

Stem Cells and Regenerative Endodontics
Chesapeake 3
3:30 – 5 p.m.
Abstracts 75 – 80

Predoctoral, General Dentist and Endodontist Sessions

**Wednesday, April 30**
Instrumentation
Chesapeake 9
10:30 a.m. – 12:15 p.m.
Abstracts 8 – 14

**Thursday, May 1**
Oral Biology
Chesapeake 9
8 – 9:30 a.m.
Abstracts 45 – 50

Endodontic Materials and Obturation
Chesapeake 9
10 – 11:30 a.m.
Abstracts 57 – 62

Clinical Endodontics
Chesapeake 9
1:30 – 3 p.m.
Abstracts 69 – 74

Pain, Lasers
Chesapeake 3
3:30 – 5 p.m.
Abstracts 81 – 84

Poster Research Presentations

**Thursday, May 1**
Resident Session
Exhibit Hall
8 – 11 a.m.
Abstracts 1 – 59

Predoctoral, General Dentist and Endodontist Session
Exhibit Hall
8 – 11 a.m.
Abstracts 60 – 98

Table Clinic Presentations

**Thursday, May 1**
Resident Session
Exhibit Hall
8 – 11 a.m.
Abstracts 42 – 44

Predoctoral, General Dentist and Endodontist Session
Exhibit Hall
8 – 11 a.m.
Abstracts 42 – 44
A Novel Approach to the Treatment and Outcome Assessment of Apical Periodontitis: A Double-Blind, Randomized Clinical Trial

T. Winberg, A. Fouad, I. Bennett, P. Bellingham, L. Otis, A. Fouad
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Despite clinical advancements, teeth with apical periodontitis have a consistently poorer prognosis than those without. Direct injection of an anti-inflammatory and/or antibacterial drug into the lesion may accelerate healing. This double-blind, placebo-controlled clinical trial aimed to evaluate the healing of periapical lesions treated with periapical injection of 7.5 mg doxycycline or placebo. Healing was measured on CBCT using a novel method of volumetric analysis. Twenty necrotic teeth with periapical lesions were endodontically treated in a two-visit protocol. Each lesion was injected with either doxycycline or placebo through the apical foramen immediately prior to obturation. CBCT scans were taken following treatment and four to six months later. Volume was calculated using a novel software algorithm. As proof of concept, a pilot study was conducted in a dry-skull to ascertain the correlation between the volumes calculated by the software and those calculated by direct impression. Additionally, lesion area was measured as the mean maximal diameter in three dimensions. The pilot study demonstrated that the software-calculated volume correlated well with impression volume (Pearson’s r=0.57, p<0.05). At four to six months, the mean reduction in periapical lesion volume was greater for the doxycycline group (87.4%, SD=7.31) than placebo (80.25%, SD=11.77) (p=0.175, one-way ANOVA). The volume calculated by the software algorithm was highly correlated to the lesion area as measured three-dimensionally on the CBCT scan (Pearson’s r=0.76, p<0.05). At four to six months, the mean reduction in periapical lesion volume calculated by the software correlated well with impression volume (Pearson’s r=0.57, p<0.05). However, in teeth with a diagnosis of pulpal necrosis, achieving apical patency was determined to be statistically significant in healing at follow-up (p=0.04). Conclusion: Establishing apical patency was not associated with improved clinical outcomes. However, in teeth with a diagnosis of pulpal necrosis, achieving apical patency was determined to be statistically significant in healing at one-year follow-up. This study was supported by the AAE Foundation.

Effects of Smear Layer Removal on Endodontic Outcomes

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Introduction: Mechanical debridement during root canal treatment creates a smear layer of dentin particles, tissue remnants and bacteria. A combination of ethylenediamine-tetraacetic-acid (EDTA) and sodium hypochlorite (NaOCl) has been shown to effectively remove the smear layer. Removal is not universally practiced by all clinicians. No in vivo clinical study has been performed to determine whether removal of the smear layer will result in better healing. Purpose: This prospective, randomized, double-blind clinical trial investigated the effect of smear layer removal on healing in initial nonsurgical root canal treated teeth. A standardized protocol was conducted with the exception of the final irrigation solution. Control subjects received 1ml/canal of 0.9% saline and experimental subjects received 1ml/canal of 17% EDTA as the final irrigant. A standardized radiographic and clinical evaluation, to determine healing, was conducted no less than 12 months after treatment. The data were analyzed using Fisher's exact test (α=0.05) and logistic regression. Results: Interim analysis of 95 subjects revealed no significant difference between groups (p=0.61). Logistic regression revealed variables that negatively affected healing: males (p=0.02), diabetes (p=0.02) and multi-rooted teeth (p=0.03). Conclusion: Under the conditions of this in vitro study, smear layer removal did not affect endodontic healing; however males, diabetes and multi-rooted teeth negatively affected healing. This study, #352491-8, is IRB approved and funded by Walter Reed National Military Medical Center.

A Naval Postgraduate Dental School Analysis of Initial Endodontic Treatment

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Introduction: Initial nonsurgical endodontic treatment is associated with high healing rates and clinical success. The literature contains numerous studies examining outcomes of initial endodontic treatment. Several variables have been identified as potential contributors to healing. Objective: This retrospective study evaluated the outcome of initial endodontic treatment performed by Navy endodontists and graduate endodontic residents and identified variables that negatively affect outcomes. Methods: Clinical and radiographic data were obtained from initial endodontic treatment for each subject. A one-year clinical and radiographic examination was performed. The healed rate was determined from radiographic scoring and clinical exams. Pretreatment, interappointment, and follow-up examination data were analyzed using Fisher’s Exact test. Logistic regression and odds ratios were used to evaluate the influence of multiple variables on healing outcomes. Results: This interim analysis included 188 subjects with an overall healed rate of 71.9%. Healed was defined as the absence of a radiographic lesion and no clinical symptoms. The functional rate was 95.2%, defined by the absence of clinical signs and symptoms. Logistic regression analysis indicated a negative effect on healing for those subjects presenting with: a preoperative diagnosis of pulp necrosis, periapical radiolucency, procedural complications, treatment requiring more than one appointment or smokers. Conclusion: Preliminary evaluation indicated a healed rate of 71.9% with a functional rate of 95.2%. The presence of a necrotic pulp, periapical radiolucency, procedural complication, treatment requiring more than one appointment or smoking significantly decreased healing outcomes. This study, #352272-10, is IRB approved and funded by Walter Reed National Military Medical Center.
IRE1α and XBP1: Novel Genetic Risk Factors for Periapical Periodontitis
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1University of Pittsburgh, PA, 2University of Texas Health Science Center at Houston, TX

Objectives: Individual genetic predisposition affects patient susceptibility for periapical periodontitis by altering the balance between bacterial and host factors. Inositol-requiring enzyme 1α (IRE1α) and X-box binding protein-1 are endoplasmic reticulum (ER) stress response signaling molecules that play an essential role in regulating intracellular protein homeostasis required for cell survival and optimal functioning. We hypothesize that IRE1α and/or XBP1 polymorphisms affect susceptibility for periapical periodontitis. Methods: Saliva DNA samples of 158 patients with deep caries and no periapical lesions (controls) and 110 patients with deep caries and periapical lesions (≥3 mm or larger) were identified from the Pittsburgh School of Dental Medicine’s Dental Registry and DNA Repository and analyzed by polymerase chain reaction (PCR) assays using IRE1α and XBP1 single nucleotide polymorphism (SNP) markers. Data was analyzed using PLINK. Results: For IRE1α SNPs rs196929 and rs1869590, p-values of 0.05 and 0.03 indicated frequency differences between patients with periapical bone destruction and controls. Moreover, genotypic association tests indicated association of SNP rs2239815 and rs2097461 with periapical lesions (p=0.05). A gene-gene interaction analysis for XBP1 SNP rs2239815 demonstrated association with IRE1α variant rs116550100 (p=0.04). XBP1 SNP rs2097461 was linked to IRE1α variant rs116550100 (p=0.009). Finally, haplotype analysis indicated bi-allelic expression: IRE1α and XBP1 are novel genetic risk factors that affect patient susceptibility for periapical periodontitis. This study was supported by the CTSI Start Up Program predoctoral award through the Clinical and Transitional Science Institute and the Institute for Clinical Research Education and NIH R01-DE018914. Data for this project was provided by the Dental Registry and DNA Repository, which is supported by the University of Pittsburgh School of Dental Medicine and NIH STLI-RR024155.

FOX3P DNA Methylation Levels as a Potential Biomarker in the Development of Periapical Lesions
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1University of Texas Health Science Center at Houston, TX, 2University of São Paulo-Bauru, Brazil

Epigenetic mechanisms, such as DNA methylation, can modify gene expression patterns without changing the DNA sequence. DNA methylation is a signaling tool that cells use to lock genes in the “off” position. Errors in methylation have been correlated to a variety of human diseases. Here, we hypothesized that DNA methylation in cytokine genes may contribute to the development of periapical lesions. We analyzed the DNA methylation patterns of a panel of 22 cytokine gene promoters using a pathway-specific, real-time polymerase chain reaction array (EpiTect Methyl II) in 27 human periapical granulomas, four apical cysts and four healthy gingiva tissue samples collected post-operatively. mRNA expression analysis was also performed. Reactions were performed in triplicates. SABiosciences’s hierarchical clustering and methylation and Prisim6 software were used for data analysis. FOX3P promoter showed the highest level of methylation in comparison to the other studied genes in both periapical granulomas and apical cysts (p<0.0001). FOX3P mRNA expression was markedly increased in periapical lesions compared to healthy gingiva tissues (p<0.0001), resulting in a negative correlation with the observed FOX3P methylation levels. Additionally, FOX3P mRNA expression was positively correlated with IL-10 and TGFβ levels in inactive lesions. Accordingly, compelling evidence suggests that FOX3P acts as a master switch governing the development and function of CD4+ regulatory T cells, which may inhibit immune responses and temper inflammation. The observed differential methylation patterns of FOX3P in periapical lesions suggest that methylation patterns of Tregs may be crucial in determining its suppressive activity and may be involved in periapical lesion development.

Interleukin-1 beta (IL1β) Gene Polymorphisms and Periapical Lesion Development in Individuals With Deep Caries
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1University of Texas Health Science Center at Houston, TX, 2Rio de Janeiro, Brazil, 3University of Pittsburgh, PA, 4University of São Paulo-Bauru, Brazil

It has been proposed that individual genetic predisposition may contribute to persistent apical periodontitis. Cytokines are associated with levels of inflammation and are involved in caries, pulpal and periapical tissue destruction. In this study, we hypothesized that polymorphisms in cytokine genes may contribute to an individual’s increased susceptibility to apical tissue destruction in response to deep carious lesions. Subjects with deep carious lesions, with or without periapical lesions (≥3 mm) were recruited at the University of Pittsburgh and the University of Texas Health Science Center at Houston. Genomic DNA samples of 316 patients were sorted into two groups: 136 cases with deep carious lesions but no periapical lesions (controls) and 180 cases with deep carious lesions and periapical lesions (cases). Nine single nucleotide polymorphisms in IL1β, IL6, TNF, RANK, RANKL and OPG genes were selected for genotyping. Genotypes were generated by endpoint analysis using Taqman chemistry in a real-time polymerase chain reaction instrument. Differences in allele and genotypic frequencies between cases and controls were calculated using Fisher’s chi-squared tests. A total of 93 human periapical granulomas and 24 healthy periodontal ligament tissues collected post-operatively were used for mRNA expression analyses of IL1β. A SNP in IL1β (rs1143643) showed allelic (p=0.02) and genotypic (p=0.004) association in the individuals with deep caries and periapical lesions. IL1β was highly expressed in granulomas (p<0.0001). Variations in IL1β may be associated with periapical lesion formation in individuals with untreated deep carious lesions. Future studies could help predict host susceptibility to developing periapical lesions.

An In Vitro Comparative Analysis on Shaping Ability of Two NiTi Reciprocating Endodontic Instruments Using Micro-Computed Tomography
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The purpose of this study was to evaluate the shaping ability of two reciprocating systems in regards to the apical transportation and the volume in simulated canals using micro-computed tomography. Sixty Endo-Training blocks were divided in two groups: Group 1 (n=30) were shaped with WaveOne 25.08 and Group 2 (n=30) with Easy Prodesign. The canals were scanned pre- and post-instrumentation using the micro-CT SkyScan 1176 using an 18 μm resolution. Mann-Whitney-Wilcoxon test has been used to calculate the differences between both systems and the mean apical transportation at 1 mm was 40.46 μm for Group one and 40.87 μm for Group two (p>0.05). There was no significant difference of apical transportation between the two files. However the volume increased significantly more using Easy Prodesign (7.62 mm3) when compared to WaveOne (5.06 mm3), p<0.05. It can be concluded that the WaveOne system allows a more conservative shape when compared to Easy Prodesign. No significant differences in regards to canal transportation were found between both systems.
Preparation Shape Tendencies of Recently Developed Root Canal Preparation Systems
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Objective: The achievement of symmetric three-dimensional root canal preparation shapes is the final aim of a root canal preparation; thus, the aim of this research was to investigate the root canal final shape achieved with a reciprocating, the SAF and two conventional preparation systems. Methods: The shaping tendencies of the Reciproc® and Mtwo (VDW, Munich, Germany), SAF (ReDent, Raanana, Israel) and ProTaper® Universal (Maillefer, Ballaigues, Switzerland) systems were investigated with simulated root canals in clear casting resin blocks. A total of 20 simulated canals were enlarged according to the manufacturers’ preparation recommendations and evaluated at seven different levels (0, 2, 4, 6, 8, 10 and 14 mm from apical). The results were compared with a theoretically ideally shaped canal by means of a photographic double exposure method. The results were analyzed with Student’s t-test (p=0.05). Results: None of the systems produced an ideal preparation shape. Although a tendency to over-shape the apical third was observed, especially at the convex side of the canals, the results showed that the original canal axis was not significantly transported from its original position with any of the systems. The shapes obtained in the middle and coronal canal thirds, although they were different between all systems; they could be determined as clinically acceptable. Conclusions: The results of this study suggest that the selection of a preparation system in the daily practice can be made based only on the individual ergonomic aspects of the systems.

Influence of Glide Path in Centering Ability and Preparation Time of WaveOne™ and Reciproc® in Root Canal Treatment of Curved Canals
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Objective: Evaluate the influence of glide path in centering ability and preparation time of two systems in mesial root preparations of ex vivo mandibular molars. Methods: Sixty mesial roots of mandibular molars, with curvatures between 25° and 39°, divided in four groups (n=15). Root length was established in 12 mm and patency obtained with #10 K file, WL was 1 mm shorter. G1: WaveOne™ Primary + Glide Path, G2: WaveOne™ Primary, G3: Reciproc® R25 + Glide Path, G4: Reciproc® R25. All groups were initially irrigated with 5 mL of 2.5% NaOCl. Manual glide path was performed with manual K-files 10, 15 and 20. Reciprocating preparation was done in three-peeking motion with 3–4 mm amplitude. After each movement, instrument was cleaned with gauze and patency checked. Movements were repeated until reaching WL. A 2 mL irrigation of NaOCl was done between instruments, both in glide path and instrumentation. Final 5 mL irrigation with EDTA was done. Preparation time was recorded in seconds, from initial patency until final irrigation. Initial and final X-rays were taken to analyze the amount of dentin removed. An apparatus was built to line roots. With Photoshop CS5, images were superimposed and discrepancies assessed at 3, 6 and 9 mm distances from apex. Results: Kruskal-Wallis (p>0.05) presented G2a and G4a faster than G1b and G3b. No difference regarding centering ability. Manual glide path didn’t influence centering ability and increased the total time of preparation with WaveOne™ and Reciproc®.

Sophomore Student Evaluation of the ‘WaveOne™’ Technique in Preclinical Endodontics
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Purpose: To evaluate sophomore dental students’ preclinical learning of the WaveOne™ technique. Introduction: Successful endodontic instrumentation incorporates many factors. These include straight line canal access, proper working length, cleaning and shaping, maintaining canal patency, developing an apical stop and establishing a glide plane. Methods and Design: This was the initial introduction of the WaveOne™ System into the preclinical endodontic laboratory course at Meharry School of Dentistry. Students were provided evidence-based didactic information that included lectures, written protocols, scientific articles, videos and small group discussions. Additionally, an all-day lecture was presented by Professor Sergio Kuttler, an expert in endodontics. The benefits and limitations of both hand and reciprocating rotary instrumentation were reviewed. In the preclinical course, students performed the WaveOne™ technique on at least five teeth. At the end of the course, a Final Clinical Competency Exam was administered. Students anonymously completed an evaluation survey. Results: The survey revealed that students understood the benefits of this technique (88%). Moreover, 81% felt that their WaveOne™ clinical experiences during their junior and senior years could be incorporated into private practice. Survey responses identified that more emphasis should be placed on hand instrumentation (63%), specifically maintaining canal patency (58%) before the introduction of the WaveOne™ reciprocating file. It was also discovered that there was limited knowledge transfer between students (58%). This study was supported by DENTSPLY Tulsa Dental Specialties.

Force and Torque Induced by Two Single-Length Rotary Systems Along the Three Root Canals Thirds
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Objectives: To compare the force and torque induced by the instruments of two single-length rotary systems, ProTaper® Universal (PTU) and ProTaper® Next (PTN), along the three root canal thirds. Material and Methods: Each independent canal of six mesial roots of mandibular molars were randomly assigned to be prepared with a new set of either PTU or PTN after a glide path was verified. The tests were run in a standardized fashion in a torque-testing platform. Value for torque (Ncm) and force (N) induced along the full length of root canal length were registered. Analysis of Variance and Tukey’s post-hoc tests were applied to compare the peak torque and maximum force induced by each instrument in the apical, middle and coronal third. Results: All instruments induced a significantly higher peak torque (p<0.001) and maximum force (p<0.002) when shaping the apical third of small root canals, compared to the other two thirds. The mean peak torque developed by PTN varied from 0.04 Ncm in coronal or 0.4 Ncm in middle to 3.7 Ncm in apical third and the mean maximum force from 0.5 N in coronal to 17 N in apical third. The mean peak torque developed by PTU ranged from 0.07 Ncm in coronal or 0.5 Ncm in middle to 2.8 Ncm in apical third and the mean maximum force from 1.1 N in coronal to 14.5 N in apical third. Conclusions: Single-length rotary systems induced a significantly higher peak torque and maximum force in the apical than in the coronal or middle third.
OR13

Micro-Computed Tomographic Analysis of Molars Prepared Using ProFile® NiTi Rotary Instruments and Reciproc® Single File Technique
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Aim: To evaluate root canal morphology after instrumentation with Reciproc® and ProFile® instruments assessed by micro-computed tomography.
Methodology: Ten maxillary and 10 mandibular first molars were randomly selected. Specimens were randomly divided in two groups and scanned before and after root canal preparation to a size 25.06 ProFile® and R25 Reciproc® respectively. Three-dimensional models were reconstructed and the total volume of dentine removed and the volume of the coronal, middle and apical thirds were calculated, as well as the average deviation of the root canal axis in the three thirds and the values compared. Student’s t-test was used to determine the difference between the two experimental groups (p<0.05). A qualitative evaluation of root canal preparation was also performed. Results: Only one instrument fractured in the ProFile® group. Deformations occurred in two ProFile® instruments. No statistically significant differences were noted between the groups in the total volume of dentine removed, except for the volume of the coronal third (p<0.05) and the volume of the apical third (p<0.05) of the distobuccal canal of maxillary and in the mesial canals of mandibular molars, where ProFile® instruments produced less canal volume enlargement in the apical third and more canal volume enlargement in the coronal third (p<0.05). No statistically significant differences were found in the root canal axis deviation. Conclusion: Under the conditions of this study, both the systems analyzed were able to prepare molar teeth with similar amount of dentine removal and low risk of procedural errors.

OR14

Cutting Efficiency of Reciproc® Instruments and WaveOne® Instruments Used With Different Reciprocating Motions
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The aim of the present study was to evaluate the cutting efficiency of two new reciprocating instruments, Reciproc® and WaveOne®. Methods: 20 Reciproc® R25 (VDW, Munich, Germany) and 20 WaveOne® Primary (DENTSPLY Maillefer, Ballaigues, Switzerland) were activated by using a torque-controlled motor (Silver Reciproc, VDW, Munich, Germany) divided in four groups (n=10): Group 1: Reciproc® instruments activated with Reciproc® ALL movement; Group 2: Reciproc® instruments activated with WaveOne® ALL movement; Group 3: WaveOne® instruments activated with Reciproc® ALL movement; Group 4: WaveOne® instruments activated with WaveOne® ALL movement. The device used for the cutting test consisted on a main frame to which a mobile plastic support for the handpiece is connected and a stainless-steel block containing a plastic block against which the cutting efficiency of the instruments has been tested. The length of the resin block cut in one minute was measured in a computerized program with a precision of 0.1 mm. Mean and standard deviations of each group have been calculated and data were statistically analysed with a one-way ANOVA and Bonferroni test (p<0.05). Results: Reciproc® R25 reported a higher cutting efficiency than WaveOne® Primary for both the movements used (p<0.05); in particular, Reciproc® instruments used with their proper reciprocating motion (Group 1) presented a statistically significant higher cutting efficiency than WaveOne® instruments used with their proper reciprocating motion (Group 4) (p<0.05). There was no statistically significant difference between the two movements for both instruments (p>0.05). Conclusion: Reciproc® instruments demonstrated statistically higher cutting efficiency respect to the WaveOne® instruments.

OR15

Fluid Dynamics in Conventional and Two Ultrasonic-Assisted Irrigation Methods: A Novel Experimental Approach
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Introduction: A thorough understanding of the irrigation dynamics of different modes of irrigation, and corresponding capacity for biofilm elimination, will support improved disinfection strategies. Aim: This study aimed to develop a clinically realistic, standardized model that allows real-time analysis of fluid dynamics, and correlates fluid-flow characteristics with biofilm elimination.
Methodology: Experiment 1: Soft lithography-based, microfabricated models were created from micro-CT scans of an instrumented maxillary incisor to study the fluid dynamics generated by two ultrasonic-assisted irrigations: continuous (CUAI) and intermittent (IUAI), and a syringe-based irrigation (SI). Real-time imaging of fluid movement was recorded using Particle-Image-Velocimetry. Experiment 2: Three-week-old Enterococcus faecalis biofilm was grown in the microfabricated models. The antibiofilm capacity of CUAI, IUAI and SI was assessed using a crystal violet assay and digital image processing analysis. Results: CUAI generated the highest velocity/shear stress at 1 mm and 3 mm levels from the working length (WL). IUAI generated the most consistent levels of shear stress in the apical 3 mm. The SI produced similar magnitudes of velocity/shear stress as the CUAI at 1-2 mm from the WL. CUAI resulted in a significant reduction of biofilm with respect to the untreated control (p<0.005), while no significant difference was seen for SI or IUAI groups (p=0.435, 0.356 respectively). Conclusion: The soft lithography-based microfabricated models provided a novel method to correlate irrigation dynamics of irrigation modes with their antibiofilm capacity. CUAI showed the highest velocity/shear stress in the apical 3 mm, which correlated with the highest degree of biofilm elimination of the tested groups. None of the groups showed complete biofilm elimination. This study was supported by the AAE Foundation and the Canadian Academy of Endodontics Endowment Fund Alpha Omega Foundation of Canada Research Grants 2012.

OR16

Comparison of Apical Extrusion of NaOCl Using Four Root Canal Irrigation Systems: An In Vitro Study
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Objective: The purpose of this study was to evaluate NaOCl apical extrusion using negative apical pressure (EndoVac®), sonic agitation (EndoActivator®), conventional positive pressure (Max-i-Probe®), and Photon Induced Photoacoustic Streaming (PIPS®) laser irrigation in an in vitro gel model. Methods: Extracted mandibular and maxillary central incisors (n=18) were prepared to size 35/.04 and 55/.04, respectively. Teeth were mounted in transparent containers with clear acrylic and suspended in a color changing pH sensitive gel creating a closed-system. Using a cross-over design, each tooth was sequentially irrigated with 6% NaOCl with each device following manufacturers’ recommendations. Each tooth served as its own control. Pre- and post-irrigation clinical and proximal view photographs served to measure the longest distance of extrusion and were analyzed with ImageJ software. Mean results were analyzed using Kruskal-Wallis and Dunn’s post-hoc test (p<0.05). Results: There was no significant difference between EndoVac®, EndoActivator® and negative control (passive extrusion) groups. EndoVac® and EndoActivator® groups produced significantly less extrusion than PIPS®. Max-i-Probe® extrusion results were more variable than EndoActivator® but had no significant difference. Across all irrigation systems, there were no significant differences between teeth prepared to 55/.04 and 55/.04. Conclusions: Under the in vitro conditions of this study, EndoVac® and EndoActivator® produced significantly less apical extrusion compared to the PIPS® laser. Apical preparation size did not significantly affect extrusion of irrigant. The potential for apical extrusion may or may not correlate when techniques are used clinically. This study was supported by the Air Force Institute of Technology and the Louisiana State University School of Dentistry.
**OR17**

**Qualitative Analysis of Para-Chloroaniline on Dentin Surface and Dentine Tubules of Root Dentin Irrigated With NaOCl, CHX and QMix® Using Time-of-Flight Secondary Ion Mass Spectrometry**

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Introduction: QMix® is a novel irrigant, contains chlorhexidine (CHX); however, its chemical interaction with NaOCl within root dentin is not well studied. The Aim: This study aims to determine the presence of precipitate and para-Chloroanilnine (PCA) on the surface of root dentin or in dentin tubules (DT) when irrigated with NaOCl, EDTA and final irrigation with CHX or saline and QMix®, using of qualitative Time-of-Flight Secondary-Ion-Mass-Spectrometry (TOF-SIMS) surface analysis. Materials and Methods: Human upper molars were sectioned to obtain dentin blocks, which were embedded in resin. The flat dentin surface exposed using a cryo-ultramicrotome, were then irrigated with 2.5% NaOCl followed by 17% EDTA followed by 2.5% NaOCl. The samples were divided in two groups for their final irrigation: Group A: 2% CHX and Group B: saline and QMix®. Samples were subjected to six separate TOF-SIMS spectra analysis scans. Cross-sections of these blocks were prepared using microtome and further subjected to TOF-SIMS analyses. Results: CHX samples (Group A) were covered with irregular precipitate containing PCA and occluding dentinal tubules. QMix® samples (Group B) was evenly covered by 13-carbon chain product without precipitate and with patent dentinal tubules. No penetration of precipitate or chlorine containing product into dentinal tubules was detected in any sample. Conclusion: No precipitate or PCA were found penetrating into DT, although dentin tubules were covered with precipitate containing PCA when CHX was used after NaOCl. No precipitate was formed when QMix® was used after NaOCl and saline. This study was supported by the Canadian Academy of Endodontics Endowment Fund.

**OR19**

**Effect of Application Time on Chlorhexidine Substantivity in Root Canals: An In Vitro Investigation**

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Objectives: To determine the effect of application time on substantivity of chlorhexidine used as a final rinse in root canal treatment of maxillary anterior teeth in vitro. Materials and Methods: Forty-five extracted single-canal maxillary anterior teeth with fully developed apices were decoronated and instrumented to within 1 mm of the apex, coronally flared with Gates Glidden burs, and prepared to a size 45/.04. Three percent NaOCl was used an irrigant during instrumentation. Seventeen percent EDTA was applied for one minute after cleaning and shaping had been completed for smear layer removal. Two percent CHX rinse was applied to three groups of 45 specimens for one, two and five minutes in the canal. Paper points were used to dry the canals. The teeth were grooved longitudinally on the buccal and lingual surfaces and split with Rongeurs, providing two halves of each root and resulting in 90 samples per group. The samples were stored at 37°C under 100% relative humidity. Each group was randomly divided into three subgroups (n=30), and substantivity was evaluated after 24 hours, 7 days and 30 days of incubation. The amount of CHX (in micrograms) was measured through UV absorption at 255 nm. Statistical analysis was performed by two-way ANOVA. Results: Preliminary data indicate application time may have an effect on chlorhexidine substantivity during root canal treatment. Conclusions: The use of chlorhexidine as a final rinse in root canal treatment may be beneficial due to its substantivity nature even with limited application time.

**OR18**

**Investigation of Stem Cell Survival After Photon Induced Photoacoustic Streaming**

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The purpose of this study was to investigate the survival of stem cells from human exfoliated deciduous (SHED) teeth seeded within ex vivo root canals that had been irrigated with endodontic irrigants or plant extracts activated using Photon Induced Photoacoustic Streaming (PIPS). Extracted permanent human teeth (n=20) were sterilized and prepared to a final apical size of 20/.04 using Vortex Blue™. The root canals were randomly assigned to the following different irrigation solutions: 6% NaOCl neutralized by 4% Sodium Thiosulfate, 1.5% NaOCl, Tea Tree Oil and Morinda Citrofolia. The irrigation solutions and EDTA were all activated by the PIPS Endodontic Fiber Tip (Lares Research, Chico, CA) using an 2940 nm Er-YAG laser (Fidelis, Fotona, Ljubljana, Slovenia) for 150 seconds at 10 Hz and 50 mJ. SHED (106/mL) was added to each of the root canals, which were maintained in cell culture for seven days. SHED survival was measured using a Lactate Dehydrogenase Membrane Integrity Assay (CytoTox-ONE, Promega, Madison, WI) and analyzed by ANOVA at a significance of p<0.05. SHED survival varied between 55.7% to 85.8% for the irrigation treatments, but was statistically similar (p<0.05). Some patients and endodontists are interested in using plant extracts for root canal irrigation, but more data is needed to determine which plant extracts are the least cytotoxic for irrigating root canal systems and the most beneficial for maintaining SHED survival. This study was supported by the AAE Foundation and Nova Southeastern University. Materials were provided by DENTSPLY Tulsa Dental Specialties.

**OR20**

**Effect of Endodontic Chemicals on the Ultrastructure, Chemical and Mechanical Characteristics of Dentin Hard Tissue**

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The ultrastructural and chemical characteristics of dentin play a crucial role in the mechanical integrity of teeth. The purpose of this study was to examine the effect of chemicals used in root canal treatment on the ultrastructure, chemical composition and mechanical properties of root dentin. One hundred eighty root dentin sections were divided into six groups and treated with 1) 5.25% NaOCl, 2) 17% EDTA, 3) 5.25% NaOCl followed by 17% EDTA, 4) 5.25% NaOCl followed by 17% EDTA and 5.25% NaOCl, 5) 5.25% NaOCl followed by QMix® and 6) water. The ultrastructure of dentin was analyzed by Scanning Electron Microscopy, the chemical composition (amide/phosphate) were determined qualitatively using Attenuated-Total-Reflectance Fourier-Transform-Infrared-Spectroscopy and the mechanical properties (toughness, strength and elastic modulus) were measured using compressive testing. The data was analyzed by one-way ANOVA and Tukey’s post hoc test (p<0.05). The SEM analysis showed that the irrigation sequences in groups three to five resulted in complete removal of smear layer, whereas in group four, erosion of peritubular and intertubular dentin was observed. The amide/phosphate ratio determined by the ATR-FTIR analysis for group four was lower than that of groups three and five. The groups treated with EDTA (groups two and three) showed reduction in the compressive strength and significant reduction in the elastic modulus, while the groups treated with NaOCl (groups one and four) showed reduction in the toughness properties when compared to the untreated control. The findings of this study highlight that the application of different chemical protocols lead to distinct changes in the dentin ultrastructure, amide/phosphate ratio and mechanical properties of dentin. This study was supported by the University of Toronto, Startup Fund.
OR21

Effect of Repeated Use and Sterilization on the Cutting Efficiency of Hyflex® CM™ NiTi Rotary Files

K. Sabey

Abstract

Four, five and six sterilizations of Hyflex® CM™ NiTi rotary files showed no significant decrease in efficiency. A significant decrease in efficiency was found following seven, eight sterilizations, and a return to no significant difference following four, five and six sterilizations. No significant difference was detected following the first sterilization. Cutting efficiency was determined by measuring the load required to maintain canal anatomy around severe curvatures, even after multiple uses. Preloading of distortion angles of size 40/04 on TYP CM files had reduced the fatigue resistance. Preloading of distortion angles of size 40/04 on TYP files would not be detrimental to the file's ability to fatigue resistance. Moderate or extensive preloading of distortion angles of size 40/04 on TYP CM files had reduced the fatigue resistance. Conclusions: TYP CM instruments have higher fatigue resistance than TYP instruments with preloading of distortion angles and without preloading of distortion angles. Fatigue resistance of TYP CM and TYP instruments seems affected by preloading of distortion angles respective of the file size. This study was supported by the AAE Foundation and the Canadian Academy of Endodontics. Files were donated by Clinical Research Dental.

OR22

Influence of Previous Angular Deformation on Cyclic Fatigue Resistance of K3XF Instruments

A. Riyahi

Abstract

The fractured files in the precycling groups showed the typical pattern of fatigue failure. Conclusions: A high amount of precycling of distortion angle reduces the fatigue resistance of K3XF and K3 files. The fatigue resistance of K3XF files is higher than that of K3 files in all preloading and no-preloading groups. This study was supported by the AAE Foundation and the Canadian Academy of Endodontics. Files were donated by SybronEndo.

OR23

The Effects of Repeated Use and Sterilization on the Cutting Efficiency of Hyflex® CM™ NiTi Rotary Files

S. Seago

Abstract

Recent NiTi manufacturing processes have resulted in an alloy which remains in a twinned martensitic phase at operating temperature. This alloy has been shown to have increased flexibility with added tolerance to cyclic and torsional fatigue. These improvements are touted to allow files to safely maintain canal anatomy around severe curvatures, even after multiple uses. Objective: The current study evaluated the effect of repeated use and sterilization upon the cutting efficiency of 35/04 Hyflex® CM™ NiTi rotary files. Methods: Cutting efficiency was determined by measuring the load required to maintain a constant feed rate through 5 mm of bovine cortical bone. Files (n=30) were autoclaved following each use according to manufacturer recommendations. Files were tested through 10 uses and nine sterilizations, with the control group not subjected to sterilization before testing. Mean data were analyzed using multiple-factor ANOVA and Dunnett's post-hoc test (p<.05). Results: There was no statistically significant difference in cutting efficiency between sterilization cycles or use cycles. Conclusion: TYP CM instruments have higher fatigue resistance than TYP instruments with preloading of distortion angles and without preloading of distortion angles. Fatigue resistance of TYP CM and TYP instruments seems affected by preloading of distortion angles respective of the file size. This study was supported by the AAE Foundation and the Canadian Academy of Endodontics. Files were donated by Clinical Research Dental.

OR24

Evaluation of Apical Transportation in Curved Canals Instrumented With Austenite and Martensitic Files

D. Andrade

Abstract

Recently, a new concept in rotary file system has been introduced and commercialized in the market as new rotary files called martensitic files. Martensitic files are made of specific NiTi alloys that receive a special thermal treatment to undergo a non-diffusive transformation from an austenitic to a martensitic lattice. Due to their physical characteristics, martensitic files are thought to be more advantageous in maintaining root canal morphology in curved canals than austenitic files. The aim of this study was to evaluate in vitro apical transportation in curved canals instrumented with one austenite rotary system: EndoSequence® (Brasseler, Savannah, GA) and three martensitic rotary systems: 10 Series™ (D&S Dental, Johnson City, TN), Vortex Blue™ (DENTSPLY Tulsa Dental, Tulsa, OK) and Hyflex® CM™ (Coltene/Whaledent, Cuyahoga Falls, OH). Sixty maxillary molars with curved mesiobuccal roots were randomly divided into four groups (n=15). Mesiobuccal canals were instrumented up to a size .04/25 of each system according to the manufacturers' recommendations. A double-digital radiographic technique was used, and the apical transportation at 0.0 - 4.0 mm from the working length was measured with AutoCAD (Autodesk Inc, San Rafael, CA). Statistical analysis was performed with one-way analysis of variance, and significance was set at .05. There was no statistically significant difference between the groups at any levels (0.0 mm, p=.436; 1.0 mm, p=.094; 2.0 mm, p=.782; 3.0 mm, p=.501; 4.0 mm, p=.156). The results indicate that three martensitic files had the same effectiveness as the austenite file in regards to apical transportation when curved canals were instrumented up to a size .04/25.
Objectives: To determine the ability of Reciproc®-25 file to reach full-canal length in MB2 canals of maxillary first molars with root canal treatment indication. Materials and Methods: Twenty maxillary first molars with root canal treatment indication were included in this study. Cavity access was made on each tooth and root canals were located under microscope magnification. After locating MB2 canal opening, and the ability to reach full-canal length was recorded, as well as the number of cycles required for each file to reach full-canal length. Results: Seven teeth were assigned to the patent canal group and 13 teeth were assigned to the non-patent canal group. A new Reciproc®-25 file was used to prepare each one of the MB2 canals in both groups, and the ability to reach full-canal length was recorded, as well as the number of cycles required for each file to reach full-canal length. Conclusions: The Reciproc®-25 file is able to reach full-canal length in MB2 canals of maxillary first molars disregarding canal patency.

Purification and Biological Activity of Serine Lipids of Porphyromonas endodontalis
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Porphyromonas endodontalis has been shown to populate necrotic root canal systems and may contribute to apical bone loss. Recent work has shown that P. endodontalis synthesizes a novel serine lipid class (Lipid 654) similar to that produced by P. gingivalis. Objective: The purpose of this study was to isolate P. endodontalis Lipid 654 in very high purity and characterize its structure and biological activity in RAW 264.7 cells and HEK293 cells. Methods: P. endodontalis (ATCC 35406, type strain) was grown under anaerobic conditions and centrifuged. P. endodontalis total lipids were recovered using the Bligh and Dyer phospholipid extraction procedure and were fractionated by semipreparative normal phase HPLC using hexane-isopropanol-water (6:8:0.75, vol/vol/vol). Fractions containing Lipid 654 were identified by mass spectrometric (MS) analysis and were pooled, refractionated using an acidic HPLC solvent, and tested for biological activity. Results: MS analysis revealed that repurified Lipid 654 contained <0.1% contaminating lipids. TNF-α secretion from RAW 264.7 cells was promoted equally by both P. endodontalis and P. gingivalis Lipid 654 preparations and was significantly attenuated by anti-mouse TLR2 neutralizing antibody. Treatment of HEK293 cells, stably transfected to express TLR2 and an NF-κB-dependent secretory alkaline phosphatase reporter, revealed that both P. endodontalis and P. gingivalis Lipid 654 preparations engage human TLR2 with equal potency. Conclusions: Our results show that P. endodontalis Lipid 654 engages TLR2 with stimulation of TNF-α secretion from macrophages and activation of the transcription factor NF-κB, and these responses may contribute to apical bone destruction associated with necrotic pulps. This study was supported by R01 DE021055.

Role of PGRP-S/DnaK Complex in Pathogenicity of Fusobacterium nucleatum
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Introduction: Fusobacterium nucleatum (Fn) is one of the most prevalent endodontic pathogens. It has been shown that Fn is able to induce apoptotic cell death, and this ability appears to be mediated through the immune cells being aggregated. PGRP-S is a member of Peptidoglycan recognition protein (PGRP) and an important host innate immunity arm capable of peptidoglycan and allied bacteria recognition. Hsp70 (heat shock-binding protein 70) is a member of the large stress-induced protein family which makes stable complexes with PGRP-S and induces apoptotic cell death. DnaK homologues (prokaryotic Hsp70) has been identified and characterized in H. pylori. Hsp70 interacts with Fn and its role in cell death induction. Materials and Methods: Jurkat T cells were co-cultured with Fn (PK1594) with or without antibodies (Abs) to block DnaK and PGRP-S. Flow cytometry and Caspase-Glo3/7 assay were used to evaluate the binding and apoptosis. Nonspecific IgG antibodies were used as controls. ANOVA with post-hoc Tukey’s test was used for statistical analysis. Results: After neutralizing the DnaK/PGRP-S complex formation, flow cytometry revealed significant decrease in binding and aggregation between Jurkat T cells and Fn (J + Fn = 64.3 ± 1.5, J + Fn + Abs = 18.7 ± 2.9, p<0.05). In addition, caspase assay showed a significant decrease in the amount of apoptosis (J + Fn = 1,700 ± 479, J + Fn + Abs = 664 ± 161, p<0.05). Conclusion: Our results showed that PGRP-S/DnaK Complex may play a significant role in the aggregation and apoptosis of Jurkat T cell induced by Fn. This study was supported by NIH NIDCR 5K08DE016872.
OR29
Efficacy of Propolis Against *Fusobacterium nucleatum* Biofilm
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Objective: This study aims to demonstrate the efficacy of propolis against *Fusobacterium nucleatum* common in primary endodontic infections and biofilm formation to support its potential use as an intracanal medicament. Materials and Methods: Propolis (Ecuadorian Rainforest, LLC) was added to cultures of *F. nucleatum* in microtiter plates ranging from 390 mg/ml to 50,000 mg/ml. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and the minimum biofilm inhibitory concentration (MBIC) were determined after a 48-hour period. The MBIC was determined by fixing biofilm to the wells and using crystal violet staining with spectrophotometry. The MBC was examined by plating solution from each concentration sample well. Results & Statistical Analysis: MIC of *F. nucleatum* was 6,250 mg/ml and MBC was 1,562.5 mg/ml. There was no MBC at any of the test concentrations. Propolis appeared to inhibit bacterial growth and biofilm formation but did not appear to be bactericidal at any of the tested concentrations. Group comparisons were performed using one-way ANOVA, followed by pair-wise comparisons among groups using Tukey’s method to control the overall significance level at 5%. Conclusions: The results of this study indicate that propolis has an MIC and MBIC when tested *in vitro* against *Fusobacterium nucleatum* although does not show an MBC. There appears to be potentially significant interaction of propolis with biofilm. The results may contribute to the ability to develop a proper concentration of propolis to use *in vivo* when treating endodontic infections.

OR30
The Antibacterial Activity of Biodentine™ and MTA Against Enterococcus faecalis
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The purpose of this study was to evaluate and compare the antibacterial activity of Biodentine™ and MTA against *Enterococcus faecalis*. The antibacterial activity of Biodentine™ and MTA were evaluated by the agar diffusion method against three different strains of *E. faecalis*: two lab strains (OG1RF and ATCC 19434) and one clinical isolate (EF 11-2). *E. faecalis* strains were diluted to obtain a suspension of approximately 5 x 10^8 concentrated colony-forming units/ml in sterile trypticase soy broth (TSB). Three different strains of *E. faecalis* were seeded on Mueller-Hinton agar plates with sterile cotton swabs. Kirby-Bauer wells were filled with freshly manipulated Biodentine™ MTA and 6 mm paper disks with Vancomycin or PBS. They were immediately placed on the seeded *E. faecalis* agar plates. This was done three times independently under aerobic conditions. After pre-diffusion of the test materials for two hours at room temperature, test plates were incubated at 37°C and evaluated at 24 hours. The antimicrobial activity of the materials were evaluated by measuring zones of inhibition by digital photography using ImageJ software. The results showed that Biodentine™ and MTA did not exhibit any antibacterial activity against three different strains of *E. faecalis* when grown aerobically. This study was supported by the AA Foundation.

OR31
Evaluation of the Antifungal Biofilm Activity of EndoSequence® Root Repair Material Compared to MTA
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*Candida albicans* is the most numerous commensal and potentially pathological yeast in the human oral cavity. *C. albicans* is able to penetrate dentinal tubules of the root canal system and is able to create a biofilm associated with chronic periapical lesions. The purpose herein was to investigate the ability of *C. albicans* to form a biofilm on EndoSequence® Root Repair Material Putty (ERRM-Putty) compared with mineral trioxide aggregate (MTA). Methods: ERRM-Putty, Gray-MTA and White-MTA were packed into sterilized IV tubing and allowed to set for three or 24 hours, and then challenged with *C. albicans*. To analyze the antifungal activity mechanism, culture media was conditioned first with tested materials for 24 hours, before transferring to wells containing test samples. Results & Statistical Analysis: MIC of *C. albicans* was 6,250 mg/ml and MBIC was 1,562.5 mg/ml. There was no MBC at any of the test concentrations. Propolis appeared to inhibit bacterial growth and biofilm formation but did not appear to be bactericidal at any of the tested concentrations. Group comparisons were performed using one-way ANOVA, followed by pair-wise comparisons among groups using Tukey’s method to control the overall significance level at 5%. Conclusions: The results of this study indicate that propolis has an MIC and MBIC when tested *in vitro* against *Fusobacterium nucleatum* although does not show an MBC. There appears to be potentially significant interaction of propolis with biofilm. The results may contribute to the ability to develop a proper concentration of propolis to use *in vitro* when treating endodontic infections.

OR32
Antimicrobial Effectiveness of Medicaments, Irrigating and Chelating Agents on *E. faecalis*
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The purpose of this study was to compare antimicrobial effectiveness of endodontic irrigating solutions on the growth of an endodontic pathogen, *Enterococcus faecalis*. Sodium hypochlorite (NaOCl), BioPure™ MTAD (MTAD) and Chlorhexidine gluconate (CHX) solutions were tested in a serial dilution series in Brain-Heart Infusion broth. Each dilution was replicated three times from the stock concentration down to 0.001% for a total of 109 samples, including five negative and five positive controls. 50ug/ml of *E. faecalis* (ATCC#29212) containing 10^5 cells were added to a volume of 10 ml per test-tube for each irrigating solution dilution. Growth of *E. faecalis* in the diluted irrigating solutions was measured using spectrophotometry at wavelength of 600 nm. Raw data was analyzed using Analysis of Variance statistical tests at significance of p<0.05. Differences were found in the antimicrobial effectiveness (p<0.05). Rank order from most to least was: CHX, MTAD and NaOCl. The results suggest care is needed when diluting irrigating solutions for root canal disinfection. NaOCl should not be diluted below 3%, MTAD below 1.1% and CHX below 0.05% to retain their ability to completely disinfect *E. faecalis*. This study was supported by the AA Foundation and Nova Southeastern University. Materials were provided by DENTSPLY Tulsa Dental Specialties.
Objective: To compare the physical properties of grey MTA (GMTA) and Biodentine and the effect of two developed accelerant formulations on their setting time, pH and compressive strength. Materials and Methods: GMTA and Biodentine were mixed with distilled water and supplied liquid respectively as control groups. Two accelerator formulations were used for experimental groups, formulation L2 and formulation L2-PO4 for both materials. For pH measurement, five samples from each group were tested for 30 minutes from the start of the mix. For the setting time measurement, five samples from each group were tested using a Gilmore needle apparatus. The compressive strength was tested on 10 samples from each group after 24 hours from the start of the mix. Comparisons between the experimental groups were performed using one-way ANOVA at a 5% level of significance and the nonparametric tests were performed using the Kruskal-Wallis test. Results: The two accelerator formulations did not affect the setting pH for both tested materials, GMTA and Biodentine. Also, there was no significant difference between GMTA and Biodentine pH value. The final setting time of GMTA mixed with formulation L2 or L2-PO4 was significantly shorter than that mixed with distilled water (p<0.00). In addition, Biodentine showed significantly shorter final setting time than GMTA (p<0.00). The compressive strength values of GMTA mixed with formulation L2 or L2-PO4 were significantly greater than that mixed with water (p<0.00). Biodentine showed superior compressive strength values compared to all GMTA groups (p<0.00).

Introduction: In tissue regeneration research, bioactivity was initially used to describe the resistance to removal of a biomaterial from host tissues after intra-osseous implantation. Hydraulic calcium silicate cements (HCSCs) have preliminarily been accepted as bioactive materials, as exemplified by the increasing number of publications reporting that these cements are capable of forming an apatite-rich surface layer after they come into contact with simulated body fluids. Methods: In this review, the same definitions employed for establishing in vitro bioactivity in glass-ceramics, and the proposed mechanisms involved in these phenomena, are used as blueprints for investigating whether these activities are identifiable in HCSCs. Results: The literature abounds with evidence that HCSCs exhibit in vitro activity. There is a general lack of stringent methodologies for characterizing the calcium phosphate phase precipitated on HCSCs. Nevertheless, the mechanisms responsible for in vitro bioactivity of these cements may be generally described in stages that are analogous to those exhibited by bioactive glasses. Although in vivo bioactivity has been demonstrated for some HCSCs, a fibrous connective tissue layer is frequently seen along the bone-cement interface that is reminiscent of the responses observed in bio-inert materials, without accompanying clarifications to account for such observations. Conclusion: As bone-bonding is not predictably achieved, there is presently insufficient scientific evidence to substantiate that HCSCs are indeed bioactive. Objective, universally acceptable appraisal criteria should be developed in the future to guide manufacturers and scientists in more accurately defining the bioactivity profiles of novel HCSCs introduced for clinical use.

Physical Properties of Two Improved Experimental Root End-Filling Materials, MTA and Biodentine

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OR35

Are Hydraulic Calcium Silicate Cements Really Bioactive?

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OR34

Biodentine Reinforcement of Endodontically Treated Teeth

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Microtensile Bond Strength of Composite Resin to Dentin After Seven Days of Bleaching Followed by Application of 35% Sodium Ascorbate

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Objective: To compare the root fracture resistance of maxillary anterior teeth filled with gutta-percha and reinforced with either composite resin (LuxaCore® Dual) or calcium silicate-based cement (Biodentine®). Forty extracted permanent maxillary anterior teeth were equally divided into four groups (n=10). One non-endodontically treated group served as the control. The teeth in the other groups were accessed, instrumented and root filled with either gutta-percha, LuxaCore® Dual or Biodentine® Root fracture strength was tested using a three-point bending test. A load was applied and measured with a universal testing machine at a crosshead speed of 0.5 mm/min until fracture. One-way ANOVA was used to analyze and compare the mean loads at fracture between the groups. Post-hoc comparisons amongst groups were performed using Tukey’s multiple comparisons at the 0.05 level of significance. The control, gutta-percha, LuxaCore® Dual and Biodentine® groups had mean fracture loads of 2.20 ± 0.72; 1.86 ± 0.60; 2.39 ± 0.70 and 2.26 ± 0.70 kilonewtons respectively. The results indicated no statistically significant difference among the groups (p=0.358). The comparison between the gutta-percha and LuxaCore® Dual groups demonstrated the greatest difference in mean fracture loads, which resulted in a large effect size (d=0.82). A larger sample size (n=25) would have provided more robust power (0.80) to detect the significance of this effect. The LuxaCore® Dual and Biodentine® groups had similar mean fracture loads and appear to be suitable materials for root canal reinforcement of endodontically treated maxillary anterior teeth. This study was supported by the AAE Foundation.
Biocompatibility of Commercially Available Calcium Hydroxide Pastes
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Several studies have shown that calcium hydroxide (Ca(OH)_2) based medicaments have a cytotoxic effect on human cells. The purpose of this study was to evaluate the cytotoxicity of several Ca(OH)_2 products on primary periodontal (PDL) cells. Materials: Calcium hydroxide powder (Avantor Performance Materials Inc.), Calasept (Nordiska Dental AB), Metapaste (Meta Biomed Co., Ltd.), Vitapex (Neo Dental International Inc.), Ultradent (Ultradent Products, Inc.), and Pulpdent (Pulpdent Corporation) products were tested. Primary human PDL fibroblasts were exposed to various concentrations of Ca(OH)_2, from each product (1 mg/mL, 5 mg/mL, 25 mg/mL, and .125 mg/mL respectively). Cell viability was measured after 24 hours and 48 hours by Cell Proliferation Assay MTS. Results: Products with a 1–5 mg/mL concentration had cytotoxic effects statistically more significant when compared to the negative control. Regardless of concentration, Metapaste was the most cytotoxic at both 24 and 48 hours. The .25-.125 mg/mL concentration of Ultradent and Pulpdent had nearly 100% cell viability at 24 hours. Conclusion: All Ca(OH)_2 products showed evidence of cytotoxicity on PDL cells, with Metapaste being the most cytotoxic. The cytotoxicity was related to concentration and exposure time. Pulpdent and Ultradent had excellent biocompatibility at lower concentrations.

Removal of Root Filling With Different Instrument Techniques and Ultrasound: A Micro-Computed Tomography and SEM Study
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Introduction: Endodontic retreatment is indicated when infection persists or recurs after treatment. Nonsurgical endodontic retreatment can be an advantageous option, especially if the existing root filling is technically deficient. Nevertheless, the success of nonsurgical retreatment depends on adequate cleaning and shaping of the root canal, and thus special attention should be paid to the technique used to remove the filling material. Methods: One hundred eleven human lower incisors were instrumented with the rotary technique using the BioRaCe™ system. All the teeth were filled using the Tagger hybrid technique. The teeth were randomly divided into three groups according to the retreatment technique used: Group 1- hand files and Gates Glidden drills; Group 2- ProTaper® Universal Retreatment System (ProTaper® UR); Group 3- D-RaCe retreatment system. In each of these groups, half of the specimens were additionally subjected to ultrasonic activated irrigation. After finishing the instrumentation, micro-computed tomography scans and SEM images were taken to detect possible root-filling residue in the canal. Results: Less root-filling material was left in canals after D-RaCe than after ProTaper® UR or hand files. D-RaCe removed the root filling better than the other methods in all three areas of the canal. The use of an ultrasonic file reduced the amount of residual root filling in all groups.

A Survey of Cone Beam-Computed Tomography Use Amongst Endodontists in the United States
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The application of CBCT in endodontics has many potential benefits, including the detection of missed canals, periapical lesions, root fractures, perforations and anatomical landmarks for surgical procedures. The majority of studies are university-based. Little is known about actual CBCT usage in private practice. This study assessed the accessibility and usage of CBCT technology among actively practicing endodontists in the United States. A Web-based survey focused on CBCT accessibility and usage was sent to 2,258 active AAE members. Besides demographics, participants were asked questions regarding practice setting, access to a CBCT and, if yes, the location and field of view (FOV) of the CBCT. Furthermore, the survey asked to list the frequency of use for specific clinical situations, including calcified cases, missing canals, immature teeth, internal or external resorptions, the identification of periradicular lesions, differential diagnosis, preoperative analyses for nonsurgical and surgical retreatments and healing assessment. Of 402 (17.8%) respondents, 38% reported to have access to a CBCT on site and 42% at an off-site location. Of these CBCT units, 55% were Limited/Localized FOV (≤5cm); however, 23% of practitioners were unaware of the volume size. CBCT use was primarily reported for differential diagnosis and the identification of periradicular lesions. The most common reason a practitioner would not utilize CBCT technology in his/her office was due to cost (46%), while 28% felt CBCT technology provided no necessary benefit to the practicing endodontist. This survey demonstrated that CBCT technology has become both widely accessible and also a regularly utilized tool by endodontic practitioners.

In Vitro Biocompatibility and Oxidative Stress Profiles of Different Hydraulic Calcium Silicate Cements
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Introduction: MTA Plus® (Avalon Biomed Inc., Bradenton, FL) is a new calcium silicate cement with unknown cytotoxicity characteristics. The objectives of this study were to examine the effect of MTA Plus® on the viability, apoptosis/necrosis profile and oxidative stress levels of rat odontoblast-like cells. Methods: MDPC-23 cells were exposed to gray and white MTA Plus® (GMTA, WMTA), gray and white Pro-Root MTA (GMTA, WMTA) cements or their eluents. The cells were evaluated for (1) cell viability by using XTT assay, (2) apoptosis/necrosis by using flow cytometry and confocal laser scanning microscopy and (3) oxidative stress by measuring reactive oxygen species. Results: XTT assay showed that all test cements exhibited marked initial cytotoxicity that decreased with time. By the end of the third week, GMTA and WMTA exposed cells were comparable to untreated cells (negative control) in terms of cell viability, whereas WMTAP and WMTA exposed cells exhibited significantly reduced viability than untreated cells. Apoptosis/necrosis profiles of cells exposed to WMTAP and GMTAP were not significantly different from untreated cells, whereas cells exposed to WMTA and GMTA showed significantly less viable cells. All experimental groups exhibited reduction of intracellular reactive oxygen species formation compared with untreated cells, although cells exposed to WMTA were not significantly different from untreated cells. Conclusions: Both the gray and white versions of MTA Plus® possess negligible in vitro cytotoxic risks that are time and dilution dependent. They enrich the spectrum of hydraulic calcium silicate cements currently available to clinicians for endodontic applications.
Effect of Canal Preparation on Fill Length in Straight Root Canals Obturated With GuttaCore®
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One adverse effect commonly seen when using thermoplastic obturators is having gutta-percha overextend the root canal into the periapex. A protocol is needed to predictably prepare a root canal which prevents overextension of the obturation material beyond the desired working length (WL). This study compares straight canals instrumented with two different preparation protocols and obturating with a new thermoplastic product, GuttaCore® (GC). The protocols are: 1) 0.04 standard taper preparation (STP); and 2) a varied taper preparation (VTP). Eighty extracted mature and patent human teeth with single canals were selected and randomly divided into two experimental groups (n=40). Group A was instrumented to WL with a master apical file size 40/0.04 via the STP technique. Group B was instrumented to WL with a master apical file size 40/0.02 via the VTP technique. All samples were obturated with Ribbon® sealer and GC. Three-dimensional fill was evaluated radiographically using the bisecting angle technique, and extension of fill was determined clinically by microscope (5×). All samples were examined by a blinded evaluator. Data was assessed ordinally to analyze fill overextension, with acceptable being +/- 1.0 mm within WL and overextension > 1.0 mm beyond WL. Preliminary assessment revealed that STP has a 50% (5/10) incidence of overextension, while VTP has a 10% (1/10) incidence of overextension. The difference between groups is statistically significant at p>.05. These results indicate that VTP is a more predictable outcome for avoiding overextension of fill when using GC in teeth with straight root canals. This study was supported by the AAE Foundation. DENTSPLY donated materials.

Invasive Cervical Root Resorption: A Review of Clinical Cases
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A retrospective analysis of records from patients with cervical resorption at the University of Iowa was performed. Numerous variables were analyzed, from patient descriptive factors, to history of various dental treatments. Data was analyzed to better understand the etiology of invasive cervical root resorption, and classify this current case series. Fifty cases were investigated from patients aged 14 to 86. Only data readily available in the records was used, therefore missing information was encountered. Forty-six percent of the patients with cervical resorption had undergone orthodontic treatment, while 16% reported no orthodontic treatment; the remainder of the data was missing. Eighteen percent reported history of trauma to the associated teeth, while 10% reported no trauma history. Thirty-two percent reported periodontal therapy to the associated tooth, while 14% reported none. Thirty-two percent reported no known associated risk factors, while 30% reported multiple associated risk factors. Twenty-six percent of cases were treated by extraction, 18% were repaired without root canal treatment (RCT), 22% were repaired following RCT and 28% were monitored. Six percent of patients elected no treatment. Only two cases were diagnosed as class I, while 17 were class IV. Attempting to look at risk factors for cervical resorption is difficult in a retrospective manner, due to missing information in patient records. The issue is quite complex, as some cases have no known risk factors and some appear to be multifactorial. A prospective examination may prove beneficial to further understand risk factors associated with this process. This study was supported by the University of Iowa College of Dentistry.

A Panoramic Survey of Air Force Basic Trainees: How Research Translates Into Clinical Practice
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Introduction: Dental research in all its forms tries to answer the question “what works?” Applied research leads to improved treatment practices and long term successful treatment outcomes. How research translates into practice is a question that should concern our profession. The purpose of this study was to examine how frequently treatment practices associated with success, as cited in the literature, are found in a young adult population in the United States. Methods: Panoramic radiographs taken of all basic trainees (BMTs) entering the U.S. Air Force during 2011 were evaluated. Panoramic radiographs that showed posterior teeth with root canal treatment (RCT) were evaluated by two Board-certified endodontists to consider the presence or absence of a cuspal coverage restoration, the quality of existing restorations, the density, taper and length of obturation and evidence of procedural errors. Results: A total of 35,811 panoramic images were evaluated. Ten percent of BMTs had existing RCT while more than 5% showed a need for RCT. The total number of posterior teeth with RCT was 3,455. Nearly half of these teeth had no cuspal coverage or had an unacceptable cuspal coverage restoration. Almost one-third of the existing RCT was considered unacceptable based on “successful” treatment practices cited in the literature. Conclusion: Factors cited in the literature as being associated with endodontic treatment success are lacking in clinical practice in this population.

Evaluation of the Prevalence and Clinical Characteristics Associated With Intrapulpal Cracks Utilizing a Novel Classification System
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Much research has focused on cracks involving the coronal tooth structure. However, few studies have investigated cracks involving the pulp chamber walls or floor. The purpose of this study was to create a classification system for intrapulpal cracks and analyze associated clinical characteristics. Retrospective analysis of all teeth requiring NSRCT or retreatment within the Virginia Commonwealth University Graduate Endodontic Practice for one year beginning July 1, 2012 (n=52) was completed. Intrapulpal cracks were identified by microscopic evaluation. The classification system describes the crack based upon its location within the pulp walls (I= one wall, II= two walls) and floor (A= crack terminates at floor-wall junction with no orifice involvement, B= extends into orifice with no floor involvement, C= traverses partially across the floor with no orifice involvement, D= traverses entire floor with no orifice involvement). Documentation consisted of demographic, subjective and objective data including diagnostic testing (e.g., bite test, transillumination), radiographs, crack location and orientation, classification and microscopic findings. Chi-squared analysis tested associations with the intrapulpal crack classifications. Ninety-two percent of intrapulpal cracks were repaired without root canal treatment (RCT), 22% were repaired following RCT and 28% were monitored. Six percent of patients elected no treatment. Only two cases were diagnosed as class I, while 17 were class IV. Attempting to look at risk factors for cervical resorption is difficult in a retrospective manner, due to missing information in patient records. The issue is quite complex, as some cases have no known risk factors and some appear to be multifactorial. A prospective examination may prove beneficial to further understand risk factors associated with this process. This study was supported by the AAE Foundation.
**OR45**

**Platelet Rich Fibrin Enhances the Regenerative Potential of Immature Permanent Teeth With Necrotic Pulp (Animal Study)**

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Objective: The aim of this study was to assess the regenerative potential of immature teeth with necrotic pulp following different treatment protocols including the application of platelet rich fibrin (PRF). Methods: Nine young dogs with 16 immature premolars each with total number of 144 teeth were used in this study. Access cavity was opened and induction of infection was done. Teeth were disinfected using triple antibiotic paste and randomly divided into three groups according to the evaluation periods, one week, three weeks and three months, respectively. Each group was further subdivided into three subgroups: REG Subgroup, regeneration was done using the regular regenerative protocol where only bleeding induction was applied; PRF Subgroup, where a modified PRF preparation and delivery protocol was used; PPP Subgroup, where platelet denaturated plasma fluid in combination with collagen type I non cross linked were used. Teeth were evaluated and compared radiographically in each group for the following: root length, root thickness and apical diameter, preoperatively and post-operatively. Animals were scarified and evaluated histologically with H and E stain for inflammatory cell count, soft tissue ingrowth inside the canal, evidence of new mineralized tissue, bone resorption and apical closure. Data were statistically analyzed using ANOVA and Duncan’s multiple range test. Results: PRF showed better healing potential than other techniques without changing the cellular content in both the three-week and three-month periods. Conclusion: The use of PRF instead of blood clot enhanced the tissue quantity without improving the tissue quality.

**OR46**

**Comparison of Vehicles to Collect Dentinal Fluid for Molecular Analysis**


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Introduction: The purpose of this study was to test the hypothesis that a material with higher water absorbency than polyvinylidene fluoride (PVDF) could increase the yield of target molecules from exposed dentin. Methods: In a series of standard tests, different cellulose membranes were compared to a PVDF counterpart for their ability to absorb water and release protein. In a subsequent randomized clinical trial, the cellulose material with the most favorable values was compared to PVDF regarding the levels of MMP-2 that could be collected from exposed dentin of healthy human teeth during filling replacement. MMP-2 levels were determined by enzyme-linked immunosorbent assay (ELISA). Data from the laboratory experiments were compared between materials by one-way ANOVA followed by Tukey’s HSD test. The frequency of cases yielding quantifiable levels of MMP-2 was compared between materials by Fisher’s exact test. The level of significance was set at 5%. Results: The cellulose membrane with the largest pore size (12–15 µm) absorbed significantly more (p<0.05) more water than PVDF. It showed a protein release that was similar to that of PVDF, while the cellulose membranes with smaller pore size retained significantly more protein (p<0.05). Using the large-pore cellulose membrane, MMP-2 could be collected at a quantifiable level from the dentin of healthy teeth in 9/13 cases, compared to 1/13 with the PVDF membrane (p<0.05). Conclusions: Under the current conditions, a large-pore cellulose membrane yielded more of a molecule of diagnostic value compared to a standard PVDF membrane.

**OR47**

**Cell Instructive Scaffold for Dental Pulp Regeneration**

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There is a significant clinical need for bioactive scaffolds for vital pulp therapy. The goal of this study is to evaluate the potential of a biosynthetic hydrogel based on polyethylene glycol and fibrinogen (PEG-F) for dental pulp regeneration. Experimental Design: Human dental pulp cells were cultured in PEG-F hydrogels with different fibrinogen concentrations: 0 (no fibrinogen: PEG-diacylate (PEG-DA)), 7.7, 8.5 and 9 mg/mL. At two, seven, 14, 21, 28 and 35 days, cell viability/morphology, plus cell proliferation, collagen and alkaline phosphatase (ALP) activity, along with gene expression (Col I, III, dentin sialophosphoprotein), were determined. ANOVA and the Tukey-Kramer post-hoc test were performed at p<0.05 for statistical analysis. Results: Cells in PEG-F hydrogel remained viable and spread over time, with cell number and network formation increasing linearly with fibrinogen content. By day 42, cell number in the 9 mg/mL group was the highest and it increased significantly over time. While no detectable collagen production was found in PEG-DA hydrogels, a significant increase in collagen content was evident in all PEG-F gels. Interestingly, both collagen/cell and ALP activity were significantly higher in the 9 mg/mL group by day 21 and day 28, respectively. Human dental pulp cells exhibited physiologically relevant morphology and matrix production in PEG-F hydrogel and these responses are enhanced with higher fibrinogen content. These results demonstrate that the PEG-F hydrogel is a promising scaffold for dental pulp regeneration. Future studies will focus on scaffold optimization and in vivo evaluation of the scaffold for pulp regeneration. This study was supported by the Presidential Early Career Award for Scientists and Engineers and the Royal Thai Government Scholarship.

**OR48**

**Effects of Seeding Density on Dental Pulp Cell Response in Polyethylene Glycol-Fibrinogen Hydrogel Scaffold**

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The goal of this study is to evaluate the response of human dental pulp cells in a composite gel of polyethylene glycol and fibrinogen (PEG-F) as a function of cell density and culturing time. Methods: Human dental pulp cells were seeded in PEG-F at three densities: 1.6x10⁶, 3.2x10⁶ and 4.8x10⁶ cells/mL and maintained in fully supplemented medium. Samples were analyzed for cell viability, cell morphology, cell proliferation, collagen content and alkaline phosphatase (ALP) activity. The expression of human dental pulp phenotypic markers collagen I, collagen III and dentin sialophosphoprotein (DSP) was determined using RTPCR. ANOVA and Tukey-Kramer post-hoc test were used for all pair-wise comparisons (p<0.05). Results: Cells within PEG-F remained viable and began to spread within the hydrogel over time. No significant change in cell number was measured after day two for the 1.6 and 4.8 million density groups. Interestingly, for the 3.2 million density group, cell number increased significantly by day 35. A significant increase in collagen content was measured on day 35 at the highest seeding density. ALP activity remained at basal levels and expression for DSP, collagen I and III were maintained in all groups. Human dental pulp cells maintained physiologic morphology, viability and phenotypic response over time in PEG-F hydrogels, demonstrating the potential of this novel gel system for guiding pulp regeneration. Increasing cell density promoted the formation of a collagen-rich matrix, suggesting that there is a critical cell density for optimal biosynthesis in these hydrogels. This study was supported by the Presidential Early Career Award for Scientists and Engineers and the Royal Thai Government Scholarship.
OR51

Dynamics and Mechanisms of Fibronectin-Coated Nanoparticles and Microbeads Uptake by Rat-2 Fibroblasts

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Nanoparticles are widely used to deliver biologically active molecules that could facilitate dentin disinfection and pulp repair in endodontic treatment. Particle size affects cellular interactions, particle fate and biological impact. Our objective was to compare the dynamics and mechanisms of uptake of nanoparticles (0.05 µm diameter) with much larger microparticles (1 µm diameter). By dot blot analysis, we found that fibronectin was strongly bound by nanoparticles and by microparticles. There was rapid uptake of fibronectin-coated nanoparticles by Rat-2 fibroblasts within one hour, which plateaued by one day whereas the internalization of microparticles was much slower. By confocal microscopy and electron microscopy, we found that two hours after incubation with cells, nanoparticles were uniformly distributed in the cytoplasm whereas microparticles were restricted to focal aggregates. Similar to microparticles, fibronectin-coated nanoparticles were internalized partially by receptor-mediated endocytosis, as uptake was decreased by cold incubation (at 4°C) and by depletion of ATP (with sodium azide). As measured in pulse-chase experiments, there was minimal exocytosis of nanoparticles and microparticles. The internalization of nanoparticles and microparticles was dependent on actin assembly as the actin filament stabilizer jasplakinolide and the actin monomer sequestering toxin latrunculin B inhibited internalization. Flow cytometry analysis showed that co-incubation of cells with propidium iodide and nanoparticles or microparticles did not affect the integrity of cell membranes, indicating the low toxicity of nanoparticles for biomedical applications. Our data indicate that nanoparticles can deliver the extracellular matrix molecule fibronectin to the cell interior and that particle diameter affects their intracellular destination. This study was supported by the Canadian Academy of Endodontics.

OR52

Presence of Metastatic Human Osteosarcoma Cells in the Dental Pulp

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The OS521 human osteosarcoma tumor cell line had been shown to exhibit metastatic characteristics when cell suspensions were subcutaneously xenografted into NOD SCID mice. The aim of this study was to investigate if cells from OS521 human metastatic osteosarcoma tumors would seed the dental pulp of subcutaneously xenografted NOD SCID mice. OS521 cells were cultured and stably transfected with a plasmid containing the human Oct-4 promoter driving a green fluorescent protein (GFP) reporter to generate the transgenic line OS521Oct-4p. Cells were trypsinized, washed, counted and divided into 100 microliters aliquots at a concentration of 3x10^4 cells. Twenty NOD SCID mice were lightly anesthetized with isoflurane and subcutaneously injected in their dorsum between the scapulas with OS521Oct-4p cells. Untransfected cells served as negative control. Tumors were allowed to develop until ≥1.0cm or 12 weeks post-injection. Mice were sacrificed and both mandibular central incisors were extracted under microscopy. Pulpal tissue was extirpated using a #10 broach inserted into the apical end of the tooth. Pulpal tissue was extirpated using a #10 broach inserted into the apical end of the tooth. Amputated tissue was placed in 0.05% PBS and observed under fluorescent light microscopy. Presence of metastatic human osteosarcoma cells in the dental pulp of subcutaneously xenografted NOD SCID mice. Our data suggest that osteosarcoma cells are able to metastasize to the dental pulp.

* — Abstract Presenter
OR53

ER Stress Signaling Molecule IRE1α is a Biological Regulator of Periapical Bone Formation

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Objectives: Osteoblasts and odontoblasts are professional secretory cells that produce bone and dentin, respectively. Inositol-requiring protein 1α (IRE1α) plays a multi-faceted role in the endoplasmic reticulum (ER) stress response, including regulating protein maturation and trafficking and determining cell survival and/or death. However, the role of IRE1 in regulating bone and dentin formation remains largely elusive. Methods: Transgenic mice that carry genetic deletion of IRE1α in both osteoblast and odontoblast lineage cells were generated and characterized by histological staining, calcein labeling and μCT analysis. Results: The IRE1α conditional knockout (CKO) mice displayed compromised bone formation in the mandibles compared with their age- and gender-matched wild type (WT) counterparts, as shown by the hematoxylin and eosin (H&E) stains. Furthermore, the CKO mice displayed approximately 10% reduction in the mineral density and 50% reduction in the thickness of the root dentin of the mandibular molars compared with those of their WT counterparts, as shown by μCT and H&E staining analyses. Consistent with these observations, the dynamic calcein labeling assay demonstrated that the CKO mice have reduced formation rate of both bone and dentin compared with their WT littermates. Finally, the CKO mice exhibited delayed tooth eruption compared with the WT controls. Conclusions: Our results, for the first time, revealed that IRE1α, one of the most ancient ER stress signaling molecules, plays an essential role in regulating periapical bone and dentin formation, as well as tooth eruption. This study was supported by MMRF grant (to H.J.O.) and NIH/NCI R21CA161150.

OR54

Ultrasound Imagery for Endodontic Diagnosis and Treatment Planning in a Porcine Model

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Objectives: Current methods of locating and measuring bony endodontic defects covered by soft tissue, specifically narrow dehiscences caused by vertical root fractures or draining sinus tracts, and fenestrations caused by granulomas, may be imprecise or invasive. The purpose was to demonstrate the feasibility of two-dimensional (2-D) ultrasound imaging of soft and hard tissues for endodontic diagnosis and treatment planning. Materials and Methods: A sector scanning ultrasound system was applied to detecting fenestrations and narrow dehiscences. Four representative clinical features (long narrow dehiscence, short wide dehiscence, fenestration and mental foramen) were created or identified in each of five porcine jaws, which were then covered with soft tissue and imaged in an acoustic water tank. Results: All four model features, as well as soft tissue and bone surfaces, in all five jaws, were clearly imaged. Most objects were visible over a large range of positions and angles. Each feature was defined by a specific acoustic signature, with the same signature recurring for each object type among all five of the jaws. The acoustic signatures of mental foramina and fenestrations differed from one another, as did narrow long and broad short dehiscences. Features were easily located and measured (±0.2 mm) beneath at least 3 mm of soft tissue. Conclusions: A 2-D sector scanning ultrasound system was capable of accurately imaging representative features for endodontic treatment planning in a porcine model; long narrow dehiscence, short wide dehiscence, fenestration, and mental foramen. Specific acoustic signatures for these features were defined. This study was supported by NIH/NIDCR R44DE021326. Martin Culjat, Eric Johnson and Rahul Singh are employed by Farus LLC.

OR55

Histological Evaluation of the Effects of Variable Extraoral Dry Times and Enamel Matrix Protein (Emdogain™) Application on Replanted Dogs' Teeth

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Extraoral dry time (EODT) is considered the most important factor affecting the outcome of replanted teeth. Current guidelines set the EODT threshold at 60 minutes, but the critical time interval may be shorter. Also, the effects of Emodogain™ (EMD) in preventing replacement root resorption in replanted teeth are not well understood. This study evaluated the periodontal healing of replanted dogs’ teeth after 20 and 60 minutes EODT, and the effects of EMD applied before replantation. Eighty mature premolar roots in four beagle dogs were extracted, endodontically treated and replanted after an EODT of 20 minutes (Groups 1 and 2) and 60 minutes (Groups 3 and 4). EMD was applied on the roots of Groups 2 and 4 before replantation. Positive (90 minute) and negative (10 minute) control groups were included. After four months, the histological evaluation and measurement of the resorptive defects (mm) were performed. One-way ANOVA showed no statistical differences amongst the experimental groups (p=0.1075). All groups presented some degree of replacement resorption and periodontal ligament (PDL) regeneration was only seen in the negative control group. It was concluded that 20 minutes of EODT is as detrimental to the PDL cells as 60 or 90 minutes of EODT. The application of EMD did not prevent external replacement resorption in replanted dogs’ teeth after 20 or 60 minutes of EODT.

OR56

Effect of Neonatal Capsaicin Treatment in the Development of Periapical Lesion in Rats

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Apical periodontitis is characterized by periradicular inflammation, resulting in bone resorption and often mechanical allodynia. Dental pulp is richly innervated with peptidergic sensory neurons that, upon activation, release vasoactive neuropeptides such as calcitonin-gene related peptide (CGRP) and substance P (SP), known to mediate neurogenic inflammation. Although several chemokines and cytokines are involved in the generation and maintenance of apical periodontitis, far less is known about the role of neurogenic inflammation. In this study, we hypothesized that peptidergic neurons modulate initiation and progression of the apical periodontitis. Materials and Methods: Neonatal Sprague Dawley rats were injected with either capsaicin to cause the selective ablation of peptidergic neurons or vehicle (control group) n=6/group. The ablation of the capsaicin-sensitive subclass of nociceptors was confirmed using the eye wince nocifensive assay. Next, rats were anesthetized and pulp exposures created in the left mandibular first molars. Animals were euthanized at seven, 14, 21 and 28 days, and tissues processed for analysis. The periapical disease progression was quantified using micro-computed tomography (μCT) to measure apical lesion volume, and immunohistochemistry to characterize innervation and immune cell content. Data were analyzed using two-way ANOVA (p<0.05). Results: For all time points, selective ablation of peptidergic sensory neurons resulted in significantly larger periapical lesions as compared to control (p<0.05). Immunohistochemical analysis revealed that decrease in neuropeptide was associated with higher inflammatory infiltrate. Conclusion: These results suggest that neurogenic inflammation has an early and significant protective role and immune- modulatory function in the development of apical periodontitis. This study was supported by the AAE Foundation.
Objective: The aim of this study was to examine the influence of powder composition and morphology on the penetration of Gray and White ProRoot® Mineral Trioxide Aggregate (MTA) into dentin tubules. Materials and Methods: Extracted extract-free human posterior teeth were used to prepare dentin discs and smear layers were removed. The tested materials were then agitated on the dentin discs. Penetration of open dentin tubules was studied by scanning electron microscopy (SEM). The estimated percentage of particle-filled open tubules was calculated from the frequency distributions of open tubules for each material. Results: The cumulative percentages of the particle lengths between 0.5 and 2.0 μm, which is less than the diameters of most tubules in root dentin, were 72%, 71% and 63% for GMTA, WMTA and CH, respectively. SEM showed all three materials, when deposited and agitated on dentin discs, penetrated the open dentin tubules. Using the frequency distributions of GMTA and WMTA, CH particles, as well as that of open tubules, the expected percentage of open tubules to be filled by GMTA, WMTA and CH were 83%, 84% and 79%, respectively. Under SEM the coverage of open dentin tubules can be described by three types: (1) particles embedded within open dentin tubules or located on the surface over open dentin tubules, (2) particles aggregated with amorphous powder film, but may be covering open dentin tubules and (3) unfilled open dentin tubules. Conclusion: All particle types penetrated into open tubules when agitated on dentin discs. This study was supported by NIH UL1TR0000451 and U54GM104942.

Dimensional Changes of MTA Fillapex™ Sealer Within Dried or Moist Conditions Using Either Sodium Fluoride or Normal Saline Solutions

Objective: To evaluate setting expansion of MTA Fillapex™ within dried or moist conditions using either sodium fluoride or normal saline as solutions. Methods: A number of nine cylindrical stainless steel molds were milled measuring 20 mm height and 5 mm internal diameter. The expansion of the MTA Fillapex™ was evaluated three times for three groups: (1) in the absence of any solution, (2) with sodium fluoride solution and (3) with NaF . In order to reach the final weight ratio evaluated three times for three groups: (1) in the absence of any solution, (2) with NaF or normal saline solutions compared with the sample devoid of solution, the difference was insignificant after 1,000 seconds (p=0.023). The setting expansion continued insignificantly after 1,000 seconds (p=0.023), and 2,000 seconds (p=0.114). However, in 4,000 seconds, the sample with NaF exhibited significantly more expansion compared to the other two samples (p=0.023). The setting expansion continued until two hours and 40 minutes to reach 0.63%. Conclusion: Using the combination of sodium fluoride solution and MTA Fillapex™ increases setting expansion which may decrease the microleakage.

Organic Content, Extrusion Temperature and Shrinking Kinetics of Three Types of Backfill Gutta-Percha

Objective: To compare the organic content, extrusion temperature and shrinking kinetics between soft, regular and hard gutta-percha pellets of the same brand (B&L). Method: Organic content was determined thermogravimetrically by heating the specimens (n=5) to 1,000°C. Extrusion temperature from a B&L heat gun set to 200°C was measured using thermocouples. Finally, linear shrinkage of the three gutta-percha types from 90°C to 37°C was assessed in real time using a shrinkage measuring device with an infrared sensor at an ambient temperature of 37°C. Zirconia specimens heated to 90°C were used as negative controls in shrinkage experiments. If not indicated otherwise, experiments were performed six times (n=6). Values between groups were compared using ANOVA and Tukey’s HSD test for multiple comparisons (p<0.05). Result: Organic content and extrusion temperature were statistically similar between the regular and hard material under investigation, while these values were significantly (p<0.05) higher for the soft material. The mean organic content values and SDs were 38.9 ± 1.0 wt%, 25.9 ± 0.4 wt% and 27.3 ± 0.6 wt% for soft, regular and hard gutta-percha, respectively. The respective extrusion temperatures were 105 ± 6°C, 87 ± 2°C, and 89 ± 4°C. With a linear shrinkage after 10 minutes of 2.9% ± 0.9%, the soft material shrunk significantly (p<0.05) more upon cooling from 90°C to 37°C than the hard counterpart (1.4% ± 0.5%). Conclusion: Altering the organic content of gutta-percha appears to impact its thermally induced properties including its shrinking behavior.
OR61

**In Vitro Analysis on the Biocompatibility of Different Sealing Agents**
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Objective: The biocompatibility of sealers and other root canal materials is essential for the success rate of root canal treatment. The aim of the present study was to investigate the *in vitro* cytotoxicity of different endodontic sealing agents by means of human gingival fibroblasts and HUVEC cell proliferation. Methods: Sealapex (Kerr) SimpliSeal (Discus) and AHPlusJet (DENTSPLY, Konstanz, Germany) Pulp canal Sealer (Kerr) and Tubli Seal (Kerr) were prepared according to the manufacturers’ instructions and were allowed to set for 24 hours. They were then incubated with human gingival fibroblasts (fourth passage, 10,000 cells/ml) and human endothelial vein cells (HUVECs). The viability of the two cell lines when incubated with the different sealing agents was measured by using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide colorimetric assay and PrestoBlue® reagent. Further a migration assay (ThinCerts) was carried out. Additional fluorescence-microscope staining was carried out in order to visualize cell growth and morphology. Results: Human gingival fibroblasts proliferation seemed to be dependent upon dental material and cultivation time. After 24 and 96 hours all investigated sealing agents showed no cytotoxicity reaction while SimpliSeal resulted in a mild decrease of cell proliferation (t-test, p<0.001). Fluorescence-microscope staining was able to confirm the results of the MTT and the PrestoBlue® assay. Conclusions: The results of this study demonstrate that most of the tested materials showed no or in case of SimpliSeal only a slight cytotoxicity. Thus, these root canal materials can be rated as biocompatible materials and their clinical use be recommended.

OR62

**High Resolution Micro-CT Analysis of Root Canal Seal Defects**
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Objectives: To qualitatively analyze the nature and spatial relationship of fine defects within root canal filling materials and in interfaces with high resolution micro-computed tomography. Methods: Roots from eight human canines were instrumented to a size 55/04 and obturated using a carrier-based gutta-percha filling material (Gutta-Core, DENTSPLY, Tulsa, OK- n=4) or a warm vertical compaction technique (Calamus, DENTSPLY, Tulsa, OK- n=4) and a polymer-based sealer (2-Seal, VDW, Konstanz, Germany). The obturated roots were micro-CT imaged at 8.5 µm- (apical 16 mm), 3 µm- (5 mm) or 1 to 1.4 µm- (2.5 µm, n=2 from each group) voxel size and the size, shape and extent of gaps and voids was recorded in 2-D and 3-D viewing mode. Results: Although, as in previous studies, numerous relatively isolated defects of larger (>20 µm) size were identified in the middle and coronal parts of the canal, a network of microscopic voids (2-8 µm size, visible only in the 1 µm scans) in the apical segment was identified as well. In the vertical compaction group 10-15 µm gaps between the main gutta-percha cone and backfill thermoplastisized gutta-percha were also found (two of four roots) to potentially serve as long (>1 mm) routes for bacterial invasion, while in the Gutta-Core group 60-100 µm wide horizontal gaps between carrier and end gutta-percha was (three of four roots) observed. Conclusions: High resolution micro-CT image of obturated root canals is very informative on sealing quality and it can serve as a guide for the optimization of each obturation technique.

OR63

**Effect of Crushing Triazolam Tablets Prior to Sublingual Administration**
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A primary goal in dentistry is to successfully manage patient anxiety. Triazolam is commonly used to achieve this goal. Sublingual administration of triazolam achieves greater plasma concentration than oral administration. Anecdotal reports suggest crushing the triazolam tablet prior to sublingual placement will enhance absorption and increase efficacy. The purpose of this study was to compare the effectiveness of 0.25 mg triazolam administered sublingually as a whole tablet versus a crushed tablet. Twenty healthy subjects between the ages of 18 and 60 were sublingually administered 0.25 mg triazolam in the whole or crushed form at two separate appointments at least one week apart in a crossover design. Each patient served as their own control. The principal investigator was blinded as to the form of the triazolam the patient received. Patients were monitored for changes in alertness every five minutes for a period of three hours using the Observer’s Assessment of Alertness/Sedation, a bispectral index system monitor, and a digit-symbol substitution test. Data was analyzed by a chi-squared test with p-values adjusted using the step-down method. Results: All 20 subjects completed the study. There were no statistically significant differences between the whole tablet and the crushed tablet groups when the data was pooled. There was a trend toward the whole tablet having a faster onset of action, but it was not statistically significant. There was no difference in the anxiolytic effect of 0.25 mg triazolam when administered sublingually either whole or crushed. Therefore no benefit was derived from crushing a triazolam tablet prior to sublingual administration.

OR64

**Evaluation of the Gow-Gates and Vazirani-Akinosi Techniques in Patients With Symptomatic Irreversible Pulpitis**
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There are only a few studies that have evaluated the Gow-Gates and Vazirani-Akinosi techniques in patients presenting with symptomatic irreversible pulpitis. Therefore, the purpose of this study was to compare the degree of pulpal anesthesia obtained with the Gow-Gates and Vazirani-Akinosi techniques using 3.6 mL of 2% lidocaine with 1:100,000 epinephrine in patients presenting with symptomatic irreversible pulpitis. One hundred twenty-five emergency patients (diagnosed with symptomatic irreversible pulpitis of a mandibular posterior tooth) randomly received either a Gow-Gates or Vazirani-Akinosi injection to block the inferior alveolar nerve before endodontic access. Pulpal anesthetic success of the injection was defined as no pain or mild pain upon endodontic access or instrumentation as measured on a 170 mm visual analog scale. The results showed that subjective lip numbness was obtained 92% (60/65) of the time with the Gow-Gates technique and 63% (38/60) of the time with the Vazirani-Akinosi technique. All patients evaluated for successful pulpal anesthesia had profound lip numbness. Successful pulpal anesthesia was obtained 35% (21/60) of the time with the Gow-Gates technique and 16% (6/38) of the time with the Vazirani-Akinosi technique. This difference was statistically significant as analyzed by a chi-squared test with p-values adjusted using the step-down method of Holm. Neither the Gow-Gates or Vazirani-Akinosi techniques provided adequate pulpal anesthetic success rates for mandibular posterior teeth in patients presenting with symptomatic irreversible pulpitis. Both injections would require supplemental anesthesia.
Experimental studies have shown women have a lower acceptance of pain, fear pain more and avoid pain more than males. The role of operator gender on subject gender pain needs further study. Therefore, the purpose of this randomized, double-blind investigation was to evaluate operator gender and its influence on subject gender pain in maxillary anterior infiltrations. Two hundred subjects (100 males and 100 females) participated in this study. Each subject was seen at two appointments separated by at least two weeks. Each subject was randomly assigned to receive an infiltration over the maxillary lateral incisor. At the initial appointment, each subject randomly received an infiltration of 2% lidocaine with 1:100,000 epinephrine by one of 20 calibrated male or female operators. At the second appointment, each subject received an infiltration of the same anesthetic at the same location by an operator of the opposite gender. Immediately after each injection, subjects rated the pain of solution deposition on 170-mm visual analog scale. Dental anxiety was determined by each subject, at each appointment, using the Corah Dental Anxiety Scale. No significant differences were detected between male or female participants on the Corah Dental Anxiety Scale. A significant difference (p=0.0357) was found during the solution deposition phase among female subjects receiving injections from male operators as analyzed using factorial, repeated-measures analysis of variance. In conclusion, gender did have a statistically significant effect for solution deposition pain when a male operator administered the injection to female subjects.

Previous studies have shown 4% articaine to be superior to 2% lidocaine when given as a primary buccal infiltration injection of the mandibular first molar. However, no study has determined if a 4% formulation of lidocaine with epinephrine or 4% prilocaine with epinephrine would be similar to a 4% articaine with epinephrine formulation in a buccal mandibular infiltration. Therefore, the purpose of this prospective, randomized, double-blind study was to compare three different 4% anesthetic solutions given as a primary buccal infiltration adjacent to the mandibular first molar. Using a crossover design, 60 adults each randomly received a mandibular first molar buccal infiltration of 1.8 mL of 4% articaine with 1:100,000 epinephrine, 4% lidocaine with 1:100,000 epinephrine and 4% prilocaine with 1:200,000 epinephrine at three separate appointments. An electric pulp tester was used to test the first molar for pulpal anesthesia every three minutes for 60 minutes. Successful pulpal anesthesia was defined as two consecutive 80 readings within 15 minutes of injection and sustained the 80 reading for 60 minutes. Success was analyzed using Exact McNemar tests. Differences in pain ratings were analyzed using multiple Wilcoxon matched-pairs signed-ranks tests. Success ranged from 27% to 53% with no significant differences between the two combinations. No difference in injection pain was found. The combination of 3% mepivacaine plus 2% lidocaine with 1:100,000 epinephrine is equivalent to two cartridges of 2% lidocaine with 1:100,000 epinephrine in terms of injection pain and pulpal anesthetic success for the IANB. This study was supported by the AAE Foundation.

Three percent mepivacaine plain has a higher pH and concentration than 2% lidocaine. In theory, using 3% mepivacaine initially would increase success, decrease the pain of injection and possibly potentiate the effect of a second cartridge of lidocaine for inferior alveolar nerve blocks (IANB). The purpose of this prospective, randomized, double-blind study was to measure the degree of pulpal anesthesia obtained with a combination of 3% mepivacaine/2% lidocaine (1:100,000 epi) versus 2% lidocaine (1:100,000 epi)/2% prilocaine (1:100,000 epi) in IANBs. One hundred asymptomatic subjects were each randomly given one cartridge of 3% mepivacaine plus one cartridge of 2% lidocaine with 1:100,000 epinephrine and two cartridges of 2% lidocaine with 1:100,000 epinephrine for the IANB, at two separate appointments. Subjects rated pain of solution deposition on a Heft-Parker VAS. The first molars, first premolars and lateral incisors were tested with an EPT every four minutes for 60 minutes. Anesthetic success was considered to have occurred when subject achieved two consecutive 80/80 readings within 15 minutes of injection and sustained the 80 reading for 60 minutes. Success was analyzed using Exact McNemar tests. Differences in pain ratings were analyzed using multiple Wilcoxon matched-pairs signed-ranks tests. Success ranged from 27% to 53% with no significant differences between the two combinations. No difference in injection pain was found. The combination of 3% mepivacaine plus 2% lidocaine with 1:100,000 epinephrine is equivalent to two cartridges of 2% lidocaine with 1:100,000 epinephrine in terms of injection pain and pulpal anesthetic success for the IANB. This study was supported by the AAE Foundation.

Innervation Density in Human Periradicular Cysts and Granulomas
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Though much is known regarding pathogenesis of apical periodontitis, knowledge regarding innervation of periradicular cysts and granulomas is mostly unknown. This lack of information contrasts sharply with the extensive knowledge base that outlines the normal innervation of the pulp and changes seen in disease conditions. The purpose of this study was to evaluate the neural innervation of human periradicular cysts and granulomas to test our hypothesis that these tissues show a variable innervation with a phenotype similar to nearby pulpal afferents. Materials and Methods: Tissue samples obtained from patients undergoing periapical surgery were processed and stained with hematoxylin/eosin for diagnosis by a pathologist and with the indirect immunofluorescence method using nerve fiber marker antibodies including protein-gene-product 9.5/neurofilament heavy (NFH) for confocal microscopy evaluation. Images were obtained and percent area with nerves was quantified using NIH ImageJ. Results: Nerve fibers were present in all samples, including large nerve fiber bundles in some, but with no difference (unpaired/student-t) in innervation density of granulomas (n=10; 0.8%+/-0.17) compared to cysts (n=5; 1.44%+/-0.92). Even though there was no difference between groups, innervation density varied widely among individual specimens. In addition, most nerve fibers expressed NFH like that seen in the human dental pulp. Conclusion: Periapical cysts and granulomas are commonly innervated by sensory nerve fibers similar to those found in the dental pulp. These findings suggest that adjacent neurons sprout into lesions, possibly playing a role in the symptoms of apical periodontitis and periapical surgery anesthesia failures. This study was supported by the AAE Foundation.
Outcome of Endodontic Treatment: Factors Contributing to the Healing Time

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Objective: To investigate factors affecting the treatment outcome and their influence on the time required for apical lesions to heal, as assessed by periapical radiographs and patients' symptoms. Methods: Two hundred fifty patients (421 roots) who received endodontic treatment were followed up from six months to five years. Factors related to patients' demographics, medical health, teeth treated and performed treatment were recorded. Clinical and radiographic examinations were performed on every follow-up appointment, and each root treated was evaluated as either “successful” (healed, healing) or “failed” (uncertain, no healing) treatment. Multiple logistic regression analysis was performed to correlate the studied factors with the treatment outcome and time elapsed for teeth to heal. Results: The average healing time was 17.8 months. This time decreased in patients under 40 (p=0.02), anterior teeth (p=0.01), and in non-immune compromised patients (p=0.01). The healing time increased in roots requiring retreatment (p=0.005), roots with apical lesions (p<0.001), over extended fillings (p=0.003) and complex canal system (p=0.05). The healing time and outcome of endodontic treatment were not affected by the size of apical lesion, number of canals, smoking, apical size, presence of voids and the coronal restoration quality. The success of endodontic treatment decreased in retreatment cases (p<0.001) and in roots with faint root canal filling (p<0.001) or with procedural errors (p<0.001). Conclusion: Age, immunological condition, complex canal anatomy and retreatment procedures appear to prolong the time required to accomplish healing. The quality of endodontic treatment appears to significantly affect the healing time and outcome of endodontic therapy.

Platelet Rich Fibrin Enhances the Regenerative Potential of Immature Permanent Teeth With Necrotic Pulp

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Objective: Treatment of immature teeth with a necrotic pulp constitutes a big challenge for endodontists. Teeth are often weak and prone to fracture. Regenerative endodontics could be a promising treatment option for open apices through completion of root formation. The aim of this study was to assess the regenerative potential of immature teeth with necrotic pulp following different treatment protocols including the application of platelet rich fibrin (PRF). Methods: Thirty children with immature necrotic upper anterior teeth were included in this study. Teeth were accessed and disinfected using triple antibiotic past and randomly divided into three groups: REG Group, regeneration was done using the regular regenerative protocol where only bleeding induction was applied; PRF Group, where a modified PRF preparation and delivery protocol was used; PPP Group, where platelet denaturated plasma fluid in combination with collagen type I non cross linked were used. Patients were examined clinically and radiographically every three months for one year. The root length, thickness, apical diameter and bone density were measured post-operatively and along the follow up period. Data were statistically analyzed using ANOVA and Duncan's multiple range test. Results: PRF Group showed better healing potential than REG Group in all the evaluation periods while PPP Group failed to produce healing. Conclusions: Platelet rich fibrin showed evidence to enhance root formation and apical closure of immature permanent teeth with necrotic pulp. Both platelet rich fibrin and regular regenerative protocol are suitable approaches to enhance root formation and apical closure in immature teeth with necrotic pulp.

Evaluation of Vital Pulpotomy Treatment in Fully Erupted Permanent Teeth With Closed Root Apices

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Objective: To assess the clinical outcomes of vital pulpotomy treatment (VPT) in mature permanent teeth with closed root apices and deep carious pulp exposures in children. Vital pulpotomy, if successful, has the advantage of preserving the vitality of the dental pulp, being less invasive, and requiring single dental visits. VPT can potentially save many teeth from being extracted as a consequence of root canal treatment (RCT), especially in younger patients who have thinner roots that minimize support to the tooth structure. Methods: Thirty permanent molars with closed root apices and pathological pulp exposure were treated by VPT in 27 children ranging in age from 10 to 15 years. The infected coronal pulp tissue was removed from each tooth, leaving vital pulp tissue in the canals. The pulp orifices were covered with mineral trioxide aggregate (MTA), followed by resin restorations. All the treated teeth were scheduled for clinical and radiographic assessment appointments. Results: Twenty-eight of the 30 teeth were available for the final recall with a mean recall period of 25 months. Twenty-six teeth were clinically asymptomatic during the follow-up period. The estimated proportion of success with VPT was 92.8% (p=0.02) when compared to RCT control outcomes (68% to 85%) from a systematic review (Ng et al. 2007). Conclusion: VPT can be used successfully to maintain vitality in mature permanent teeth with carious pulp exposure in children. Lower treatment costs and less complexity have the potential to increase access to vital pulpotomy and strengthen tooth structure.

Association of Endodontic Infection With the Detection of an Initial Lesion to the Cardiovascular System in Women

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Objectives: The aim of this study was to evaluate, in the absence of cardiovascular disease (CVD) and CV risk factors, the correlation between apical periodontitis (AP) and an initial lesion to the cardiovascular system in young females. Methodology: Forty healthy women of childbearing age (18-50 years) free from periodontal disease, CVD, traditional CV risk factors and who were not on oral contraceptives were enrolled in the study. Twenty subjects had AP, and 20 acted as controls. Patients underwent complete dental examination and CV assessment. Measurement of endothelial flow reserve (EFR), levels of interleukins-2 and -6 (IL-2, IL-6), tumor necrosis factor alpha, reactive oxygen species (ROS) and asymmetrical dimethylarginine (ADMA) were obtained. Data were analyzed with the two-tailed Student's t-test for unpaired data. Correlation between instrumental and laboratory variables was assessed by Pearson t-test (p≤0.05). Results: CV assessment revealed no abnormalities in any of the subjects studied. Patients with AP displayed statistically higher serum levels of IL-2 (p<0.03) and ROS (p<0.02), while IL-6 and TNFa manifested no significant differences with respect to the control group. EFR values were at the lower limit of the normal range and significantly lower than the controls (p<0.05), while no difference was observed in the levels of ADMA for the two groups. Conclusions: Reduced EFR values and increased levels of IL-2 and ROS might suggest the existence of an early endothelial dysfunction in young females with AP. The lack of increase in ADMA seems attributable to the protective action of estrogens.
A Clinical Evaluation of Cone Beam-Computed Tomography
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Introduction: Cone beam-computed tomography (CBCT) has become a valuable diagnostic tool for endodontics. The literature generally supports the accuracy of this imaging modality. However, others report CBCT may have limitations in representing the true clinical presentation. The aim of this descriptive study was to compare presurgical limited field of view (FOV) CBCT images against the actual clinical presentation. Method: Eleven patients requiring endodontic surgery at the Naval Postgraduate Dental School were consented and enrolled. Treatment notes and clinical photographs were used to generate a clinical presentation for each patient. CBCT images were evaluated by three board certified specialists. Thirty-three questions were answered by the evaluators with information gathered from the CBCT images. Results: This cohort consisted of nine males, two females, ages 24-56 years, with a total of 13 teeth. Buccal plate perforations were correctly identified 85% of the time. Anterior buccal plate perforations were more often correctly identified when compared to posterior buccal plate perforations (89% versus 80%). Maxillary sinus perforations were identified 53% of the time. The remaining cortical bone was underestimated in every CBCT with a mean underestimation of 1.7 mm. Conclusion: Buccal plate perforations were detected more frequently when compared to sinus perforations in limited FOV CBCTs. All cortical bone levels were underestimated. Knowledge of CBCT technology combined with experience in interpreting CBCT images are critical for clinicians to correctly utilize this diagnostic tool. This study, #383684-1 is IRB approved by Walter Reed National Military Medical Center.

Effect of DynaMatrix® Membrane on Angiogenic Cytokine Expression in Human Dental Pulp Cells
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Objective: The purpose of this study was to determine the effect of the DynaMatrix® membrane, an extracellular porcine small intestine submucosa (SIS) derivative, on the expression of angiogenic cytokines by human dental pulp stem cells (HDPSCs). Methods: The experimental groups were 1) HDPSCs alone, 2) DynaMatrix® alone and 3) HDPSC seeded on DynaMatrix®. After each group was cultured in media for 72 hours, angiogenesis-related proteins were quantified using the Human Angiogenesis Antibody Array (RayBiotech Inc., Norcross, GA). Results: The angiogenesis cytokine array revealed the following: The HDPSC/DynaMatrix® group contained significantly more angiogenic factors (PDGF-BB and bFGF) and significantly less anti-angiogenic factors (IL-6, IL-8, MCP-1, TIMP-1 and TIMP-2) when compared to the HDPSC control group. There was no significant difference between the DynaMatrix® control and HDPSC/DynaMatrix® for IL-6, IL-8, leptin, MCP-1, PDGF-BB, TIMP-2 or VEGF-D. The microarray results were statistically analyzed by ANOVA. Conclusions: This results of this study suggest that DynaMatrix® supports the growth of HDPSCs and is associated with a positive angiogenic cytokine profile. The DynaMatrix® membrane may be beneficial in regenerative endodontics.

Identification and Comparison of Virulence Genes of Enterococcus faecalis From Secondary Endodontic and Periodontal Infections
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Introduction: Enterococcus faecalis, an opportunistic pathogen, has been isolated from persistent periodontal endodontic infections. Isolates of E. faecalis produce several specific virulence factors that enable the bacterium to survive and be pathogenic. E. faecalis strains UTDB 11-2 and TUSoD11 were isolated from periodontal and endodontic infections, respectively. However, the virulence gene content of these strains has not been previously investigated. The purpose of this study was to identify and compare the presence of specific virulence-associated genes in these E. faecalis isolates. Methods: E. faecalis isolates UTDB 11-2, TUSoD11 and OG1RF (laboratory reference strain) were grown on brain heart infusion agar. Isolated colonies were subjected to colony PCR using primers designed for virulence genes; agg (aggregation factor), cylB (cytolysin B), esp (enterococcal surface protein) and gelE (gelatinase). Universal bacterial 16S rRNA primers were used as an internal control. PCR products were resolved by gel electrophoresis, stained and visualized by transillumination. Results: PCR products for virulence genes agg, cylB and gelE were detected in the UTDB 11-2 isolate, while in the TUSoD11 isolate only the agg gene was detected. However, the esp gene was not detected in either isolate. Conclusions: Enterococcus faecalis isolates TUSoD11 and UTDB 11-2 displayed variable distributions of virulence-associated genes that could contribute to their survival, pathogenicity and promote persistent disease.

Behavior of Dental Pulp Stem Cells on Polysisoprene With TiO2 and MTA as Nanoparticulate Fillers
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In tissue engineering applications, the properties of the scaffold can strongly affect the behavior of cells. Previous studies demonstrate that polysisoprene found within gutta-percha can promote differentiation and biomineralization of dental pulp stem cells (DPSC) without induction. Furthermore, cell behavior is largely influenced by scaffold thickness, nanoparticle loading and modulus. The purpose of this study was to evaluate effects on cellular behavior of nanocomposites containing polysisoprene and two different nanoparticles: titanium dioxide (TiO2) and mineral trioxide aggregate (MTA). Methods: Four nanocomposites were created with nanoparticles at different concentrations: TiO2 (0.1mg/ml and 0.5mg/ml) and MTA (0.5mg/ml and 5mg/ml). Materials were spun cast and annealed on silicon wafers. DPSC were cultured and grown on the prepared surfaces. Two surface controls were utilized: tissue culture plastic (TCP) and polysisoprene (PI). A positive control for differentiation and biomineralization was used – dexamethasone. Growth curves were obtained for an eight-day time period and confocal laser scanning microscopy (CLSM) was performed at day eight evaluating cell viability. At day 28, scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDAX) was performed, evaluating biomineralization. Results: A direct relationship was observed between nanoparticle concentration and cellular growth rates in both groups (TiO2 and MTA). Increased nanoparticle concentrations resulted in enhanced proliferation rates. CLSM demonstrated viable cells at day eight for all surfaces. Biomineralization was observed on MTA surfaces in the presence and absence of dexamethasone. Conclusion: Varying nanoparticle concentration can effectively alter scaffold surface mechanics and modulus, thereby altering cellular behavior. In addition, the specific nanoparticle may also influence cell proliferation, differentiation, and biomineralization. * — Abstract Presenter
Temporal-Controlled Release of Bovine Serum Albumin From Chitosan Nanoparticles on the Regulation of Alkaline Phosphatase Activity in Stem Cells from Apical Papilla

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The controlled delivery of bioactive molecules is crucial in dentin-pulp tissue engineering. Specific bioactive molecules are important at specific stages of cellular differentiation. We hypothesize that modification in the methods of incorporation of bioactive molecules in chitosan nanoparticles (CSPn) could bring variation in release kinetics which in turn effect differentiation potential of stem cells. Objectives: 1) Synthesize and characterize bovine serum albumin (BSA) loaded CSPn 2) Evaluate temporal-controlled release of BSA 3) Evaluate the effect of temporal-controlled release on regulation of alkaline phosphatase (ALP) activity in stem cells from apical papilla (SCAP). Materials and Methods: BSA loaded CSPn was synthesized by two methods: (1) encapsulation (BSA-CSPnI) and (2) adsorption (BSA-CSPnII). After characterization of size, charge and release kinetics, SCAP was cultured in presence of nanoparticles. SCAP viability and ALP activity was analyzed to determine the effect of nanoparticles on cytotoxicity and differentiation potential. The data were analysed by two-way ANOVA and Bonferroni post-hoc test to check for statistical significance between the groups. Results: BSA-CSPnI and BSA-CSPnII presented distinct in vitro release profiles in a time-controlled manner. Cell viability was enhanced over time in the presence of BSA-CSPn and BSA-CSPnII. ALP activity was significantly higher (p≤0.01) in presence of BSA-CSPn after three weeks than in BSA-CSPnII. Conclusions: It was found that ALP activity of SCAP is affected by the characteristic release profile of BSA. This study highlighted the potential of temporal-controlled bioactive molecule release technology in dentin-pulp regeneration. This study was supported by the University of Toronto Start-Up Fund.

Evaluation of the Clinical Delivery of Mesenchymal Stem Cells to the Root Canal Space of Necrotic Mature Teeth

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Introduction: Regenerative endodontic procedures are viable alternatives for treating immature teeth. The transition of these procedures to treat mature permanent teeth depends, among other factors, on the availability and delivery of mesenchymal stem cells (MSCs) into root canals. Although evoked-bleeding results in a substantial influx of MSCs into root canal spaces in immature teeth, its efficacy in mature teeth has never been evaluated. We hypothesized that evoked-bleeding delivers MSCs into root canals of mature, fully formed, teeth. Methods: A total of 18 patients referred for nonsurgical root canal therapy due to pulpal necrosis in fully mature permanent teeth participated in this study. Following root canal chemomechanical debridement, blood samples were collected from canals after over-instrumenting through the apex and achieving intracanal bleeding from the periapical tissues. A positive blood aspirate (control systemic blood sample) was also collected in the cartridges during local anesthetic injection. Total RNA was isolated and used as template in qRT-PCR reactions using MSC-specific PCR arrays. Data were normalized to a housekeeping gene and then to systemic blood levels for each gene, and analyzed using one-way ANOVA. Results: Evoked-bleeding technique resulted in increased expression of MSCs markers CD73, CD90, CD105 and CD146 with median fold regulation values of 4, 16.6, 8.7 and 9, respectively. In addition, there was a decrease in the expression of the negative marker CD145 (median = -9.1). Conclusion: These findings suggest that evoked-bleeding technique can be a potential method to deliver undifferentiated MSCs clinically in regenerative endodontic procedures in patients with mature teeth.

Effect of Small Molecules on Human Dental Pulp Stem Cell Properties Via Altering Signaling Pathways

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One ultimate concern regarding stem cells for regenerative medicine is the maintenance of stem cell stemness. Objective: To test whether small molecules (Pluripotin (SC1), 6-bromoindirubin-3-oxime (BIO) and rapamycin) can enhance stem cell properties of human dental pulp stem cells (hDPSCs) by targeting certain signaling pathways. Methods: Primary cultures of hDPSCs were exposed to optimal concentrations of these small molecules. Treated hDPSCs were analyzed by immunocyto staining, flow cytometry analysis, qPCR and western blot analysis for their proliferation, the expression levels of pluripotent and MSC markers, differentiation capacities (odont/oosteogenic, adipogenic and neurogenic) and intracellular signaling activations. Results: Small molecule treatments decreased cell proliferation and increased the expression of STR0-1 and pluripotent stem cell markers NANOG, OCT4 and SOX2, while diminishing cell differentiation into odonto/oosteogenic, adipogenic and neurogenic lineages in vitro. These effects involved Ras-GAP, ERK1/2, and mTOR signaling pathways, which may preserve the cell self-renewal capacity, while suppressing differentiation. Conclusion: Small molecules appear to enhance the immature state of hDPSCs in culture, which may become a promising potential for adult stem cell maintenance in regenerative clinical applications. This study was supported by the AAE Foundation and NIH R01 DE019156.

Clinical and Radiographic Study of Traumatized Immature Permanent Necrotic Teeth After Revascularization/Revitalization Therapy

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Revascularization/revitalization therapy of immature permanent necrotic teeth not only restores vitality but can also promote increased thickness of the canal walls and continued root lengthening, unlike traditional apexification procedures. The purpose of this prospective study was to investigate the responses of traumatized immature permanent necrotic teeth after revascularization/revitalization therapy. Material and Methods: Twenty immature permanent traumatized necrotic anterior teeth were treated with a standardized revascularization protocol supported by the American Association of Endodontists. The Turbogrip plugin within the ImageJ software package was used to standardize the preoperative and follow-up periapical images before making measurements of apical closure, root length and root thickening. The mean or median difference in the measurements at three, six, nine and 12 month follow-up films relative to baseline were analyzed statistically using McNemar's test and paired t-test. Results: The radiographic responses to revascularization/revitalization therapy, such apical closure, continued root development, and thickening of the canal walls varied greatly. Ninety percent of cases showed complete resolution of periapical lesions at 12 month follow-up examination. Significant changes in mean root length (mm) and in mean thickness of the canal walls (mm) compared to baseline were observed at the three month and six month follow-up radiographs. In some individual cases, the changes in thickness of the canal walls and the continued root development after different follow-up periods are very small and are not discernable with visual examination. Conclusion: The radiographic changes observed after a standardized revascularization procedure are varied; further studies are needed.
Preliminary Evaluation of a Model to Study Barodontalgia

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Barodontalgia is defined as tooth pain caused by changes in ambient pressure. Although numerous articles have been published on this potentially debilitating condition, there is a lack of scientifically based in vitro studies from which objective data can be drawn for future prospective clinical studies. The purpose was to obtain objective data concerning dental structures when exposed to increased as well as decreased pressure. Extracted human molar teeth (n=7) were instrumented to an apical size of 25. Root apices and external root surfaces were sealed and inserted into impression material to the level of the cemento-enamel junction. A barometric pressure transducer was placed in the pulp chamber and the access was filled with cotton. Sensors were calibrated by placing the specimens into a pressure chamber with a second transducer to monitor ambient chamber pressure. The specimens were subjected to a pressure change protocol involving a slow ascent to 25,000 feet above sea level back down to 5,000 feet, followed by a rapid ascent to 25,000 feet and a rapid descent to 5,000 feet. The access was then closed with Cavit and after 24 hours the simulated altitude pressure protocol was repeated. Real-time pressure from both transducers were recorded with mean results showing that pressures between the sensors correlated closely (r^2=.97-.99). Greater pressure differences were noted during descent (mean=5.52 torr), but differences were largely specimen dependent with the differential never exceeding 16 torr (0.41 psi). The results demonstrate the ability to obtain objective data in an in vitro barodontalgia model.

Efficacy of Clonidine as an Additive to Lidocaine on Post-Operative Pain for Inferior Alveolar Nerve Block Anesthesia After Single-Visit Root Canal Treatment in Teeth With Irreversible Pulpitis

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Objective: Pain control after root canal treatment is important in endodontic practice. The purpose of the present study was to investigate efficacy of an admixture of lidocaine with clonidine on post-operative pain and the use of analgesics after root canal treatment. Methods: In a randomized, double-blind clinical trial, 60 patients (30 per group) having first or second mandibular molars with irreversible pulps randomly received either 1.8 ml of 2% lidocaine with clonidine (15 microg/ml) or 1.8 ml of 2% lidocaine with epinephrine (12.5 microg/ml), using a conventional inferior alveolar nerve block. After single-visit root canal treatment, each patient recorded their pain score on a visual analogue scale at six, 12, 24, 36, 48 and 72 hours after treatment. Data were analyzed by Mann-Whitney, c2, Cochran Q and t-tests as well as Pearson correlation analysis (p<.05). Results-data and statistical analysis: The results indicate that patients who received clonidine/ lidocaine had significantly lower pain scores at six and 12 hours after root canal treatment compared with the patients who received lidocaine alone (p<.05). The use of analgesics in the clonidine patients was significantly lower than in the lidocaine group (p<.05). Conclusion: Patients who received clonidine/lidocaine as the anesthetic agent for single-visit endodontic treatment of irreversible pulps in mandibular molars had significantly less early post-operative pain and used fewer analgesics than those who had lidocaine as the anesthetic.

Post-Operative Pain After Instrumentation With Two Different Rotary NiTi Instruments: A Prospective Randomized Clinical Trial

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Aim and Objective: To evaluate the post-operative pain after instrumentation with ProTaper® and Mtwo in teeth with asymptomatic irreversible pulpitis. Materials and Methods: Eighty-four patients of asymptomatic irreversible pulpitis and patients requiring intentional root canal therapy in mandibular first molar were used in this study. The working length was confirmed by an electronic apex locator and periapical radiographs. Canals were instrumented with the instruments as decided by the randomization. The final apical size was F1 for ProTaper® and 20-6% for Mtwo group in the mesial root and F3 for ProTaper® and 35-6% for Mtwo group, respectively. A total volume of 30 ml of 4% hypochlorite was used as irrigant throughout the procedure using 30 gauge side vented needle placed upto 2 mm short of the working length. Final irrigation was done with saline and closed using Glass ionomer cement. Pain was recorded as no pain, slight, moderate or severe bidualy up to five days. The pain on percussion score was also recorded at the end of one, three and seven days. Results and statistical analysis: The findings were statistically analysed using Mann-Whitney U-test to compare two independent samples. ProTaper® instrumentation produced higher post-operative pain when compared to Mtwo until 84 hours post-instrumentation with a p-value less than 0.05 (significant). With regard to tenderness to percussion, ProTaper® instrumentation produced higher tenderness on first, third day and not on seventh day with a p-value less than 0.05 (significant). Conclusion: Instrumentation using rotary ProTaper® produced more pain and tenderness to percussion when compared to Mtwo.

Temperature Development on the External Root Surface During Laser-Assisted Endodontic Treatment Applying an Innovative Chopped Mode of a 980 nm Diode Laser

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We investigated the temperature increase on the external root surface, while simulating a laser-assisted endodontic treatment using a diode laser (980 nm) in microchopped mode. Ten freshly extracted, human maxillary incisors with mature apices were collected, prepared to an apical size of F4 (ProTaper®), DENTSPLY Maillefer, Switzerland), mounted to a holder and irradiated (using spiral movements in coronal direction) with a diode laser (GENTLEray 980 Classic Plus, KaVo, Germany) with a 200 μm fiber in four different treatment modalities: Group 1 (control group) was irradiated in six cycles of 5 seconds irradiation/20 seconds pause with 2.5 W in pulse mode. Groups 2 to 4 were irradiated accordingly in microchopped mode (Group 2- 1.6W; Group 3- 2.0W, Group 4- 2.5W). Canals were kept moist by sterile saline irrigation in between irradiations and temperature changes were continuously measured using a thermal imaging camera. The highest mean of temperature rise of 1.94 ± 1.07°C was measured in Group 4, followed by Group 3 (1.74 ± 1.22°C) and Group 2 (1.58 ± 1.18°C). The lowest increase occurred in Group 1 (1.06 ± 1.20°C). There was a significant difference (p<0.05) between the groups. Significant differences were found between Groups 1 and 4 (p=0.007) and 1 and 2 (p=0.035). Additionally, a marginal significant difference between Groups 1 and 2 (p=0.052) was noted. There was no significant difference between Groups 2, 3 and 4. Within the limitations of this study, it can be concluded that microchopped diode laser irradiation is a safe treatment option in laser-assisted endodontic treatment, as the critical temperature threshold of 40°C was never exceeded. Further studies are required to investigate the bactericidal properties of the microchopped mode.
**PR01**

**Susceptibility of Common Endodontic Pathogens to Common and Potential Intracanal Antibiotics for Regenerative Endodontics**

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Introduction: The most commonly used root canal space medicament for regenerative endodontic procedures is the triple antibiotic paste (TAP) which is a mixture of ciprofloxacin, minocycline and metronidazole. The currently used technique to prepare the triple antibiotics into a creamy paste results in a high concentration of antibiotics which has been shown to be toxic to the stem cells of apical papilla (SCAP). Therefore, the aim of this study is to determine the minimum bactericidal concentrations (MBCs) and minimum inhibitory concentrations (MICs) of TAP as well as doxycycline, Augmentin and tigecycline against four common endodontic pathogens. Methods: *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Streptococcus intermedius* were grown in specific media and incubated in anaerobic chamber. *Enteroctococcus faecalis* was incubated in aerobic chamber. Optical density of tested microorganisms was adjusted to 0.5 McFarland. MICs and MBCs of tested antibiotics were determined for the test organisms, using E-test susceptibility method. Each test was performed in triplicate for a total of 72 experiments.

Results: All tested microorganisms were sensitive to tested antibiotics except for metronidazole, which did not inhibit the bacteria at concentrations up to 256 µg/mL. For all tested microorganisms, ciprofloxacin had significantly higher bactericidal concentration compared to other tested antibiotics (ANOVA and Tukey's tests, p<0.05). Conclusion: The minimum bactericidal concentrations of tested antibiotics were lower than concentrations previously shown to be toxic to stem cells; therefore, these low concentrations should be considered for disinfection in regenerative endodontic procedures.

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**PR02**

**Treatment Outcome in Endodontics: A Retrospective Comparative Study Between Undergraduate Dental Students and Endodontists**

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Objectives: This study assessed the outcome of initial endodontic treatment on patients from the dental school of University of Mainz, Germany treated either by supervised dental students or by graduated endodontists in the last 10 years. Methods: A total of 336 teeth were examined clinically and radiographically by an independent examiner: 139 teeth (mean age 54 ± 16.9) by dental students and 197 teeth (mean age 61.6 ± 20.8) by endodontists. We compared the healing rate (healing was considered as no clinical symptom or signs and periapical index < 3) time between specialized dentists (endodontists) and dental students by obtaining Kaplan-Meier curves. In order to assess the joint influence of treatment type, status at root canal treatment, we fitted a proportional hazard model (Cox regression model). Results: The healing rate after three years was 84.47% (students) and 85.59% (endodontists). The five-year healing rate was 76.54% (students) and 72.3% (endodontists) whereas the 10-year healing rate was 69.7% (students) and 65.38% (endodontists). The most striking influence was the presence of apical lesions (hazard ratio=33.6, p<0.001).

Conclusions: No statistically significant difference of healing rate was noted between endodontists and dental students. The success rate of patient treated by dental student was slightly higher. Undergraduate students do not treat complicated cases and this might be the reason for the difference in the groups. This study confirmed apical periodontitis as the main predictors of outcome in initial endodontic treatment.

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**PR03**

**Comparison of Distortion Resistance of Nickel-Titanium Rotary Instruments in a Single Simulated Curvature in Two Different Rotary Systems**

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A concern when using rotary NiTi is the possibility of file distortion and separation. The proprietary design of Vortex Blue (VB) allows for reduced shape memory with less stress and distortion. EndoSequence (ES) has an electropolished surface, which results in fewer surface defects and increases fracture resistance. The aim of this study was to evaluate the distortion in two rotary systems in a simulated curved canal. Eighty ES files and 80 VB files size 30/.04 at 21 mm length were selected for use in acrylic blocks. After instrumentation, files were observed under a microscope for detection of gross deformity or fractures at the five-second and 10-second time intervals. ES exhibited the most distortion at the five-second interval and VB exhibited the most distortion at 10 seconds. Upon evaluation of distortion types, at the five-second time interval ES revealed 76% unwinding, 23% both unwinding and overtwisting. There was 23% unwinding for VB and 77% both unwinding and overtwisting. At the 10-second time interval, ES revealed 85% unwinding and 15% unwinding and overtwisting; whereas, for VB group at 10 seconds, only 13% unwound and 87% were both unwinding and overtwisting. ES and VB both distorted at five-second and 10-second intervals. There was no significant difference in the amount of distortion between the two test groups.

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**PR04**

**Detection of Human Beta Defensin One, Two and Three (HBD) in the Dental Pulp by Enzyme-Linked Immunosorbent Assay (ELISA)**

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Introduction: Human β-defensin (HBD) is a small cationic antimicrobial peptide made by epithelial cells. During microbial invasion there is production of HBD which stimulates the innate immune response, contributes to adaptive immune response and shows chemotactic activity. HBDs play an important role in the early host defense against Gram-positive and Gram-negative bacteria, mycobacteria, fungi and enveloped viruses. Although the expression of HBD in the pulp has been previously reported, little is known about whether the difference in pulpal diagnosis would affect the HBD level. We hypothesized that the level of HBD is different between symptomatic irreversible pulpitis, asymptomatic irreversible pulpitis and normal pulp. Purpose: To investigate the expression level of HBD one, two and three in teeth endodontically diagnosed with normal pulp, symptomatic irreversible pulpitis, and asymptomatic irreversible pulpitis. Methods: Patients undergoing NSRCT from the Graduate Endodontic Clinic were selected. Patients grouped as the following: Group I: symptomatic irreversible pulpitis, Group II: asymptomatic irreversible pulpitis and Group III: normal pulp. Samples were collected from the pulp after access cavity preparation and transferred into Eppendorf tubes. Expression level was assessed by using enzyme-linked immunosorbent assay (ELISA) and bicinchoninic acid assay (BCA). The statistical analysis was calculated using SPSS 16.0 software. Results: Expression of HBD one, two and three was significantly greater with symptomatic irreversible pulpitis > asymptomatic irreversible pulpitis > normal pulp (p<0.05). Conclusion: HBD one, two and three may contribute in pulpal host response. Further *in vivo* and *in vitro* studies are required to investigate the therapeutic, endodontic regeneration potential of HBD.
PR05

Comparison of Fill Length in Curved Root Canals Using Two Preparation Techniques and Two Thermoplastic Obturation Systems: GuttaCore and RealSeal 1®

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Thermoplasticized obturation provides three-dimensional fill and the ability to fill irregularities in root canals. However, the thermoplastic technique frequently results in overextension of material beyond the root apex. It is clinically significant to provide a predictable protocol for length control with newly introduced thermoplastic obturation systems. The purpose of this in vitro study was to evaluate overextension of RealSeal 1 (RS) and GuttaCore (GC) in curved canals instrumented with standard taper preparation (STP) and varied taper preparation (VTP). Distobuccal/distal roots exhibiting 20–40° canal curvature using Schneider's method were selected. Master apical file sizes were 40/0.04 for STP and 40/0.02 for VTP. Eighty-four mature and patent human maxillary and mandibular molar teeth were randomly divided into four groups (n=21). Group 1: STP/GC, Group 2: STP/RS, Group 3: VTP/GC and Group 4: VTP/RS. Three-dimensional fill was evaluated radiographically using the bisecting angle technique and extension of fill was determined clinically by microscope (5x). Samples were examined by a blinded evaluator and data was assessed ordinally to analyze fill overextension, with acceptable being +/- 1.0 mm within WL and overextension > 1.0 mm beyond WL. Results revealed the following incidence of overextension: Group 1: 10/21, Group 2: 2/21, Group 3: 4/21 and Group 4: 0/21. This difference between groups is statistically significant at p<.05 for both the type of preparation technique and for the GC/GC systems. This study was supported by the AAE Foundation, DENTSPLY Tulsa Dental Specialties and SybronEndo.

PR06

The Effect of Demographics on the Desire for Sedation and the Relationship Between Specific Endodontic Terminology and Patient Anxiety

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Few studies have looked at patients' desire for sedation while undergoing endodontic treatment. Our purpose was to 1) investigate whether patients' desire for sedation was related to their demographics and 2) determine how specific terms in endodontics affect patient anxiety. We hypothesized that demographics would influence the desire for sedation in endodontics and that terminology would influence anxiety. Adult patients who presented for consultation to the graduate endodontic department were surveyed about their age, income, gender, level of education and level of interest in sedation for root canal treatment. Patients were asked about their anxiety levels related to the specific terms “root canal” and “endodontic therapy.” Adult patients under age 35 were more likely to report interest in sedation for root canal treatment as compared to patients over 35. Patients who reported annual income levels below $30,000 had more interest in being sedated than those above $30,000. Females were more interested in sedation than males. Patients reporting lower levels of education had greater interest in sedation. Patients associated less anxiety with the specific term “endodontic therapy” than “root canal.” Only 52.5% associated moderate to severe anxiety with “endodontic therapy” while 70% reported moderate to severe anxiety with a “root canal.” The level of interest in sedation in endodontics may be related to patient demographics. Using the term “root canal” may elicit more anxiety than using the term “endodontic therapy.” Understanding patient demographics when considering use of sedation as well as careful use of terms may improve endodontic treatment plan acceptance.

PR07

In Vitro Effect of Nanodiamond on Oral Bacterial Biofilm

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Objective: This study was initiated to investigate the effects of nanodiamonds (NDs) on formation and viability of oral bacterial biofilms. Methods: The bacterial species used in the in vitro biofilm model were Streptococcus mutans, Fusobacterium nucleatum and Enterococcus faecalis. Bacteria were cultured in the presence of various levels (50-200 µg/ml) of NDs for one day under single-species biofilm formation in 48-well tissue culture plates. We utilized the minimal inhibitory concentration (MIC) test, crystal violet staining assay, and colony forming unit counting method to quantify the effects of the ND particles on biofilm formation. Biofilm morphology was also observed with live/dead staining via epifluorescence microscopy. We also used fluorescent agent-labeled NDs to probe their interaction with bacterial cells. Results: NDs did not inhibit the growth of bacteria based on the MIC and live/dead staining data. ND administration resulted in minimal effects on the viability of the bacterial species tested. Interestingly, presence of NDs led to cell clumping in the bacterial cell culture, presumably resulting from ND attachment to the bacterial cell wall and subsequent crosslinking of the cells. In the presence of ND particles, bacterial biofilm formation was notably impaired and the biofilm could be easily detached from the plastic surface. Conclusions: These data indicate that NDs may interfere with bacterial cell-to-surface interaction via its binding onto bacterial cell walls. This study was supported by the Jack Weichman Endowed Fund and the UCLA School of Dentistry’s Seed Grant program.

PR08

Evaluation of a New Biomimetic Cement (GEMOSIL) for Use in Endodontic Therapy as Compared to the Widely Used Mineral Trioxide Aggregate (MTA)

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Mineral trioxide aggregate (MTA) has proven to be an effective material for endodontic therapy; however, due to its long setting time and dentinal/gingival discoloration, its use is limited. Objective: To assess the hypothesis that a new biomimetic cement, GEMOSIL, has physical-biological properties comparable or superior to MTA in endodontic therapy. Design/Materials/Methods: Mechanical strength of GEMOSIL was evaluated using cylindrical samples under uniaxial compressive load and disc samples with an Instron machine for biaxial flexure strength. Biological properties of both materials were tested using a viability test of pulp cells and antimicrobial culture to measure the response of the zone of inhibition against S. mutans and E. faecalis. Both materials were placed in pulp chambers of extracted teeth for discoloration testing and in vivo as retrograde fillings in rat incisors to assess biocompatibility. Results: The setting time of GEMOSIL is approximately three to six minutes. Compressive strength of GEMOSIL reached 28 MPa after two hours of water immersion and 93 MPa fully dried. Flexure strength reached 59 MPa fully dried. These properties were superior to those of MTA. S. mutans was susceptible to both GEMOSIL and MTA, but E. faecalis was only susceptible to GEMOSIL. GEMOSIL caused no cytotoxicity and promoted proliferation of pulp cells (p<0.05). It demonstrated less discoloration than MTA in pulp chambers of extracted teeth (p<0.05) and showed biocompatibility for in vivo retrograde fill in rat incisors. Conclusion: GEMOSIL has superior physical and biological properties as compared to MTA; thus GEMOSIL may be used as a viable substitute for MTA. This study was supported by the AAE Foundation, NIH/NIDCR R44DE022218 and NIH/NIDCR K08DE018695.

* — Abstract Presenter
PR09

**Cone Beam-Computed Tomography – Anatomic Analysis of Mandibular Posterior Teeth: Impact on Endodontic Surgery**

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Objective: The purpose of this study was to use cone beam-computed tomography (CBCT) measurements to investigate root thickness (B-L) of posterior teeth, the dimension of the buccal and lingual bone over the root, and the relative location of the mandibular canal (MC) to the tooth. Methods: CBCT scans from 106 patients were used to evaluate measurements from 960 teeth and respective tooth areas. Bone and root thickness were measured at the preferred root resection level of 3 mm from root apex. Results: Buccal bone was thinnest over the root of the first premolar (2.07 mm) and thickest over the distal root of the first molar (3.0 mm). Lingual bone thickness ranged from 4.15 mm (first molar distal root) to 4.5 mm (second premolar). Root width (B-L) at the resection level averaged 5.28 mm, 5.77 mm, 4.4 mm and 4.3 mm for the first molar distal root, first molar mesial root, second premolar and first premolar, respectively. The MC location was most often seen to the buccal of the second molar distal root (58%) while it was most often seen to the lingual of the root at the level of the mesial root of the first molar (31.5%). The MC was inferior to roots of posterior teeth in 38-58% of the time. Conclusion: Knowledge of the mandibular posterior tooth dimension for apical resection is beneficial to the endodontist. The root width and its relative position to the mandibular canal can aid the surgeon performing the root resection. This study was supported by the AAE Foundation and the Canadian Academy of Endodontics. Files were donated by SybronEndo.

PR10

**Evaluation of Root Canal Curvature in Distolingual Root of Three-Rooted Mandibular Molars Using Cone Beam-Computed Tomography**

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The presence of the distolingual root in permanent mandibular first molars is considered as a normal morphologic variation for Mongoloid populations. The endodontic management of this root variation may present challenges for the clinicians. The objective of this study was to assess the root canal curvature of distolingual root of three-rooted mandibular molars in Korean populations. Method: CBCT images of the mandible were collected from patients who had undergone CT scanning at Wonkwang University Daejeon Dental Hospital, Daejeon, Korea, in 2012. A total of 109 mandibular molars from 69 patients were selected. Using AsahiVision software, we recorded the center of distolingual root canal space of each image slice and 3-D coordinate system was constructed individually. Calculation of the gradient between adjacent coordinates and statistical analysis was performed by using the Excel (Microsoft Corp., Redmond, WA) 2010 software package. Results: Most of three-rooted mandibular molars have severe root canal curvature in buccolingual plane than that of mesiodistal plane. In buccolingual plane, 60% of a position of inflection was middle third of the root canal and a position of maximum gradient was apical third (59%). In mesiodistal plane, 89.6% of a position of inflection was apical third of the root canal, and a position of maximum gradient was coronal third (42%). But, it was not statistically significant. Conclusions: Three-rooted mandibular molars have 3-D canal curvature mainly in buccolingual plane. The inflection point was middle third of root, and the position of maximum gradient was apical third. Curvature in mesiodistal plane was not significant.

PR11

**Clinical and Radiographic Evaluation of MTA Pulpotomies: A Retrospective Study**

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Introduction: Vital pulp therapy is indicated for exposed pulps in the young permanent tooth to preserve pulp vitality. Historically, calcium hydroxide (Ca(OH)₂) was the material of choice for a vital pulpotomy. Recently, mineral trioxide aggregate (MTA) has been used as a pulp sealing material because of its biocompatibility and its ability to induce hard tissue barrier formation. The purpose of this retrospective study was to evaluate the clinical and radiographic outcome of pulpotomy procedures performed with MTA. Methods: All patients receiving MTA pulpotomies at Virginia Commonwealth University School of Dentistry Graduate Endodontic Practice between November 2009 and August 2013 were recalled and evaluated for presence or absence of clinical symptoms, pulp vitality, continued root development and pulp canal obliteration. Results were analyzed descriptively. Chi-squared analysis was used. Results: At time of treatment caries was found to be the most common etiologic factor. Sixty-two percent of cases were asymptomatic at time of treatment (38% symptomatic). Forty-six percent of the teeth presented with immature apices (54% with radiographically closed apices). Overall recall rate was 37%. At recall all teeth were clinically asymptomatic. Twenty-nine percent of teeth tested responded normally to cold. Forty-three percent showed pulp canal obliteration. There was a correlation between pulp canal obliteration and teeth that were symptomatic at time of original treatment. All teeth with immature apices showed continued root development. Conclusion: MTA pulpotomy is a predictable treatment modality for young vital permanent teeth affected by caries. This study was supported by the AAE Foundation.

PR12

**Involvement of IL-13 and IL-15 in Pulpal and Periradicular Inflammation**

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Objectives: Pulpal inflammation is mediated, in part, through secretion of various pro- and anti-inflammatory cytokines. To determine the cytokine network during pulp inflammation, we used a dental pulp stem cell (DPSC) and monocyte (THP-1) co-culture model exposed to lipopolysaccharide (LPS). Methods: The cells were cultured in the presence of 1ug/ml LPS for 24 hours, and the soluble factors in the conditioned medium were profiled using cytokine antibody array. The resulting data was further verified by time-course experiments in which the level of cytokine gene expression was determined by qRT-PCR. The cytokine array data was confirmed using an animal model in which mandibular first molar pulp was exposed and inoculated with common endodontic pathogens. Results: LPS treatment of the DPSC/THP-1 co-culture led to induction of pro-inflammatory cytokine gene expression, including IL-1, TNF-a, IL-6 and IL-8. In addition, there was strong induction of IL-13 and IL-15, as evidenced by the cytokine array and qRT-PCR for intracellular mRNA levels. Time-course assay revealed transient induction of IL-13 expression at six hours post-LPS treatment while IL-15 expression was delayed until 48 hours, suggesting differential temporal expression of these two cytokines in DPSCs after LPS treatment. The mouse pulp exposure model successfully led to periapical bone destruction, indicative of apical periodontitis, within four weeks post-exposure. The correlation between IL-13/15 induction and the extent of apical periodontitis in the animal model is presented. Conclusions: This study identifies two cytokines, IL-13 and IL-15, both of which may play crucial roles in the pathobiology of bacteria-induced apical periodontitis.
Comparing Canal Transportation and Remaining Dentin Thickness of WaveOne™ Reciprocating Files with TF adaptive™ Rotary Files by Using Micro-Computed Tomography

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The purpose of this study was to evaluate the preparation abilities of WaveOne™ and TF adaptive™ in curves of mesial root canals of mandibular molars (20–45°) using micro-CT. The parameters that were measured included root canal straightening or transportation, amount of unprepared dentin, as well as changes in volume of dentin. Eighteen extracted mandibular molars with mesiobuccal and mesiolingual canals with separate foramina were used. Pre-instrumentation scans of all teeth were taken, and the teeth randomly divided into two groups. In Group 1, the canals were instrumented with WaveOne™ files, and in Group 2, the canals were instrumented with TF adaptive™ files. Postinstrumentation scans were performed, and the two scans were compared to determine canal transportation and remaining dentin thickness. Data was analyzed with ANOVA statistical method. Preliminary data shows that there was no significant difference in canal transportation and centering ability between the two file systems at 1, 3, 5 and 7 mm levels from the apex. Both file systems are clinically effective in moderately curved mesial canals of mandibular molars. This study was supported by Nova Southeastern University Health Professions Division and DENTSPLY Tulsa Dental Specialties.

Mutagenic Effects of a New Irrigant Mixed With Commonly Used Irrigants in Endodontic Therapy

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A number of irrigants are commonly used in endodontic therapy to disinfect and debride the root canal system. The quest is continuous to find new irrigants and methods to improve disinfection of the root canals. QMix is a new irrigant that has been tested for its antibacterial and smear layer removal properties. Six percent NaOCl and 2% Chlorhexidine in combination have been shown to have potential mutagenicity. However, there have been no studies that investigate the mutagenic potential of the solutions of QMix combined with other commonly used irrigants. The objective was to assess the mutagenicity of endodontic irrigants in conjunction with QMix. We hypothesized that the combinations of QMix with Chloroform, 17% EDTA, 6% NaOCl and 2% Chlorhexidine would not have a mutagenic effect. The solutions were divided into six groups: QMix + 6% NaOCl, QMix + 17% EDTA, QMix + 2% Chlorhexidine, Positive Control and a Negative Control, and were transferred into 50mL tubes, mixed with sterile water, reagents and a mutant bacterial strain. The Muta-ChromoPlate kit was used to evaluate mutagenicity. After culturing and incubation, the number of mutagenic wells was recorded. Environmental Bio-Detection Products Inc. was used for statistical analysis to determine the level of mutagenicity. The Positive Control plate showed mutagenic effects, however zero of the 96 wells in the samples and Negative Control plate showed mutagenicity. QMix demonstrated no mutagenicity when used in combination with 6% NaOCl, 17% EDTA, Chloroform and 2% Chlorhexidine, therefore it is not necessary to rinse with sterile water between these irrigants to avoid mutagenic effects.

Biocompatibility Screening of Endodontic Regeneration and Repair Biomaterials

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The aim of this study was to measure the in vitro biocompatibility of endodontic regeneration and repair biomaterials using Mouse (1929) cells and human periodontal stem cells (hPSCs). The test biomaterials (n=200 samples) were: Biodentine™, both white (WMTA) and grey (GMTA) ProRoot™ mineral trioxide aggregate, calcium hydroxide USP, Intermediate Restorative Material™ (IRM), SuperEB™, Geristore™, Bis-Core™, EndoSequence™ Root Repair Putty and Paste were prepared according to manufacturer instructions. The test biomaterials were packed into sterile tubing creating 1 mm by 1 mm samples, which were placed in tubes, mixed with sterile water, reagents and a mutant bacterial strain. The Muta-ChromoPlate kit was used to evaluate mutagenicity. After culturing and incubation, the number of mutagenic wells was recorded. Environmental Bio-Detection Products Inc. was used for statistical analysis to determine the level of mutagenicity. The Positive Control plate showed mutagenic effects, however zero of the 96 wells in the samples and Negative Control plate showed mutagenicity. QMix demonstrated no mutagenicity when used in combination with 6% NaOCl, 17% EDTA, Chloroform and 2% Chlorhexidine, therefore it is not necessary to rinse with sterile water between these irrigants to avoid mutagenic effects.

MirRNA-181a Negatively Regulates Toll-Like Receptor Agonist-Induced Interleukin-8 Production in Human Dental Pulp Fibroblasts and Macrophages.

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Pulpitis represents an immune response to bacteria and is the most common reason for patients seeking emergency dental care; however, the regulatory mechanisms governing pulpal inflammation are not completely understood. Our preliminary data using human dental pulp fibroblast (HDPF) showed that miR-181a expression is modulated by toll-like receptor activation in a time- and dose-dependent manner and that IL-8 expression levels exhibit an inverse correlation with miR-181a expression. To date, the mechanisms by which miRNA-181a may regulate IL-8 is yet to be investigated. Objectives: 1) To investigate potential miRNA-miRNA regulatory mechanisms on IL-8, and 2) to determine if this mechanism also exists in macrophages, a resident immune cell of the dental pulp. Methods: Primary HDPF and U937 macrophages were exposed to LPS, with or without transfection with miR-181a mimic or antagonim, and supernatant IL-8 levels examined by ELISA. Unchallenged and nontransfected cells served as controls. MiR-IR-181a/IL-8 3’UTR mRNA alignment was predicted using miRWalk and this interaction analyzed using dual-luciferase assays in HEK293 cells. Results: Transfection with miR-181a mimic resulted in increased IL-8 production in both HDPF and macrophages (p<0.05) while its antagomir significantly reduced supernatant levels in both cell types (p<0.05). In-silico analysis identified a miR-181a binding site on the 3’UTR of IL-8 which was confirmed by dual-luciferase assays. Conclusion: miR-181a directly binds to the 3’UTR of IL-8, an important inflammatory component of the pulp immune response, and modulates its levels. This is the very first report demonstrating miR-181a regulation of IL-8. This study was supported by University of North Carolina at Chapel Hill School of Dentistry, NIDCR T90DE021986 and NIH/NIDCR DE021052.
**Effectiveness of Irrigating and Chelating Agents with Photon Induced Photoacoustic Streaming**  
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The purpose of this study was to investigate the effectiveness of irrigating and chelating agents activated with Photon Induced Photoacoustic Streaming (PIPS) to disinfect root canals in *ex vivo* teeth. Fifteen extracted permanent human teeth were infected with 50 microliters of *E. faecalis* (ATCC 4082) for 28 days in Brain Heart Infusion (BHI) broth. The root canals were prepared to a final apex size of 35/.04 using Vortex Blue instrumentation with 6% NaOCl irrigation, followed by 17% EDTA, QMix 2-in-1 or MTAD. The irrigants and chelating agents were activated by a PIPS endodontic fiber tip using an 2940 nm Er-YAG laser for 150 seconds at 10 Hz and 50 mJ. All the specimens were then placed in BHI broth for 72 hours, the growth of residual microorganisms were measured by BHI absorbance using a spectrophotometer at 600 nm. Data was analyzed by ANOVA at a significance of p<0.05. The residual bacterial growth was 13.7% with QMix 2-in-1, 28.2% with EDTA and 50.1% with MTAD, but because of the large variation in means the differences were not significant (p>0.05). We are in the process of collecting more data to investigate if chelating agents and irrigants can be activated with PIPS to improve the cleaning and disinfection of contaminated root canals. This study was supported by the AAE Foundation and Nova Southeastern University. Material support provided by DENTSPLY Tulsa Dental Specialties.

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**Antibiotic Significance With Immediate Implant Placement Into Sites With Apical Pathology of Endodontic Origin**  
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Objectives: Antibiotic resistance is a growing concern in our population. While there are not always exact guidelines for their indications, many clinicians are quick to prescribe with surgical procedures in the hope that it will decrease the chance of failure. One such example is the replacement of a nonrestorable tooth that also has apical pathology with an immediate implant. This study explored the use of antibiotics in improving the outcome of immediate implant placement following extraction of teeth with apical periodontitis. Methods: Subjects who signed an informed consent were randomly assigned to the antibiotic (n=10) or a placebo group (n=10) prior to surgery. Pain, discomfort and level of healing were evaluated at follow-up appointments using a visual analog scale. CBCT and impressions taken at pre-op and six-month recall were used to evaluate bone and soft tissue healing. Results: One hundred percent and 80% survival was observed in the antibiotic and placebo groups, respectively. Of the survival group we found that antibiotic coverage did not make a statistically significant difference in post-op pain, healing and integration. One failure was found to have debris wedged between the implant and socket, the second was a patient who struggled with periodontal disease in the past. Conclusion: As there are currently no clear guidelines on this topic, and with the growing risk of antibiotic resistant bacterial strains, this study will provide relevant information on the effect of antibiotic coverage for immediate implant surgery. This study was supported by the American Academy of Implant Dentistry Foundation and the American College of Prosthodontists Educational Foundation.

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**The Prevalence of Pulpal Necrosis and Symptomatic Irreversible Pulpitis in Teeth Restored With Crowns, Composites and Amalgams Within One Year of Restoration**  
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Most teeth are not pulp tested prior to placement of new restorations. Some may require NSRCT even before the tooth is prepared for restoration. There are limited studies that evaluate the pulp diagnosis prior or subsequent to placement of the following permanent restorations: crowns, composites and amalgams. The purpose of this study was to investigate the prevalence of the most common pulp diagnoses in teeth within one year of their specific restoration placements. We hypothesized that there would be a difference in the prevalence of pulpal diagnoses with each restoration type. Analysis of records of all dental school patients who received permanent restorations from January 2012 until January 2013 was completed. The records were further limited to those patients that had NSRCT performed within a one-year period. Data recorded for each patient: type of restoration, date of restoration placement, endodontic diagnosis, and date of endodontic treatment. Chi-squared test was used for statistical analysis. The prevalence of pulpal necrosis (PN) was 50% (crowns), 53% (composites) and 17% (amalgams). The prevalence of symptomatic irreversible pulpitis (SIP) was 33% (crowns), 33% (composites) and 83% (amalgams). Furthermore, the 50% PN of crowned teeth occurred within six months, while the 83% SIP of amalgam restored teeth occurred within one year. Within the confines of this study, consideration should be given to the pulpal diagnosis of teeth at initial restorative treatment. Dentists must be aware that either certain restorations will result in a more common pulpal degeneration within a minimum time frame, or that NSRCT was required prior to restoration.
Assessment of Factors Influencing Patients’ Demands for Sedation in Endodontics

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Endodontic therapy is perceived as a procedure to be feared. Sedation is a useful method for alleviating fear and anxiety. However, there are a limited number of studies involving the demand for sedation in endodontics. The purpose of this study was to assess patients’ awareness and the potential demand for sedation in endodontics that may be influenced by their personal perceptions. We hypothesized that there would be a correlation between patients’ awareness of sedation and their demands. Patients’ perceptions and demands regarding sedation and endodontic therapy were assessed using a questionnaire that was given to all patients 18 years and older, who presented to the Graduate Endodontic Clinic. Results were collected and statistically analyzed. Results showed 48% of the patients reported their perception of sedation was being put to sleep and 35% reported it as relaxation or reducing pain. Fifty percent of the patients reported having previous experience of root canal therapy, of which 60% of them would prefer to be sedated during the procedure if sedation were available. Common concerns that patients associated with root canal therapy were as follows: pain 65%, fear of needles 28%, difficulty getting numb 16% and claustrophobia 13%. Sedation is not often offered in endodontic practice and this study demonstrated low patient awareness of sedation availability and yet high demand for sedation. Sedation may aid in providing more pleasant experiences for the patients receiving endodontic therapy. Endodontists should consider providing sedation as a useful adjunct in their practice and it may lead to more patients seeking endodontic care.

Comparison of Cyclic Fatigue Failure of Three Nickel-Titanium Rotary Files

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Cyclic fatigue causes separation of the files in the canal during root canal treatment. This study evaluated the fatigue life of three different nickel-titanium rotary files when subjected to dynamic testing in simulated root canal preparations. The instruments tested were EdgeFiles® X7 series, Profile® Vortex Blue, and Profile® ISO (tip size 35 with a .06 taper) (n=90 total, 30 per group). Each was subjected individually to rotary motion using a mounted, electric torque-controlled handpiece within a simulated canal in a metal block, with a 45° curvature, until fracture occurred. The rpm for the EdgeFiles® X7 series and Profile® Vortex Blue was set at 500, and the Profile® ISO was set at 300 as per the manufacturers’ recommendations. The time-to-fracture and the number of cycles-to-fracture (NCF) were recorded and calculated. Median NCF values per group were compared using Kruskal-Wallis one-way ANOVA, and pairwise comparisons were done using Tukey’s analysis. Fractured files were mounted with carbon tape for analysis of fracture surfaces and origins under scanning electron microscope. Testing time ranged from 61.6 to 259.7 seconds. The median NCF values were 1,277.16 for EdgeFiles® X7 series, 898.5 for Profile® Vortex Blue, and 712.6 for Profile® ISO demonstrating statistical significance among all three groups (p<.01). Microscopic analysis indicated some surface flexural fatigue striations, processing inclusions and stress concentrations at the flute pitches due to geometric differences. EdgeFile™ X7 rotary files were significantly more resistant to cyclic fatigue than the other two rotary file systems.

The Efficacy of the WaveOne Reciprocating File System Versus the ProTaper Retreatment System in Endodontic Retreatment of Two Different Obturating Techniques

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Objectives: This ex vivo study evaluated the efficacy of retrieving GuttaCore and warm vertically condensed gutta-percha in moderately curved canals with two different systems: ProTaper retreatment and WaveOne. Methods: Eighty mesial roots of mandibular molars were prepared using the WaveOne primary file. The canals were obturated with two different techniques: warm vertical or GuttaCore. The warm vertical group was obturated using a continuous wave technique of gutta-percha compaction, and the GuttaCore group was obturated according to manufacturers’ instructions. The teeth were subdivided into four experimental groups (n=20) each with the same mean root curvature: Group 1: Warm Vertical retreated with the ProTaper, Group 2: Warm Vertical retreated with the WaveOne system, Group 3: GuttaCore retreated with the ProTaper, Group 4: GuttaCore retreated with WaveOne. After allowing sealer to set, each specimen was retreated with either ProTaper retreatment files (D1-D3) or the WaveOne primary file to the predetermined working length. Time to reach working length (T1) was recorded. Instrument fatigue and failure was also recorded. Results: The preliminary results indicate that there is no statistical significance in the time to reach working length during retreatment with the two systems. Instrument failure occurred at a higher rate with WaveOne than with the Protaper instruments. Treatment of GuttaCore was faster and led to fewer instrument failures than retreatment of continuous wave with both instruments. Conclusions: WaveOne is not a safe file for use in retreatment. GuttaCore is removed more efficiently and places less stress on instruments than continuous wave. This study was supported by the AAE Foundation.

A Comparison of Bacterial Growth After Conventional and Aseptic Endodontic Techniques

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Introduction: Microorganisms are the essential cause of pulp and periapical disease. Assuming “vital teeth” are sterile, endodontic outcomes should approach 100%. Instead, some studies of vital outcomes report 88 to 93%. Aim: #1. We propose that microorganisms can be introduced to and survive in treated root canals of previously sterile extracted teeth. #2. To compare whether a Conventional Aseptic Technique (CAT) or an Aseptic Endodontic Technique (AET) will yield differences in the occurrence of surviving microorganisms. We hypothesize AET will result in fewer surviving microorganisms. Materials and Methods: In this pilot study, 96 extracted, sterile teeth/media/vial, assemblies were divided into two experimental groups (36 teeth each) and 24 sterilization controls. Thirty six teeth in the CAT group were instrumented and filled in a typical clinical manner. For the 36 teeth in the AET group, instrumentation and filling were completed using a strict aseptic technique. Appropriate controls were employed at all steps. Microbial growth was observed at zero and 30 days and after crushing followed by aerobic and anaerobic subculture. Results: Two of 24 (8.3%) of the sterilization controls demonstrated growth. Day 0 CAT: six of 18 grew; Day 0 AET: two of 14 grew p=0.413; Day 30 CAT: 10 of 18 grew; Day 30 AET: zero of 17 grew p=0.003. Conclusions: Microorganisms can be introduced by technique and survive in previously sterile root canals. Strict aseptic techniques result in fewer detectable microorganisms after endodontic therapy. This study was supported by the Krakow Harvard/Forsyth Endowed Endodontic Research Fund.
**Analysis of Sequential Osteoblastic Marker Expressions in Human MG63 Cells**

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Introduction: The aim of this study was to investigate the effect of Conditioned Medium (CM) on differentiation of MG63 stem-like cell. Methods: MG63 cells were grown and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with fetal bovine serum and antibiotics at 37°C in a 5% CO₂ humidified atmosphere. Apical bud cells (ABCs) were isolated from murine incisors at postnatal day seven, and cultured in DMEM with the same condition. MG63 cells and ABCs were co-cultured for 10 days. RNA extracted from MG63 cells were subjected to real-time PCR and Western blot analyses to assess sequential osteoblastic marker gene expressions. Results: In co-cultured samples, alkaline phosphates and type I collagen significantly increased from day three to day seven. Bone sialoprotein (BSP) mRNA expression was dramatically increased approximately 230-fold in comparison to the expression level of the control. BSP protein expression showed a similar pattern to that of BSP mRNA expression. Conclusions: Based on findings from this in vitro experiment, CM from ABCs promoted osteoblastic marker expression during the differentiation of human MG63 cell.

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**Disinfection Efficacy of Current Regenerative Endodontic Protocols in Simulated Necrotic Immature Permanent Teeth**

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The lack of mechanical debridement and reduced concentrations suggested for chemical debridement to maintain stem cell viability, calls into question the disinfection efficacy of current regenerative protocols. The purpose of this study is to determine if simulated immature teeth infected with Enterococcus faecalis can be completely disinfected by following current standardized regenerative protocols. Only the concentration and type of antibiotic medications used will be varied. This study compares canal disinfection with known stem cell toxicity. Sixty-six caries-free maxillary incisors were used. S1 sampling protocols were validated in both a negative control group and positive control group via culture and SEM. All teeth, except the negative controls, were inoculated with E. faecalis. The teeth were divided into the following groups: Group 1: 24 teeth: triple antibiotic paste (ciprofloxacin:metronidazole:minocycline) at concentrations of 10, 1, 0.1 mg/mL. Group 2: 24 teeth: double antibiotic paste (ciprofloxacin:metronidazole) at concentrations of 10, 1, 0.1 mg/mL. Group 3: eight teeth: Ultraclal™ XS (Ca(OH)₂). Controls: four negative controls and six positive controls. Current regenerative protocols recommended by the AAE were followed. S2 sampling was performed and tested for bacterial presence via culturing and SEM analysis. Findings show that Ca(OH)₂, and the current recommended antibiotic concentrations are not capable of completely eliminating bacteria from simulated necrotic immature permanent teeth. Overall, this study focuses on the need to re-evaluate the balance between stem cell toxicity and bacterial elimination in order to determine the appropriate concentrations and medicaments for successful regenerative endodontic procedures. This study was supported by the AAE Foundation.

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**Effect of Calcium Silicate-Containing Materials on Stem Cells From the Apical Papilla**

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Mineral trioxide aggregate (MTA) has been exclusively used as an overlying material to the scaffold in regenerative endodontics. Recently, to improve MTA’s drawbacks such as discoloration and long setting time, Biodentine™ was introduced as an alternative. This study aimed to evaluate the effect of calcium silicate-containing cements (MTA or Biodentine™) on the proliferation and Alkaline Phosphatase (ALP) activity of stem cells from the apical papilla (SCAP). SCAP were placed into the wells of culture plate to undergo differentiation stimulation and 1 mg/mL Biodentine™ or MTA was added to the osteogenic/dentinogenic medium. Untreated cells served as control. Cell viability was assessed by MTT assay at three days and 14 days. ALP activity from the cells cultured for three days and seven days was determined with p-nitrophenyl phosphate as a substrate. Absorbance was measured at 410 nm using a spectrophotometer. The results showed that cell viability was higher in Biodentine™-treated cells on day three than other groups (p<0.01, ANOVA, Tukey’s HSD test, n=12), whereas that with the MTA was significantly lower at 14 days as compared to both Biodentine™ and control groups (p<0.01). Compared with untreated control cells, cells treated with Biodentine™ or with MTA exhibited decreases in ALP activity at both three and seven days (p<0.001, n=4). In addition, ALP activity was decreased in all culture conditions at seven days. Within the condition of this study, Biodentine™ can be considered as alternative material because it demonstrated higher cell viability than MTA. Both calcium silicate-containing materials decreased the ALP activity of SCAP.

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**The Critical Time Period Between Various Restoration Placements and Subsequent Endodontic Intervention**

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There are studies regarding the cause of pulpal inflammation due to restorative treatment. However, a thorough search of the literature failed to show what is the critical time period between the restorative placement and pulp inflammation. We hypothesized that when endodontic treatment was required following restoration, it would be needed within 12 months. We further hypothesized that full-coverage restorations would more likely require endodontic treatment compared to amalgams or composites. Therefore, the aim of this study was to investigate the time lapse of endodontic treatment subsequent to restorative treatment: amalgam, composite and full-coverage restorations. A comprehensive computerized analysis of all dental school patients who received restorations (n=10,666) from 2012 to 2013 was obtained. Data was collected and statistically analyzed using chi-square and odds ratio calculations. Of the population that required NSRCT following restoration, it would be needed within 12 months. Treatment rates were 10.4%, seven months (15.9%), eight months (7.14%) and 12 months (7.1%). Furthermore, composite restorations were 1.83 times more likely than amalgam restorations to require root canal treatment within a one-year time period. Three out of four patients who required NSRCT following restoration placement needed it within four months, some as early as one week. Dental practitioners should perform pulp vitality testing before (and after) all restorative treatment. This will save time and costs for patients by preventing the compromise of a new restoration and/or the need for an additional restoration.
The Prevalence of Intrapulpal Cracks in First and Second Mandibular Molars Requiring Nonsurgical Root Canal Treatment

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Introduction: Few studies report the incidence or prevalence of cracked teeth with pulp involvement. No attempts have been made to evaluate the prevalence or clinical predictors for intrapulpal cracks. The purpose of this study was to investigate the prevalence of intrapulpal cracks in first and second mandibular molars and to determine if clinical findings are predictive for the existence of intrapulpal cracks. Methods: One hundred ninety mandibular molars requiring nonsurgical root canal treatment at the Virginia Commonwealth University Graduate Endodontic Practice between February 15, 2013, and August 15, 2013, were analyzed, retrospectively. Teeth were transilluminated, stained and inspected for intrapulpal cracks using a dental microscope. Characteristics included: demographic data, subjective and objective information regarding the chief complaint to include bite stick test, probing depths greater than 4 mm, existing restorations and diagnosis. Chi-squared and logistic regressions were performed (p<0.05). Results: The prevalence of intrapulpal cracks in mandibular molars was 9% (17/190, 95%CI = 5.7% to 13.9%). Deep probing depths and age over 40 were jointly significant for predicting intrapulpal cracks. Staining of the pulp chamber identified only one of 17 intrapulpal cracks. Clinical findings predictive for the existence of intrapulpal cracks other than age and pocket depth were inconclusive. Conclusions: Age and deep probing depth were found to be significantly associated with intrapulpal cracks. Staining of the pulp chamber did not significantly increase the ability to identify intrapulpal cracks. This study was supported by the AAE Foundation.

Comparison of Mineral Trioxide Aggregate, Bioaggregate, Biodentine™ on Pulp Response in Rat Molar

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The objective of the present study was to assess the capacity of three calcium silicate-based cements (Mineral trioxide aggregate, Bioaggregate and Biodentine”) to induce pulp healing in a rat pulp injury model, when compared with MTA. Pulp exposure was prepared on maxillary first molars of eight nine-week-old male rats, and exposed pulp was capped with Bioaggregate, Biodentine™ and MTA. Cavities were sealed with flowable resin. Animals were sacrificed at four weeks. Samples from eight rats were used for micro-CT evaluation. Histologic examination (hematoxylin and eosin staining) and immunohistochemistry were performed. MTA showed complete dentin bridge formation with normal pulp histology. Homogeneous dentin bridge formation was also observed in Bioaggregate and Biodentine™. Immunoreactivity to the anti-DSP was detected on all experimental groups. It appeared that Bioaggregate and Biodentine™ induced dentinal bridge formation and it had similar characteristics with MTA.

A Survey for Endodontists in Today’s Economy: Exploring the Current State of Endodontics as a Profession and the Relationship Between Endodontists and Their Referral Base

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Objective: The purpose of this study was to assess the perceptions, referral trends and practice patterns of practicing endodontists in the United States. Methods: A 22-question survey was formulated and sent via www.surveymonkey.com to 3,255 active members of the American Association of Endodontists. Participants were assured of confidentiality of their responses. Overall, 875 participants completed the survey, a response rate of 27%. Results: The average number of treatment cases per day was 5.7. Average work hours per week were 34.3 for males and 30.7 for females (p<0.05). Among all treatment cases, 46% were nonsurgical retreatment and 7.2% were apical surgical procedures. Procedural misadventure accounted for 10.8% of all treatment cases with the most common referral reason being “unable to locate canals” (75.0%) followed by “separated instruments” (15.3%). Of all respondents, 49.9% performed regenerative endodontic procedures and 7.7% placed implants. Among endodontists who practice in urban areas, 69.7% felt there were too many endodontists and 50% have delayed their retirement plans due to recent economic impact, compared to their suburban and rural counterparts at 66.1% and 38%, 25.9% and 33.1%, respectively (p<0.05). Fifty-nine percent of respondents were optimistic about the future of endodontics as a specialty but those who have practiced more than 20 years were more pessimistic than those with less experience (p<0.05). Conclusions: Recent economic impacts appear to have had an effect on the perceptions of active endodontists regarding practice success, the future of the specialty and their retirement plans. This study was supported by the AAE Foundation.

Effect of EndoSequence Root Repair Material on Differentiation of Human Dental Pulp Cells, a Potential Material for Pulp Capping

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Pulp capping techniques aim at preserving the vitality of pulp tissue, thus avoiding more invasive endodontic procedures. EndoSequence Root Repair Material (ERRM) has been introduced as a potential pulp capping material. It is hypothesized that ERRM promotes the healing of dental tissue, thus maintaining integrity and vitality of the pulp after pulp capping procedures. However, the exact mechanism by which ERRM affects the pulp after pulp capping procedures is still not delineated. Therefore, the aim of this research is to delineate the mechanisms by which ERRM protects the dental pulp and promotes healing. The effects of ERRM on the dental pulp cells (DPCs) were analyzed using various assays such as Trypan blue staining, WST-1 proliferation assay, VEGF (Vascular Endothelial Growth Factor) ELISA and Fluorescent Microscopy staining. Cells in contact with Mineral Trioxide Aggregate (MTA) were used as the positive controls. Results were analyzed using an ANOVA with a post-hoc Tukey’s test and the level of significance set at p≤0.05. The results demonstrate that the DPCs showed higher levels of trypan blue positive cells and lower levels of proliferation as compared to the control cells and the MTA group. The cells in contact with the ERRM, however, did secrete higher levels of VEGF, an angiogenic factor necessary for cell survival and function. Overall, this study demonstrates decreased survival and proliferation of DPCs when in contact with ERRM and would need to be investigated further before using it for pulp capping procedures. This study was supported by the AAE Foundation and the Douglas Morrell Grant, University of Washington.
Apical transportation, ledging and zipping during instrumentation of root canals are undesirable consequences that can negatively impact the success of endodontic therapy. The purpose of this study was to investigate the occurrence of apical transportation in root canals after instrumentation with two different rotary files, EndoSequence and Vortex Blue. Forty extracted mandibular first and second molars were selected. After working lengths were determined, pre-instrumentation radiographs were taken with a #15 hand file. The mesial canals were instrumented using either EndoSequence or Vortex Blue rotary files. Post-instrumentation radiographs were taken with the master apical rotary file. An apparatus was utilized which allowed the teeth to be radiographed in an identical position prior to and after instrumentation. The images were superimposed digitally in Adobe Photoshop CS6 and a double-digital radiographic technique was utilized to evaluate apical transportation using AutoCAD. The Student’s t-test was used to analyze the data. For the EndoSequence group, the mean value of apical transportation was 0.20 mm. For the Vortex Blue group, the mean value of apical transportation was 0.21 mm. There was no statistical difference between the two groups. In conclusion, instrumentation with EndoSequence rotary files exhibited slightly less apical transportation than Vortex Blue rotary files, but there was no statistically significant difference in apical transportation between the two files.

Elimination of biofilm-forming pathogenic microorganisms and prevention of recurrent infections through a chemomechanical approach are important goals in endodontics. Since the residual presence of *E. faecalis* has been associated with endodontic failures, irrigants such as 6% sodium hypochlorite (NaOCl), 0.12% chlorohexidine gluconate (CHX), QMix®, and Biopure™ MTAD are currently used as antimicrobials. The objective of this study was to assess the efficacies of these agents: (1) in removing preformed single- and dual-species biofilms of *E. faecalis*, *S. gordonii* and *S. mutans* and (2) in inhibiting biofilm growth on irrigant-coated surfaces. Biofilms were formed in 96-well microtiter plates by using 107 CFU/mL of bacteria. Biofilms exposed to irrigants were used as controls. CFU-based plating methods were utilized to quantify residual viable cells after irrigant treatment. Crystal Violet staining with OD590 measurements was used to quantify biofilm formation on pre-coated surfaces. Statistical analysis was performed using Student’s t-test. Consistently, NaOCl and QMix® eradicated all bacteria (100 ± 0.1%) in single- and dual-species biofilms whereas CHX and MTAD were less efficient (95-98 ± 0.5%) in eliminating *E. faecalis* from biofilms. *E. faecalis* colonies were present at 60-70 ± 0.5% level compared to the total bacteria in dual-species biofilms. In the irrigant-coated experiments, all four irrigants were ineffective (60-100% residual *E. faecalis*; p<0.001) allowing *E. faecalis* to sustain and colonize. These results suggest the efficacies of currently used irrigants are less desirable and the need for better antimicrobial and biocompatible irrigants is warranted. This study was supported by the Rutgers School of Dental Medicine Department of Endodontics and the Office of Research.

Introduction: Antibiotics are empirically administered in patients with severe endodontic infections. Expanding our current knowledge on microbial flora, their virulence factors and antibiotic susceptibility is essential to promote effective management of these infections. Purpose: The purpose of this study was to identify the bacteriological differences between hospital inpatients and outpatients diagnosed with endodontic infections. Methods: A total of 15 subjects diagnosed with acute apical abscesses were included. Six subjects were hospitalized patients at the University of Maryland Medical Center (UMMC), and nine patients were treated as outpatients in a dental school’s endodontics or oral surgery clinics. Results: The data collected from inpatient subjects showed an average hospitalization of four-and-a-half days and higher systemic signs of infection than the control group. The number of microorganisms cultured and identified was not significantly different between the two groups (Student’s t-test; p=0.9). The most commonly cultured bacterial genera in both groups were: *Streptococcus spp.*, *Staphylococcus spp.* and *Pepotostrepococcus spp.*, for which results were not statistically significant between the groups (Fisher’s exact test; p≥0.5). One case from an outpatient had methicillin-resistant *Staphylococcus aureus*. Conclusion: In this cohort study, the analysis of microbiological parameters of outpatients and inpatients diagnosed with endodontic infections did not reveal statistically significant differences. This study was supported by the AAE Foundation.
Quantitative Analysis of the Antimicrobial Efficacy of Two Irrigation Protocols

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It is well established that mechanical instrumentation alone does not clean all the canal walls. Irrigation is one of the most important phases of endodontic therapy. The aim of this study was to evaluate the efficacy of two irrigation protocols on biofilm removal and correlate with the size of the apical preparation. Fifty-four mandibular molars were instrumented with rotary files. A standardized amount of Enterococcus faecalis and Fusobacterium nucleatum was inoculated at the isthmus of mesiobuccal and mesiolingual canals. Samples were incubated for 10 days to allow biofilm formation. Three teeth were used as negative control (without bacteria inoculation) and three teeth were used as positive control (biofilm without irrigation). Forty-eight teeth were divided into four groups: 1) Conventional irrigation, apical size preparation 25; 2) QMix® apical size 25; 3) Conventional irrigation, apical size preparation 30; 4) QMix® apical size 30. Conventional group received irrigation with 5 ml of 6.15% NaOCl and QMix® following manufacturer specifications. Samples were obtained pre- and post-irrigation with paper points, MB2.

Background: The presence of a second canal in the mesiobuccal root (MB2) of maxillary molars is well documented in the literature. The purpose of this retrospective study was to assess the frequency of location/treatment of the MB2 canal in maxillary molars and variables affecting location frequency in a postgraduate endodontic program. Methods: Records and radiographs of patients treated in the postgraduate endodontic clinic between July 1, 2006, and June 30, 2013, were reviewed by two observers. Data collected included: treating resident code, treated in the postgraduate endodontic clinic between July 1, 2006, and June 30, 2013, were reviewed by two observers. Data collected included: treating resident code, tooth number, patient age and gender, presence of preoperative crown, pulp vitality status, presence/absence of MB2, initial treatment or retreatment. MB2 was considered present if noted in record and radiographs. Pearson’s chi-squared test was used for statistical analysis. Results: Six hundred or retreatment. MB2 was considered present if noted in record and radiographs. Results were analyzed using ANOVA with a post-hoc Tukey’s test and the level of significance set at p≤0.05. Teeth were then longitudinally sectioned and observed under the confocal microscope to correlate the results from the micro-CT. This study demonstrates that the percentage of residual filling material remaining was greater in BC Sealer groups than in AH Plus groups. This study was supported by the AAE Foundation.

The Anti-Inflammatory Effect of GV1001 Peptide on P. gingivalis

LPS-Induced Inflammatory Cytokine Production and Its Mechanism in Human Dental Pulp Cells

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Introduction: Reverse transcriptase subunit of telomerase (hTERT) has been an attractive target for cancer vaccines. GV1001 peptide, which is a peptide corresponding to amino acids 611-626 of hTERT (EARPILTSRLRFIPK), has been developed as a vaccine against various cancers and has been reported to have the ability of penetrating into various cells including cancer cell lines and primary blood cells. Objectives: The purpose of this study was to confirm the cell penetrating function of GV1001 peptide in human dental pulp stem cells (hDPSC) and to investigate its ability in regressing inflammatory cytokines induced by LPS which can be responsible for pulpitis and periodontitis. Methods: The intracellular distribution of GV1001 was analyzed by confocal microscopy. RT-PCR was performed to determine the expression level of TNF-α and IL-6. The phosphorylation of MAP kinases (ERK and p38) and Jnk-α expression were examined using western blot analysis. Effect of GV1001 peptide on hDPSC viability was measured by MTT assay. Results: GV1001 was predominantly located in hDPSC cytoplasm. The peptide inhibited LPS-induced TNF-α and IL-6 production in hDPSCs without significant cytotoxicity. GV1001 treatment markedly inhibited the phosphorylation of MAP kinases (ERK and p38) and significantly reduced NF-κB activation in LPS-stimulated hDPSCs. Conclusions: GV1001 has the ability to penetrate into the cell. It suppresses the LPS-induced production of inflammatory cytokines by blocking NF-κB and MAP kinase (ERK and p38) activation. These findings provide mechanistic insights into anti-inflammatory actions of GV1001 peptide in LPS-stimulated pulpitis and periodontitis without significantly affecting cell viability.
Endodontic irrigants and solvents commonly come in contact with each other during biomechanical preparation. Although recent studies have investigated the interactions between them, contradicting results regarding the formation of mutagenic precipitates lead to an under-emphasis of the potential hazards of their contact with periapical tissues. Objective: The purpose of this study was to determine mutagenicity of precipitates/solutions formed by different combinations of irrigants using a modified Ames test. We hypothesized that the precipitates/solutions formed by 6% NaOCl, 17% EDTA, 2% CHX and Chloroform in different combinations are not mutagenic. Methods: Irrigants were titrated in the following combinations: 6% NaOCl and 2% CHX, 6% NaOCl and 17% EDTA, 6% NaOCl and Chloroform, 2% CHX and Chloroform. The precipitates formed were dissolved in 100% Dimethyl Sulfoxide and cultured with Mutant Salmonella typhimurium strains (MSTS) TA100 and TA98 and essential reagents in 96-well plates at 37°C. Two known mutagens, 2-Nitrofluorene and Sodium Azide, were used as positive controls while sterile water served as the negative control. MSTS when exposed to mutagenic solutions under certain conditions underwent reverse mutation from histidine auxotrophy to prototrophy, allowing growth of the bacteria. This resulted in turning of the blue-purple wells on the microtitre plate to yellow. The test was repeated for accuracy and the number of revertant colonies per plate were entered into the EBPI (Environmental Biodetection Products Inc.) database for statistical analysis. Results: The precipitates formed by 6% NaOCl, 17% EDTA, 2% CHX and Chloroform in the above combinations did not show mutagenic potential. Conclusion: Our results suggest that they can be safely used within the root canal system.
The Effect of Smear Layer Removal on Microtensile Bond Strength of Bioceramic Sealer
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Smear layer removal may improve the micromechanical retention of some sealers. However, for bioceramic (BC) sealers, maintaining smear plugs in dentinal tubules may improve the chemical bonding of BC sealers to the canal wall. This study assessed the bond strength of a BC sealer in the presence or absence of smear layer. Forty 3 mm dentin discs were cut from roots of 20 extracted teeth. After instrumentation with a BioRace-0 file, discs were sectioned in half. To ensure stability, the halves were repositioned and held tightly by hemostats. The slices were immersed in different irrigants: group (NaOCl) (n=15) in 3% NaOCl (10 minutes); group (NaOCl/EDTA) (n=15) in 3% NaOCl (10 minutes) and then 17% EDTA (one minute); group (NaOCl/NaCl) (n=10) in 3% NaOCl (10 minutes) and then saline (five minutes). Canal spaces were filled with EndoSequence BC sealer (Brasseler, Savannah, GA). The samples were incubated for seven days at 37°C and 100% humidity. Microtensile bond strength was measured using a Microtensile Tester (Bisco, Schaumburg, IL) at 2 mm/minute. The data was analyzed using one-way ANOVA and Tukey’s HD tests. Mean values for microtensile bond strength were: group (NaOCl) = 0.48 (SD±0.04) MPa, group (NaOCl/EDTA) = 0.54 (SD±0.06) MPa, group (NaOCl/NaCl) = 0.55 (SD±0.05) MPa, which was statistically significant for all groups (p<0.005, ANOVA). There was a significant difference between group (NaOCl) and group (NaOCl/EDTA) (p<0.05) and between group (NaOCl) and group (NaOCl/NaCl) (p<0.01) (Tukey’s HD). Smear layer removal did not improve the bond strength of BC sealer NaOCl as a final irrigant may have adverse effect on the bond strength of BC sealer.

The Use of Cone Beam-Volumetric Tomography to Identify the Second Mesiobuccal Canal in Maxillary Molars: A Clinical Study
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A retrospective clinical study was carried out to determine if cone beam-volumetric tomography (CBVT) would aid the practitioner in locating the second mesiobuccal canal (MB2) in maxillary molars during endodontic treatment. CBVT scans of 50 patients who received root canal treatment at a private dental practice limited to endodontics were reviewed by the principal investigator (TR) for the presence or absence of MB2. The clinical notes and post-operative radiographs were then reviewed by the principal investigator (TR) to determine whether an MB2 was located and treated by the practitioner. A single experienced endodontist (MF) carried out all clinical diagnostic and treatment procedures. The treating clinician had the following diagnostic aids available to help identify MB2: preoperative CBVT (Kodak 9000, Carestream Dental, Atlanta, GA), surgical operating microscope (Global Surgical Corp., St Louis, MO), and ultrasonics. The treating clinician (MF) was uninvolved in data collection to reduce operator bias. The prevalence of MB2 in the CBVT images in this study was 92%. The prevalence of MB2 that was clinically identified was 89.1%. Statistical analysis showed a strong positive correlation (r=0.629) between the presence of MB2 in CBVT images and clinical findings, indicating CBVT accurately portrays actual anatomy. Furthermore, the clinical prevalence of MB2 was higher than previous in vivo studies using conventional two-dimensional radiography, suggesting that the use of CBVT can increase the clinical identification and treatment of MB2. This study was funded by the Endodontics Department at the University of Illinois-Chicago College of Dentistry.

Probiotics: The Nano Army of Endodontics
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Objective: The concept of probiotics has not yet been evaluated in endodontics although it has proven successful in periodontal disease. The aim of this study was to evaluate the effectiveness of a probiotic cocktail against Enterococcus faecalis (E.f) and Candida albicans (C.a) which are associated with apical periodontitis. Methods: Five groups (G I, II, III, IV and V) of commercial probiotics were selected and evaluated based upon numbers and concentration of organisms. Pathogenic test organisms were C. albicans (ATCC 10231) and E. faecalis (ATCC 47077) set to a one McFarland standard challenge. Testing was conducted by a disc diffusion assay test to evaluate Zones of Inhibition (ZOI) in millimeters of the selected probiotics against the E.f and C.a. Microorganisms from probiotic samples were extracted and placed on sterile discs. A five probiotic disc template on a blood agar plate was inoculated with a lawn of either E.f or C.a and incubated at 37°C for one week. Sterile disc templates on blood agar plates with a lawn of E.f and C.a were run parallel as a control. Results: Based on a one-way ANOVA analysis, Group I, IV and V showed the most statistically significant results (p<0.05) with a mean ZOI of 7.2 mm, 8.6 mm, 10.2 mm for C.a and a mean of 6.4 mm,11.1 mm and 12.5 mm for E.f, accordingly. L. acidophilus, L. casei, L. rhamnosus and B. longum were all common strains in the groups. Conclusion: Recognizing probiotics act differently based on composition and concentration, our pilot study suggested that the above mentioned organisms are effective against E. faecalis and C. albicans and suggests further evaluation.

Intervention: LL37 is an antimicrobial peptide formed from the last 37 amino acid residues of the C-terminus of human cationic antimicrobial peptide 18, which is the only human cathelicidin. LL37 shows antimicrobial activity against bacteria, fungi and viruses, and inhibits lipopolysaccharide bioactivity. Hypothesis: We hypothesized that there is no difference in LL37 levels between symptomatic irreversible pulps, asymptomatic irreversible pulps and normal pulp tissues. Objective: To assess the levels of LL37 in teeth diagnosed with symptomatic irreversible pulps, asymptomatic irreversible pulps and in normal pulp. Materials and Methods: Patients undergoing nonsurgical root canal treatment from the Graduate Endodontic Clinic were randomly selected. Pulpal and periradicular status were assessed the bond strength of a BC sealer in the presence or absence of smear layer. Forty 3 mm dentin discs were cut from roots of 20 extracted teeth. After instrumentation with a BioRace-0 file, discs were sectioned in half. To ensure stability, the halves were repositioned and held tightly by hemostats. The slices were immersed in different irrigants: group (NaOCl) (n=15) in 3% NaOCl (10 minutes); group (NaOCl/EDTA) (n=15) in 3% NaOCl (10 minutes) and then 17% EDTA (one minute); group (NaOCl/NaCl) (n=10) in 3% NaOCl (10 minutes) and then saline (five minutes). Canal spaces were filled with EndoSequence BC sealer (Brasseler, Savannah, GA). The samples were incubated for seven days at 37°C and 100% humidity. Microtensile bond strength was measured using a Microtensile Tester (Bisco, Schaumburg, IL) at 2 mm/minute. The data was analyzed using one-way ANOVA and Tukey’s HD tests. Mean values for microtensile bond strength were: group (NaOCl) = 0.48 (SD±0.04) MPa, group (NaOCl/EDTA) = 0.54 (SD±0.06) MPa, group (NaOCl/NaCl) = 0.55 (SD±0.05) MPa, which was statistically significant for all groups (p<0.005, ANOVA). There was a significant difference between group (NaOCl) and group (NaOCl/EDTA) (p<0.05) and between group (NaOCl) and group (NaOCl/NaCl) (p<0.01) (Tukey’s HD). Smear layer removal did not improve the bond strength of BC sealer NaOCl as a final irrigant may have adverse effect on the bond strength of BC sealer.

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Background/Purpose: In 2009, the American Association of Endodontists accepted a classification system of six pulpal and five periapical diagnoses. The purpose of this study was to investigate the prevalence of each of these revised diagnoses among patients undergoing endodontic treatment in a postgraduate clinic and to assess correlation of each diagnosis with clinical diagnostic findings. Methods: A record review of patients treated in the postgraduate endodontic clinic over two years was performed. Variables included patient age and gender, treated tooth number, treatment procedure performed, pulpal and periapical diagnosis, presence of a periapical lesion or condensing osteitis, antibiotic treatment during treatment, post-treatment flare-up and number of treatment appointments. Descriptive statistics and odds ratio in SPSS version 21 were performed for analysis. Results: Data was collected from 416 treated teeth. The most common pulpal diagnosis was symptomatic irreversible pulpitis (SIP) (41.6%) followed by pulp necrosis (PN) (36.1%). The most common periapical diagnosis was symptomatic apical periodontitis (SAP) (58.2%) followed by normal apical tissue (21.6%). Forty-two-and-a-half percent of necrotic teeth with SAP had a periapical radiolucency. A diagnosis of SIP presented with SAP 76.3% of the time. A flare-up occurred in 2.2% of cases. There was no significant difference in flare-up incidence between treatment in one or multiple appointments (2.2% versus 2.1%, p=.95). Conclusions: In this postgraduate clinic, SIP was the most frequent pulpal diagnosis. It was accompanied by SAP in over 75% of cases.

Introduction: Our current understanding of the distribution and frequency of periapical pathoses include biopsies submitted by all specialists and general dentists. As a result, they do not accurately reflect the distribution seen by endodontists. This retrospective chart review aims to determine the prevalence of periapical pathoses and associated demographics from biopsies submitted by endodontists over 30 years. Methods: All biopsy reports submitted to the Virginia Commonwealth University Oral Pathology Diagnostic Service from January 1, 1983, to December 31, 2012, were reviewed. Only reports submitted by verified endodontists and those with a periapical location were included. The following data was recorded from each report: submission date, referring endodontist, sex, age, race, biopsy location, tooth number and histologic diagnosis. Results were calculated using chi-squared and logistic regression analysis (significance p<0.05). Results: Nine thousand two hundred thirty biopsy reports fulfilled the inclusion criteria for an overall distribution of 23.99% cysts, 73.6% periapical granulomas, 1.73% scars and 0.68% other pathologies. Findings include a significant association between sex, location and diagnosis. An association with race or age was not seen. Significantly more cysts were seen in males and in the anterior. Conversely, significantly more periapical granulomas were seen in females and in the posterior. Significantly more other pathoses were found in the mandible and more scars in the anterior. Conclusions: Approximately three-fourths of biopsies submitted for evaluation by endodontists are diagnosed as periapical granulomas and one-fourth as cysts. Other pathoses and scars make up less than 3% of diagnoses. This study was supported by the AAE Foundation.

The purpose of this study was to evaluate the cytotoxic effects of a commonly used endodontic sealer combined with selenium (Se) against Enterococcus faecalis and Candida albicans. Bacteria were grown aerobically from frozen stock cultures overnight in Brain Heart Infusion (BHI) to late logarithmic phase in 10-12 hours at 37°C. Cells were placed in direct contact with normal and Se-containing material, allowed to dry and resuspended in BHI broth. Bacterial growth was determined spectrophotometrically to an optical density of 600 nm, OD=.1-.2. Bacterial growth in Se- and non-Se-containing materials was compared for their antibacterial effect. Pilot studies indicate that at 1% Se content, no bacterial growth was seen on Se-containing sealer while sealer containing no Se showed florid growth. The data in the pilot study thus far indicate that dental sealer containing Se displays active cytotoxic effects against E. faecalis and C. albicans.

Background: A question often posed to endodontists is, “when should orthodontic treatment (OT) be initiated or resumed following dental trauma (DT)?” According to the literature, initiation or resumption of OT after DT should be delayed from a few weeks to as much as one to two years depending on the type and severity of the DT. Aim: To determine the opinion of endodontists on the appropriate timing of OT after recent DT of varying severity. Methods: An online survey was distributed to members of the American Association of Endodontists (AAE). Part I created a basic profile of the clinician. Part II presented scenarios on OT of varying severity and assessed recommendations by the endodontist for resumption or initiation of OT. Descriptive statistics and nonparametric tests in SPSS version 21 were used for data analysis. Results: Four hundred fifty-three clinicians responded to the survey, 83% male and 17% female. Maximum concern for subsequent OT occurred after avulsion (74%), horizontal root fracture (HRF) (68%) and intrusion injuries (54%) compared to concussion (3%) and subluxation (7%). After HRF, 34%-39% (nonsurgical root canal therapy to fracture line or vital coronal segment respectively) recommended no future OT compared to only 3% after avulsion. Conclusions: Data indicate that avulsion and HRF generated the most concern for subsequent OT. Endodontists appeared more concerned with OT following horizontal root fracture than with avulsion. Orthodontic movement of teeth post-dental trauma is an area requiring more evidence-based research.
**PR53**

The Correlation Between Periapical Healing With Diabetes and HbA1c Levels

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The aim of this study was to evaluate the healing of periapical lesions in teeth with preoperative periapical lesions in diabetic and non-diabetic patients. Fifty diabetic and 50 non-diabetic patients treated in the Advanced Endodontics Program at the University of Maryland were invited for follow-up evaluations at two to four years following treatment. Only cases judged radiographically to have adequate treatment were included. There was an attempt to match diabetics and non-diabetics by age and gender, and only one tooth per patient was included. The immediate post-operative and recall periapical images were compared by two blinded endodontists who described each case as healed, healing or not healed. Differences among the examiners were discussed and a consensus was reached. At the recall time each patient’s glycosylated hemoglobin (HbA1c) was determined. Patients who responded to the recall attempts included 18 self-reported diabetics and 10 self-reported non-diabetics. Each patient was categorized as having controlled glycermia or uncontrolled glycermia where an HbA1c of six-and-a-half was the cut off. Fisher’s Exact test was used to analyze the data. The percentage of healed cases in self-reported non-diabetics was higher than self-reported diabetics (p=.77). Also the controlled glycermic group had a greater percentage of healing than the uncontrolled glycermic group (p=.34). These differences were not statistically significant, which may have been due to the small sample size. A power analysis determined a sample of 88 patients was needed to achieve a power of 80% at alpha error 0.05. This study was supported by the AAE Foundation.

**PR54**

Elimination of Intracanal Tissue and Debris Through a Novel Laser-Activated System Assessed Using High Resolution Micro-Computed Tomography

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Introduction: Laser-activated irrigation to remove organic debris from canal isthmuses was investigated using X-ray micro-focus computed tomography. Methods: A total of 14 extracted human mandibular molars were used. The mesial canals were prepared to a standardized instrumentation protocol. Two groups (n=7) underwent final irrigation using either standard needle irrigation (SNI) or photon initiated photoacoustic streaming (PIPS). Canal volumes were reconstructed from µCT scans before and following irrigation to assess removal of organic tissue and inorganic debris by quantitative analysis of the superimposed volumes. Comparisons of the volumes were made using two-way ANOVA and Tukey’s HSD, with statistical differences considered significant at the α=0.05 level. Results: Debris removal and increase in canal volume for the laser-activated PIPS group was more significant (p<0.001) than for SNI (p=0.04). Irrigation using PIPS increased canal volume and eliminated debris from the canal system 2.6 times greater than SNI. Conclusion: Eliminating debris from complex canal spaces found in mandibular molars was achieved at a significantly greater level using laser-activated PIPS irrigation compared to SNI.

**PR55**

Comparison of Two Continuous Ultrasonic Irrigation Devices in the Removal of Root Canal Debris

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Introduction: The purpose of this in vitro study was to compare two continuous ultrasonic irrigation devices (VPro StreamClean System [VSS], Vista Dental; and ProUltra PiezoFlow [PPF], DENTSPLY) when used for the final irrigation procedure to debride the apical region of the root canal. The null hypothesis was that there was no difference between the two devices. Methods: Root canals of matched pairs (n=20) of extracted human teeth were prepared to an apical size of F3 (ProTaper; DENTSPLY) with 6% NaOCl irrigation. One tooth of each pair was randomly assigned to receive final irrigation with either the VSS or PPF. Experimental constants were irrigation cycles (5 mL NaOCl, 5 mL 15% EDTA, 5 mL NaOCl), irrigant flow rate (5 mL/min), and needle depth placement (2 mm from WL). Serial sections taken 2 mm and 4 mm from WL were stained with hematoxylin-eosin and viewed at 100× magnification for the presence of debris. The percentages of debris in each canal lumen after VSS and PPF were compared using Wilcoxon matched-pairs test with significance level at p<0.05. Results: Percentage remaining debris ranged from 0.0-4.2% for VSS and 0.0-17.8% for PPF at 2 mm from WL, and from 0.0-0.1% for VSS and 0.0-6.0% for PPF at 4 mm from WL, with no difference in-group median values. The null hypothesis was accepted. Conclusions: Final irrigation with the VSS and PPF continuous ultrasonic irrigation systems resulted in no significant difference in the removal of root canal debris at 2 mm and 4 mm from the WL. This study was supported by the the AAE Foundation and the Leslie A. Morgan Endodontic Endowment Fund. The study was loaned the VPro StreamClean System from Vista Dental and the ProUltra PiezoFlow System from DENTSPLY Tulsa Dental Specialties.

**PR56**

An In Situ Animal Model for Dentin-Pulp Regeneration

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Tissue regeneration requires an interaction of stem cells and growth factors in a bioactive scaffold. Previous preclinical research does not present an in situ model for regenerative procedures following pulp space infection, as they would be used on patients. The objective of this study was to characterize the ferret canine model for stem cell approaches in regenerative endodontics. The advantage of using ferret canines as experimental animal models is that they have similar root canal anatomy to human teeth than rodents, with less cost and ethical objections than cats, dogs or primates. Ferret pulp tissues from immature canines were removed and processed to harvest dental pulp stem cells. The cells were passaged three to four times, tested for presence of stem cell biomarkers using immunofluorescence microscopy and tested for odontogenic/osteogenic and adipoigenic differentiation potential. Ferret antibodies are not available, so the following were tested for cross-reactivity: mouse anti-human STR0-1 IgMA; mouse anti-rat, mouse, rabbit Thy1 mab IgG1; rabbit anti-human CD105 polyclonal; rabbit anti-rat, human, mouse CD146 mab Ig6; and mouse anti-human, mouse, rabbit CD146 IgG1c. Results showed that the ferret cells were positive for the following stem cell markers: STR01, CD90 and CD105, but negative for CD146. When cultured in osteo/odontogenic medium, they showed strong mineralization potential as stained with Alizarin Red. The cells showed weak adipogenic differentiation potential. Following osteo/odontogenic stimulation, dentin sialophosphoprotein (DSPP) levels were not significantly higher than controls. Based on these results, ferret dental pulp cells stained positive with antibodies for stem cells and showed mineralization but not dentin formation in differentiation studies.

* — Abstract Presenter
Correlation Between the Presence of Apical Periodontitis and Symptoms in Endodontic Patients Using Cone Beam-Computed Tomography and Periapical Radiographs

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Cone beam-computed tomography (CBCT) is a valuable adjunct to endodontic practice. Among the many uses of CBCT is diagnosis of apical periodontitis, often in cases where there is no evidence of pathosis identified by conventional imaging. The purpose of this study was to correlate the presence of apical periodontitis of teeth evaluated with 2-D periapical (PA) radiographs and 3-D CBCT volumes with clinical signs and symptoms. Approval was obtained through the University of Washington Human Subjects Division. Charts were reviewed from patients examined at the University of Washington Graduate Endodontics Clinic and included clinical exam, PA radiographs and limited field of view CBCT scans. Of 498 cases, 75 fulfilled the inclusion criteria and were evaluated for apical periodontitis and symptomology. CBCT slices and PA radiographs were evaluated by two Board-certified endodontists and a Board-certified oral and maxillofacial radiologist for the presence of apical periodontitis. Forty-eight of 75 teeth showed the presence of apical periodontitis on PA radiographs and on CBCT, while 14 teeth had no evidence of apical periodontitis on PA radiographs or on CBCT. Thirteen cases showed the presence of apical periodontitis visible on CBCT that was not visible on PA radiographs. Of 498 cases, 75 fulfilled the inclusion criteria and were evaluated for apical periodontitis and symptomology. CBCT slices and PA radiographs were evaluated by two Board-certified endodontists and a Board-certified oral and maxillofacial radiologist for the presence of apical periodontitis. Forty-eight of 75 teeth showed the presence of apical periodontitis on PA radiographs and on CBCT, while 14 teeth had no evidence of apical periodontitis on PA radiographs or on CBCT. Thirteen cases showed the presence of apical periodontitis visible on CBCT that was not visible on PA radiographs. Presence of apical periodontitis in CBCT slices and PA radiographs was correlated with clinical signs and symptoms, including chief complaint. This research has important implications to prevent over-treatment, and to provide treatment for those patients with persistent symptoms lacking proper diagnosis based on conventional (2-D) radiographs. This study was supported by the AAE Foundation.

Mesenchymal Stem Cells in the Periapical Environment: Evidence From Human and Experimental Periapical Lesions

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Previous studies describe contrasting molecular profiles of active and inactive periapical granulomas characterized by distinct expression of cytokines, osteoclastogenic factors and wound healing markers. Although the molecular mechanisms underlying such dichotomy remain unknown, in this present study we investigated the potential involvement of mesenchymal stem cells (MSCs) in determining human and murine periapical lesions activity and outcome. Periapical granulomas (n=85) and control samples (n=24) were comparatively assessed for the expression levels of 11 MSCs markers through RealTimePCR. Experimental periapical lesions induced in mice (pulp exposure and bacterial inoculation) were evaluated for MSCs markers expression and effects of AMD3100 treatment (a chemokine receptor antagonist for CXCR4 and CXCL12-mediated chemotaxis) on lesions outcome.

Analysis of Factors Related to Extraction of Endodontically Treated Teeth

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Objective: The purpose of this study was to analyze the reasons for extraction of endodontically treated teeth. Methods: Data were collected over a six-month period at the time of extraction of endodontically treated permanent teeth (n=228) that were deemed unsuitable for further treatment. The patients’ age and gender, tooth type and the reasons for extraction (restorative, peridontic, endodontic, orthodontic and vertical root fracture) were recorded. Results: The major reason for extraction was restorative (71.4%), followed by endodontic (14.9%) and vertical root fracture (7%). Mandibular first molars (27.6%) and maxillary second premolars (17.9%) were the most common teeth subjected to extraction. Conclusion: Endodontically treated teeth were prone to extraction mainly due to restorative-related factors and, to a lesser extent, to endodontic-related reasons.

Reduction in Bone Density Necessary for the Detection of Periapical Pathosis

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Objective: The aim of this study was to determine the percent loss of bone density necessary for detecting periapical lesions. Additionally, we compared the results obtained between and within the groups of evaluators. Methods: Digital radiographs from nine cadaver jaws were altered to simulate a lesion at the apex of a single root. Each jaw produced images at seven different modification levels, correlating with the percentage of bone loss for each lesion. This resulted in a total of 63 different images for participant evaluation. Images were presented in a PowerPoint slide show in a randomized order. Participants indicated whether a lesion was definitely present, definitely absent or possibly present on the identified tooth. Results: Images were evaluated by 12 participants, including four endodontic faculty, four endodontic residents and four radiology residents. Preliminary data suggest there is a significant positive correlation between bone loss and lesion detection. With 6% bone loss, approximately 8% of participants were able to detect a lesion, while at 12% and 18% bone loss, approximately 43% and 77% were able to detect a lesion, respectively. There was no significant difference in responses between the groups of evaluators, although there was a significant difference between evaluators within each of the three groups. Conclusions: The ability to detect a periapical lesion on a digital radiograph depends on the extent of bone loss. In addition, there is significant subjectivity in radiographic interpretation of the presence or absence of periapical pathosis, not only among different evaluators but among the same evaluators. This study was supported by the AAE Foundation.
PR61

**In Vitro Comparative Assessments of an Experimental Polyolefin-Based Core and Two Commercial Cores for Carrier-Based Root Canal Fillings**

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Aim: The first aim of this paper was to compare the push-out strength of the gutta-percha coating of two commercially available carriers (Thermofil and GuttaCore) with that of gutta-percha used to coat an experimental hydroxyapatite/polyethylene (HA/PE) obturator. The second aim was to compare the thickness of gutta-percha around the carriers of GuttaCore and HA/PE obturators using micro-computed tomography (µCT). Methodology: Ten (size #30) 1 mm thick samples of each group (Thermafil, GuttaCore and HA/PE) were prepared. An orthodontic wire with a diameter of 0.5 mm was attached to the plunger of an Instron machine in order to allow the push-out test of the gutta-percha coating. Five samples of (GuttaCore and HA/PE) were scanned using µCT to determine the thickness of gutta-percha around the carriers. The data obtained was analysed with one-way analysis of variance and Tukey’s post-hoc test, and the statistical significance was set at p<0.05. Results: HA/PE obturators exhibited significantly higher push-out strength (p<0.001) determined at 6.84±0.63 than those of GuttaCore around 3.75±0.75 and Thermofil at 1.5±0.63. GuttaCore demonstrated significantly higher bond strength than Thermofil (p<0.001). µCT imaging revealed that the volume of gutta-percha around the GuttaCore carriers was uneven and void formation was evident. However, the volume of GP around the HA/PE carrier was homogeneously distributed. Conclusion: The push-out strength of gutta-percha coated HA/PE carriers was superior to that of gutta-percha coated GuttaCore and Thermofil obturators. The µCT observations demonstrated that the thickness of gutta-percha around the HA/PE was more uniform than that of GuttaCore carrier.

PR62

**Comparison of Push-Out Bond Strength of Three Calcium Silicate-Based Endodontic Materials**

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The purpose of this study was to evaluate the push-out bond strength of Biodentine™ compared with two available calcium silicate-based materials, Bioaggregate (BA) and WMTA. Methodology: One hundred twenty-three root dentin slices were obtained. The specimens were stored for 72 hours. The highest force applied to materials at the time of dislodgement was recorded. The push-out bond strength was calculated in megapascal. Slices were then examined under a stereomicroscope at 40× magnification to determine the nature of bond failure. Statistical Analysis: ANOVA test was used to compare means of push-out bond strength. Post-hoc test was then accomplished for multiple comparisons. Chi-squared test was used to determine if there is significant association between the type of material and type of failure. Results: The mean push-out bond strength ± SD values of WMTA, BA and Biodentine™ were 23.26 ± 5.49, 9.57 ± 3.45, 21.86 ± 6.9, respectively. There was no significant difference between the means of WMTA and Biodentine™ (p=0.566), but the mean of BA was significantly lower than those of WMTA and Biodentine™ (p=0.000). WMTA and BA showed a majority of mixed type of failure than cohesive, where Biodentine™ showed the opposite. No adhesive failure was observed in any of the three materials. Conclusion: Biodentine™ showed comparable results to WMTA, whereas BA had the lowest push-out bond strength to root dentin.

PR63

**Effect of Selenium on RANKL-Induced Osteoclastogenesis**

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Selenium is an essential trace element required to generate antioxidant selenoproteins in the body. In inflammation, it is thought that the supply of selenium needed to generate antioxidant selenoproteins may be attenuated, promoting, in turn, tissue damage caused by oxidative inflammation. It is also true that antioxidants can suppress RANKL-mediated osteoclastogenesis. The purpose of this study was to explore the effects of sodium selenite on the RANKL-mediated osteoclastogenesis without causing any cytotoxicity. It was found that sodium selenite markedly inhibited the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells in RAW 264.7 cells. Also, using pit hole formation assay showed that sodium selenite markedly suppress osteoclasts’ function. As a conclusion, selenium can suppress RANKL-mediated osteoclastogenesis and bone resorption activity mediated by mature osteoclasts. This study was supported by King Abdulaziz University – Deanship of Graduate Studies. Dr. Laila Bahammam was working at the Forsyth Institute but paid by the Saudi Cultural Mission and King Abdulaziz University.

PR64

**Evaluation of the Effect of Volume and Concentration of Lidocaine on Pulpal Anesthesia in Human Inferior Alveolar Nerve Block**

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Introduction: Profound pulp anesthesia is important for successful completion of dental procedures. The purpose of this study was to evaluate the success rates of dental pulp anesthesia with an increased volume and concentration of lidocaine in human inferior alveolar nerve block. Methods: Thirty adult volunteers participated in this double-blind study. Each subject was injected with one of three solutions at three appointments: (A) 1.8 ml of 2% lidocaine with 1:100000 Epinephrine, (B) 3.6 ml of 2% lidocaine with 1:100000 Epinephrine, and (C) 1.8 ml of 5% lidocaine with 1:100000 Epinephrine. Before and every five minutes up to a maximum of 60 minutes after injection, the response of the first molar, canine, lateral incisors and contralateral tooth was assessed using an electronic pulp tester. Successful pulp anesthesia was considered to have occurred with 80/80 reading with EPT. Data were analyzed by the chi-squared and Student’s t-tests. Results: There was a significant difference in anesthesia success when solution A was compared with solution B and C in the molar and canine (p<0.05). Anesthetic problems occurred in 23 to 40% of molars, 33 to 53% of canine and 43 to 56% of lateral incisors. Conclusion: Increasing the volume and concentration of lidocaine increases the success of anesthesia in molar and canine. There was a high incident of anesthetic problems in the inferior alveolar nerve block.
PR65

**CGRP Release From Human Tooth Slices: Focus on Individual Differences**

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Oral Health in America: Report of the General Surgeon (2000) lists oral-facial pain as a “chronic and disabling condition.” The most common source of oral-facial pain resulting from oral diseases is odontalgia, often caused by inflammation of the dental pulp. A better understanding of the neurobiology of the dental pulp is critical to developing novel methods to manage orofacial pain. Objectives: This study evaluated whether individual differences, both between and within teeth, affect stimulated neurosecretion from human dental pulp. Methods: Non-carious freshly extracted teeth were collected and sectioned longitudinally into 0.95 mm thick slices, exposing the dental pulp. The tooth slices from 35 patients were exposed to 60 µM capsaicin to stimulate the neurosecretion of CGRP (calcitonin gene-related peptide) from the pulp. CGRP release was normalized to the weight of the dental pulp. Patient factors analyzed: age, gender and anesthesia type (IV versus local only). Data was rank transformed and analyzed using a mixed-model ANOVA. Results: Seventy percent of the variability observed in CGRP release was attributable to consistent differences between individuals. There was no effect of IV sedation or gender on stimulated CGRP release. Subject age was associated with stimulated CGRP release (p=0.05) with a 1 mg/µg increase for every year beyond the mean age of 28 years. Conclusion: CGRP release from human dental pulp may provide a useful tool to assess pharmacological influences on human nociceptor activity. CGRP release is highly individual, and within-subject designs will be critical to the success of this translational model. This study was supported by the AAE Foundation and NIDCR 1K23DE019461.

PR66

**The SAPHO Syndrome: The Role of Endodontic Infections in Autoimmune Disease**

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The objective of this work is to describe a possible link between the autoimmune disease SAPHO syndrome and apical periodontitis. The SAPHO syndrome (acronym for Synovitis, Acne, Pustulosis, Hyperostosis and Osteitis) is a systemic disease that involves the bones, the joints and the skin. The aetiology is still uncertain and consequently there are still no univocal treatment protocols. We describe a case report of a 44-year-old woman suffering from SAPHO syndrome who presented a mandibular involvement with pain and bone alterations. We examine the possibility that this condition may have originated from a tooth subjected to different endodontic treatments and who presented an endodontic failure. This work presents a review of the literature on this systemic pathology and investigates the possible pathogenetic hypothesis of the syndrome and particularly the role of *Propionibacterium acnes* in this disease. We put in linkage of the endodontic pathology with mandibular and systemic manifestations of the syndrome analyzing the radiographic findings present in some studies. The result of our study is that there are some endodontic problems in people with SAPHO, especially in patients with the mandibular manifestations of the syndrome. We think that is possible that endodontic bacteria like *P. acnes* are involved in the aetiology of the systemic syndrome. It may be the trigger that causes the flare up of the manifestation in people with genetic predispositions.

PR67

**Influence of Cement Type and Relining Procedure on Push-Out Bond Strength of Fiber Posts to Root Dentin**

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Introduction: The aim of this study was to evaluate the influence of cement type and relining procedure on push-out bond strength of fiber posts after cyclic loading. Methods: Forty bovine incisor roots were divided into four groups: Group 1, fiber post (FP) luting with Rely X Unicem; Group 2, fiber post relined with resin composite (FPC) luting with Rely X Unicem; Group 5, FP luting with Rely X ARC; Group 4, FPC luting with Rely X ARC. Afterwards, half of the samples were exposed to 250,000 cycles in a controlled chewing simulator. With the other half of the samples in each group, the push-out test was performed 24 hours after FP luting (immediate groups). All roots were sectioned transversely, producing 1 mm thick slices, and the push-out test was performed. Statistical analysis was performed using analysis of variance and Tukey’s test for post-hoc comparisons (α=0.05). Results: FPC had higher bond strengths than FP (p<0.05). Rely X Unicem showed higher bond strength than Rely X ARC (p<0.05). Cyclic loading did not significantly affect the bond strength value (p>0.05). Conclusion: The relining procedure and the cement type are important factors for the bond strength of fiber posts to root dentin. This study was supported by the University of Passo Fundo and the State University of Campinas.

PR68

**A Comparison of Liners and Adhesive Systems in Preventing Coronal Dye Penetration in Root-Filled Teeth Subjected to Functional Forces**

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Introduction: This study was undertaken to compare coronal dye penetration of two liners and two adhesive systems in root-filled teeth whilst simultaneously subjected to functional loading. Experimental design: Seventy-six premolars with two canals were used. Root canal treatment was completed in 74 teeth. Two teeth were left intact as negative controls. Experimental teeth were divided into four groups of 18 teeth: Vitrebond™ and self-etched (Optibond FL™), Vitrebond™ and total-etched (Optibond XLR™), Ionomit™ and self-etched (Optibond FL™), Ionomit™ and total-etched (Optibond XLR™). Two teeth were left open as positive controls. Experimental teeth were filled with composite resin (Premise™). All samples were submersed in India ink and simultaneously subjected to simulated chewing for the equivalent of three months intra-oral function. Maximum dye penetration was assessed and related to the full length of the teeth. Results: No dye penetration occurred in the negative controls and positive controls had dye penetration throughout their full length. One-way ANOVA showed no statistically significant difference between the four experimental groups (p=0.051). Neither Vitrebond™ nor Ionomit™ showed significant differences in preventing dye penetration (t-test, p=0.663) but the total-etched system had significantly less dye penetration than the self-etched system (t-test, p=0.007). Conclusions: There was no significant difference between Vitrebond™ and Ionomit™ in preventing dye penetration, but the total-etched system was significantly better at preventing dye penetration than the self-etched system in root-filled teeth.
Intentional replantation is the last resort for tooth conservation when the conventional surgical access is not obtained. Many clinicians, however, feel reluctant to perform intentional replantation because of the possible complications including root resorption and ankylosis. The aims of this study were to evaluate the treatment outcome of intentional replantation and to investigate the prognostic factors contributing to the failure of the intentional replantation. The clinical database was searched for the patients with a history of intentional replantation between March 2000 and December 2010. The teeth with preoperative periodontal involvement or root defects were excluded. A total of 215 cases were included in this study. Survival analysis was performed using Kaplan-Meier method and the Cox proportional hazard regression model. Cumulative complication-free survival rates after intentional replantation were 94% for one year, 92% for two years, 91% for three years and 86% for four years or more. The Kaplan-Meier survival curves and log-rank tests revealed that the cases using MTA showed significantly lower survival rate compared to cases using IRM or SuperEBA. External root resorption was significantly more in maxilla than in mandible, and cases with no preoperative periapical lesion or extraoral time longer than 15 minutes had significantly higher ankylosis rates than the others. A Cox regression model showed that periapical lesion and extraoral time were the factors affecting the occurrence of ankylosis. The intentional replantation is a promising procedure provided the correct indications are applied. Using faster-setting retro-filling materials and reducing extraoral time may lead to better result.

**The Effects of Gutta-Percha Solvent Application and Different Removal Irrigation Times on Microtensile Bond Strength to Root Canal Dentin**

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The gutta-percha solvent application is usually a part of the retreatment procedure. Post and core cementation is important for long-term success in root canal treatment. The aim of this investigation was to evaluate the effects of the solvent (chloroform) application and ethyl alcohol irrigation times for removing residual chloroform on microtensile bond strength to root canal dentin. Forty extracted human single-rooted teeth were instrumented with a #60 file and randomly divided into four experimental groups of 10 each. Each group was treated by following procedure. Group 1: Root canals were irrigated with 99% ethyl alcohol for 60 seconds (negative control). Group 2: Roots canals were treated with chloroform for 60 seconds only (positive control). Group 3, 4: All of them were treated with chloroform for 60 seconds and irrigated with 99% ethyl alcohol for 30 seconds and 120 seconds respectively to remove residual solvent. All root canals were obturated using Luxacore Smartmix Dual. After 24 hours of storage in saline, serial 1 mm thick cross sections were cut and trimmed. Microtensile bond strength to root canal dentin were measured using universal testing machine. The results indicated that the treated roots with ethyl alcohol for 120 seconds had significantly higher than the treated roots with ethyl alcohol for 30 seconds. Gutta-percha solvents have an adverse effect on bond strength of adhesive cements to root canal dentin and sufficient removal irrigation of solvent is necessary to strong bonding.
PR73

**A Retrospective Clinical Evaluation of the Incidence of Root Canal Instrument Fractures in an Undergraduate Dental School: An EndoMuc Database Study**

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Objective: The aim of the study was to investigate the incidence of instrument fractures in the Undergraduate Dental School at the Ludwig Maximilians University of Munich (Germany) between 2009 and 2013. Methodology: This study is a retrospective analysis on the incidence of root canal instrument separation during five years of undergraduate clinical training in endodontics with the MTwo™ system in single use (one set of files for one case) and SS Handfiles for canal patency preparation. One thousand seven hundred ninety (790) endodontically treated teeth (37% molars, 34% premolars and 29% incisors and canines) were investigated. The patients’ ages ranged from 14 to 95 years (median age: 47). Instrument separation was defined as clinical and/or radiographic signs of instrument separation. Results: On 1,790 cases the incidence of SS hand instrument fracture was 0.50% and the incidence on Niti rotary instrument fracture was 1.23%. The file with the highest fracture rate was the MTwo™ 15/05 with 14 instruments out of 30. The location of the fragment was found to be in in 80% of the cases in the apical third. The highest percentage of instrument fracture occurred in mandibular molars (53%) and maxillary molars (25%). The likelihood of instrument fracture in molars was three times higher than in premolars (odds ratio: 3.04). Conclusion: Undergraduate students with the MTwo™ file system in a single use protocol are able to achieve low incidence of instrument separation.

PR74

**Micro-Computed Tomography Evaluation of the Preparation of Long-Oval Root Canals Comparing the Reciproc® and BioRace” Systems**

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Root canals that have a long-oval cross section hinder the action of rotating instruments, which cannot adapt to all extensions of the root canal walls. Newly developed single-file systems claim to prepare any type of root canal with only one instrument. The purpose of the present study was to compare the preparation of long-oval root canals with a single reciprocating system and a multiple-file rotary system using micro-computed tomography. Distal canals of 30 mandibular molars were selected and assigned into two groups (n=15): Reciproc® 40 (VDW, Munich, Germany) and BioRace™ System (FKG Dentaire, La Chaux-de-Fonds, Switzerland). Teeth were scanned before and after preparation of the root canals by a SkyScan 1172 micro-computed tomography scanner at 11 µm resolution. Morphometric variations were measured by volume increases and by the remaining untreated canal surface area in the entire canal and each third. Data was compared using the Mann-Whitney test. The Reciproc® system was found to have the highest increase in volume when analyzing both the entire canal and the apical third (p<0.5). In spite of that, it left more untouched areas (p<0.001) in the cervical and middle thirds (18.14% and 21.82%) as compared to BioRace™ (8.14% and 11.35%). In conclusion, neither technique was able to completely prepare the long-oval outline of root canals. The Reciproc® system removed more tooth structure while BioRace™ left fewer untouched dentin walls in the more oval thirds of the canal. This study was supported by the São Paulo Research Foundation (Process 11/50996-0).

PR75

**Influence of Chlorhexidine Application Time on Fiber-Root Dentin Bond Strength Durability**

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Introduction: The aim of this study was to investigate the effects of chlorhexidine (CHX) application time on the bond strength and durability of the adhesion of the fiber post relined with resin composite to the root dentin. Methods: Eighty bovine incisor roots were divided into four groups after root preparation: Group 1 (control), irrigation with physiological solution; Group 2, CHX for 30 seconds; Group 3, CHX for 60 seconds; and Group 4, CHX for 120 seconds. Fiber posts relined with resin composite were cemented with RelyX ARC. In half of the samples of each group the total-etch adhesive system Scotchbond Multi-Purpose was used, and in the other half the self-etch adhesive system Clearfil SE Bond was used. The samples were randomly divided into two subgroups: 24 hours of storage and 12 months of storage. All roots were sectioned transversely and push-out tests were performed. Bond strength means were analyzed by ANOVA/Tukey’s (a=0.05). Results: CHX pretreatment resulted in homogeneous bond strength values at 24 hours and 12 months of storage (p<0.5) irrespective of the CHX application time and adhesive system. A significant bond strength decrease was noticed after 12 months of storage when irrigation was performed with physiologic solution in the control groups (p<0.05). Conclusion: The use of CHX pretreatment could preserve the bond strength of the fiber post relined with resin composite to root dentin for 12 months irrespective of the CHX application time (30, 60 or 120 seconds) and adhesive system used. This study was supported by the University of Passo Fundo and the State University of Campinas.

PR76

**Inflammatory Potential of Root Canals Content Against Macrophages by the IL-1β and TNF-α Production in Primary Endodontic Disease**

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This study investigated the endotoxin levels in root canals with primary endodontic infection and apical periodontitis, determined their antigenicity against macrophages through the levels of IL-1β and TNF-α, before and after chemomechanical preparation (CMP) with rotary NiTi system, and after a final rinse with 17% EDTA. Samples were taken from 30 root canals: S1- before CMP; S2- after CMP; and S3- after EDTA. Two-and-a-half percent sodium hypochlorite (NaOCl) was used as irrigant, LAL assay was used for endotoxin measurement. Cytokine levels were measured by ELISA. Friedman’s test compared the endotoxin and cytokine levels at each clinical time; Mann-Whitney (p<.05) analyzed the correlation between the median levels. LAL assay was able to detect endotoxin in 100% of the samples. Endotoxin content was significantly reduced at different samples (S1>S2>S3) (p<0.05). CMP was effective in reducing endotoxin levels in 99.46% (p<0.05). The final rinse with EDTA slightly improved the endotoxin reduction (99.56%), without statistical difference. Higher levels of IL-1β and TNF-α levels were produced at the initial samples when compared to that found after EDTA. A positive correlation was found between endotoxin levels and the expression of target cytokines. It was concluded that 2.5% NaOCl + 17% EDTA was effective in reducing endotoxin load in the root canal infection from primarily infected teeth with apical periodontitis. The inflammatory potential of endodontic content decreased after root canal treatment, as shown by the levels of IL-1β and TNF-α after root canal treatment. This study was supported by FAPESP 2010/19136-1, 2013/15734-3, 2011/-49047-4; CAPES & CNPq 302575/2009-0.
A Proposal for a New Pilot Program in the Honors in Endodontics Program
Curriculum of the Fourth Year D.D.S. Program at New York University
College of Dentistry 2013-2014
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Objectives: This course is structured to obtain an outcome by defining the main objective which is to assist Honors in Endodontics students in not only formulating a diagnosis and treatment plans for specific clinical cases provided to them in the course, but also enabling the students to demonstrate their level of theoretical knowledge. Another objective is to assess how they analyze and integrate history-taking skills in combination with the patient’s concerns in formulating a diagnosis and a viable differential diagnosis. Methods: A clinical case is presented to the Learners (student dentists) involving a patient (standardized patient) with odontogenic toothache due to a variety of causes. The student dentist must be able to gather information through questioning of the patient’s chief complaint, gather further data through intra-oral examination tests to establish the source of the pain, and be able to reproduce the quality and duration of the pain. Discussion: Presently, this is a pilot program; however, I hope to broaden this project by including real patients in a clinical setting and then assessing their motor skills to evaluate each step in root canal treatment and also to evaluate how they manage the patient after the procedure, especially if the patient develops a flare-up (post-operative pain and/or swelling). The major goal of this pilot program is to gauge the effectiveness of this program through feedback commentary and through determining if the learning objectives were met.

Treatment of Traumatic Injured Teeth Using Resin Modified Calcium Silicates as a Direct Pulp Capping Material
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Resin modified calcium silicates (RMCS), a light-cured bioactive material was introduced to seal and protect the dentin-pulp complex. According to the literatures, RMCS is a calcium-releasing material that is able to form the formation of apatite. RMCS can be tried as a barrier protecting the pulp while inducing the formation of a new dentine bridge between the pulp and restorative material. The purpose of this study was to report on the use of RMCS in direct pulp capping of traumatic injured teeth. Methods: Clinical and radiographic examinations were performed preoperatively and post-operatively in patients with traumatic injuries to the injured teeth. Methods: Clinical and radiographic examinations were performed preoperatively and post-operatively in patients with traumatic injuries to the injured teeth. Results: In all cases, there were no symptoms and the number of cycles to failure (NCF) was evaluated in a simulated canal. Data were analyzed using two-way ANOVA and Duncan post-hoc, and a multiple regression analysis. The fractured fragment surfaces were examined under the SEM. Results: There was a significant effect from alloy type and the extent of torsional preloads on cyclic fatigue resistances. The 50% and 75% torsionally preloaded K3XF and ProTaper groups had higher NCFs than the other group(s). However, the only 50% torsionally preloaded WaveOne group had higher NCFs than other group(s). Most WaveOne and some K3XF after 75% preloading showed the longitudinal cracks parallel to the long axis of the file, while the 75% preloaded ProTaper did not. Distinctively, some 75% preloaded K3XF showed the longitudinal cracks were away from the fracture area. The microcracks did not seem to follow the machining grooves on the instrument surface but run irregularly. Conclusion: The alloy type of NiTi instruments made different effect on the fatigue resistance from torsional preloading. Proper degree of torsional preloads may improve the cyclic fatigue resistance of NiTi rotary instruments.

Evaluation of Sodium Fluoride as a Final Irrigant on the Apical Microleakage of Canals Filled With MTA Fillapex™ Sealer by Fluid Filtration Technique
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Objective: Purpose of this study was to analyze apical microleakage of root canals filled with gutta-percha and MTA Fillapex™ when sodium fluoride used as final irrigant. Methods: Seventy-two anterior maxillary teeth were prepared as final irrigant. Materials: Seventy-two anterior maxillary teeth were prepared as final irrigant. Results: Ten-day mean apical microleakage was as follows: group 4 (NaF, moist; 0.0344), group 2 (saline, moist; 0.0538), group 5 (NaF, dried canals; 0.0931), group 1 (saline, dried canals; 0.1031). The moist groups showed significantly less microleakage when compared with the dried groups (p<0.01). Conclusion: This study supports the clinical use of sodium fluoride as final irrigant and leaving the canals moist before obturating with MTA Fillapex™.
**Irritant Activation Protocols on Smear Layer Removal in Single Root Canals**

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This study aimed to evaluate the efficiency of two irrigant activation systems. Sixty single-rooted human teeth were prepared mechanically and randomly divided into three groups: group 1 (control) - manual needle irrigation; group 2 (EndoActivator; DENTSPLY, Tulsa, OK) - activating irrigation with sonic device; group 3 (CK file; B&L Biotech, Korea) - activating irrigation with ultrasonic device. After irrigation, longitudinal grooves were made with diamond disk on buccal and lingual surfaces of all teeth, and then horizontal grooves were made on 3 mm from the apex of all roots. Then, all teeth were split by chisel to get 5 mm sized cross-sectioned samples of apex area. Each sample was dehydrated and viewed with a SEM. The results showed that the groups treated with EndoActivator (group 2) and CK file (group 3) were significantly more effective in removing smear layer than the group treated with manual needle irrigation (group 1) (27.2; 24.1; 40.3) (Kruskal-Wallis test, p<0.05). However, there was no significant difference between group 2 and group 3 (21.3; 19.7) (Mann-Whitney U test, p>0.05). Both activating devices, EndoActivator and CK file, were more effective in removing smear layer from the apical 3 mm of the root canal than manual irrigation method.

**Effect of Extra-Oral Time on Periodontal Healing After Autotransplantation of Permanent Teeth**

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Objective: Prolonged extra-oral time after extraction of the donor tooth can damage periodontal ligament cells and lead to complications such as ankylosis of the tooth. The aim of this study was to determine the effect of the extra-oral time on the development of ankylosis in the autotransplanted teeth. Materials and Methods: The subjects consisted of 88 patients (45 women and 33 men; aged 11-63 years, the average age 36 years) who had undergone transplantation in the department of Conservative Dentistry at the Yonsei University Dental Hospital from March 2004 to March 2009. These cases were followed for six to 54 months after the surgery. We calculated an average extra-oral time of the donor tooth elapsed during the surgical procedure and divided the autotransplanted teeth into two groups on the basis of the average extra-oral time (above or below the average extra-oral time). The correlation between the extra-oral time and the development of ankylosis was analyzed with a chi-squared test. Results: The overall extra-oral time of 88 transplanted teeth ranged 3.2 to 38.2 minutes, and the mean time was 11.2 ± 4.04 minutes. Ankylosis was observed in 28 transplanted teeth. The association between ankylosis and extra-oral time considered to be statistically significant (p=0.0093). Conclusion: This study showed that the extra-oral time less than 11.2 minutes may prevent dentoalveolar ankylosis.

**Periapical Status Related to the Quality of Coronal Restorations and Root Fillings in a Korean Population**

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Introduction: The purpose of this study was to evaluate the relationship between the quality of root canal fillings and coronal restorations and the periapical status of these teeth. Methods: Full-mouth periapical radiographs at the Dental Hospital of Yonsei University were examined. A total of 1,030 endodontically treated teeth restored with full veneer crown type restorations were evaluated by two independent examiners. Teeth were classified as healthy or diseased according to the periapical status. The quality of endodontic treatment and coronal restorations were also classified via radiographic and clinical evaluation. The data were analyzed using the chi-squared test and logistic regression. Results: Forty-one percent of all endodontically treated teeth were classified as diseased. Approximately 35.6% of the teeth had endodontic treatments that were rated as adequate. The diseased teeth rate for cases with adequate endodontic treatment was 24.5%, which was significantly lower when compared with teeth with inadequate endodontic treatment (49.9%). The number of teeth with adequate coronal restorations was 706 (68.5%). Teeth with adequate coronal restorations had a significantly decreased prevalence of diseased teeth (34.7%) compared to teeth with inadequate coronal restorations (54.3%). Teeth with both adequate root fillings and restorations showed a significantly better outcome (82.3%) than the others, and teeth with both inadequate root fillings and restorations showed a significantly worse outcome (41.2%) than the others. Conclusion: The quality of endodontic treatment and coronal restorations were of equal importance and were strong independent predictors of the periapical status. This study was supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2010 – 0021281).

**Concurrent Measures of Radicular Dentin Thickness and Three-Dimensional Curvature in Mandibular First Molar Mesial Roots with Micro-Computed Tomography**

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Objectives: Radicular dentin thickness and root canal curvature can strongly affect canal instrumentation. The purposes of this study were to measure i) root dentin thickness, ii) direction of thinnest dentin thickness and iii) 3-D curvature of mesiobuccal (MB) and mesiolingual (ML) canal of mandibular first molar mesial roots using micro-computed tomography (MCT) and custom-developed software. Methods: Thirty-seven extracted mandibular first molar mesial roots were scanned by MCT (voxel size of 31.8 μm³) and surface model of roots and root canals were reconstructed. Using custom-developed software, the imaginary central axis was created in each canal by connecting the points of intersection of the major and minor axes in each MCT image slice. Then 3-D surface models of root and root canal were sectioned in 0.1 mm intervals along the central axis. In each section, dentin thickness and 3-D canal curvature were automatically measured. Results: Average dentin thickness of thinnest, mesial, distal and lateral direction were 0.89 ± 0.26, 1.15 ± 0.37, 1.03 ± 0.29 and 1.84 ± 0.57 mm, respectively. In the coronal portion, the dentin thicknesses for both canals were thicker in mesial side than those of distal side, while it was vice versa in the apex. The direction of thinnest dentin thickness were mostly disto-inside of root. The degree of canal curvatures was highest in apical region and decreased in the order of furcal and middle area. Conclusions: This anatomical information in mandibular first molar mesial root would be useful during nonsurgical endodontic therapy. This study was supported by the Seoul National University Research Fund (01-2013-0002).
Effect of Novel Light-Curable Material on Differentiation of Human Dental Pulp Cells
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The aim of this study was to evaluate and compare the effect of mineral trioxide aggregate (MTA) and novel light-curable material (TheraCal LC, Bisco Inc., Schaumburg, IL) on differentiation of human dental pulp cells. The expressions of dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1) at gene level were detected by reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time PCR. The mineralization effect was evaluated by ALP staining and Alizarin red S (AR-S) staining. The expression of DSPP and DMP-1 genes increased in the MTA group and TheraCal group compared to control group. The results of ALP staining and AR-S staining showed that MTA and TheraCal induced odontoblastic differentiation and mineralization. TheraCal appears to be able to induce odontoblastic and mineralization-associated gene expression in human dental pulp cells.

Size Change and Hardness of New Root Canal Sealer Containing Oleic Acid, Eugenol and Zinc Oxide
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The purpose of this study was to evaluate size-changing and hardening rates of a new root canal sealer (new sealer) using 15% eugenol in oleic acid. The ratio (P/L) of powder to liquid of the new sealer was 2.0. A zinc oxide eugenol sealer (CANALS™, Showa Yakuhin Kako, Japan) with 80% eugenol on the market was used as a control sealer (P/L=3.3). The size change of the new sealer was determined in accordance with the method described by the International Organization for Standardization (ISO 6876), section 7.6. For the size change experiment, the cylindrical molds (diameter of 6 mm and height of 12 mm) were filled with these sealers, covered by glass plates and stored at 100% relative humidity and 37°C. After 24 hours, the samples were stored in distilled water for one, three, six, seven, 10, 14, 21, 25 and 30 days. After the various periods, samples volumes were measured. The volume of the new sealer immersed in distilled water for 30 days, was increased by 0.15%. On the other hand, the control sealer volume was decreased by 0.34%. A statistically significant difference between the new sealer and the control sealer was p<0.05. Hardening rates of the new sealers were measured with a texture analyzer (EZ Test, Shimazu Co. Ltd., Kyoto, Japan). Hardening rate of the new sealer did not change from three to 30 days. These results show that the new sealer has good sealing ability in the apical area.

Evaluating the Effects of Different Dental Devices on Implanted Cardioverter-Defibrillator
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The Implanted-Cardioverter-Defibrillator (ICD) is an electronic device that emits electrical signals to the heart via lead wires and electrodes. It is used for cardiac rhythm monitoring and treatment. We hypothesized that dental devices may interfere with the ICD’s function. Nine devices were tested (three gutta-percha guns, heat carrier, apex locator, electric pulp tester, unipolar electrosurgery unit, electric motor and curing light) for their ability to interfere with the function of four types of ICDs (two single-chambered and two dual-chambered). For 30 seconds the ICD’s activity was monitored using the ICD programmer (an instrument used to monitor electrical signals) and EGM test strip print out. Each test was repeated three times. All ICDs captured some electromagnetic interference (EMI) with the electric motor, curing light, electric pulp tester and electrosurgery unit. No electromagnetic disturbances (EMD) impacted the clinical program function of the ICDs; however, a dual-chambered ICD (Consulta CRT-D) was affected by the electrosurgery unit and delivering therapies for fibrillation when no ventricular fibrillation was present. The devices with no electromagnetic interferences are: the three gutta-percha guns, heat carrier and apex locator. Our results suggest that the unipolar electrosurgery unit produces electromagnetic disturbances with clinically significant outcomes (unwanted therapy delivery-shock). Whereas the use of electric apex locator, electric pulp tester, electric motor, curing light, gutta-percha guns and gutta-percha heat carriers did not provide any evidence of electromagnetic interference or disturbances of the ICD function, and are thus considered safe devices for clinical use on patients, with implanted ICDs. This study was supported by Medtronic, Inc. and the University of Texas Health Science Center at Houston. Girard Guillaume is an employee of Medtronic, Inc.

The Antimicrobial Activity of Electrochemical Irrigation Against Enterococcus faecalis Biofilm
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A major goal of endodontic treatment is to disinfect the root canal system. Current methods often fail to completely remove bacteria from the canal system. To improve the efficacy of disinfection, techniques and irrigants have been developed to supplement the conventional irrigation protocol. The purpose of this study was to compare electrochemical activation (ECA) using Sterilox and QMix® on its ability to remove biofilm from the isthmus and mesial canals of lower molars. Twenty-six mandibular molars were instrumented with ProTaper rotary files. A standardized amount of Enterococcus faecalis was inoculated at the isthmus of the mesiobuccal and mesiolingual canals. Samples were incubated for 10 days to allow biofilm formation. Three teeth were used as negative control (without bacteria inoculation) and three teeth were used as positive control (biofilm formation without irrigation). Twenty teeth were divided in two groups following instrumentation and 5 ml irrigation with conventional 6.15% NaOCl: 1) ECA and 2) QMix®. Both experimental groups were irrigated following manufacturer specifications. Samples were obtained pre- and post-irrigation with paper points, total DNA was extracted, and quantitative real-time PCR was used to analyze presence of bacteria and effectiveness of irrigation protocol. Data were analyzed using one-way ANOVA with Bonferroni’s correction for multiple comparisons (p<0.05). The results showed no statistical difference on the reduction of biofilm when both irrigation protocols were compared. ECA can be an alternative for the irrigation of root canal systems.
**PR89**

**Antigenic Activity of P. gingivalis Lipopolysaccharides Clinically Isolated From Root Canal Infection Against Macrophages by Levels of IL-1β and TNF-α**

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Introduction: The aim of this study was to investigate the antigenic activity of lipopolysaccharide (LPS) purified from P. gingivalis from primary endodontic infection (PEI) against macrophages cells by levels of IL-1β and TNF-α. Methods: P. gingivalis strain was clinically isolated from PEI by using anaerobic technique. Identification of the isolates was primarily performed by using biochemical methods and confirmed by sequencing the 16S rRNA gene. Bacterial lipopolysaccharides (LPSs) from P. gingivalis and its ATCC strain were extracted by using the TRI Reagent method. Escherichia coli LPS was used as control. Macrophages (Raw 264.7) were stimulated with LPS at 100 ng/mL for four, eight and 12 hours. IL-1β/TNF-α mRNA expression and secretion were determined by RT-PCR and ELISA-assay, respectively. Results: LPS from E. coli and P. gingivalis induced significant production of IL-1β and TNF-α secretion of IL-1β occurred at higher levels than TNF-α for all incubation times (four, eight and 12 hours). LPS from E. coli exhibited the highest production of IL-1β and TNF-α (p<0.05). Higher levels of IL-1β and TNF-α production were found in macrophage supernatants stimulated with clinically isolated LPSs compared to their respective ATCC strains. Conclusion: LPSs from P. gingivalis demonstrated a potent antigenic activity against macrophages, which seems to be a susceptibility factor for initiation of the up-regulation of periapical tissue breakdown in apical periodontitis. This study was supported by CAPES, FAPESP 2013/17928-6; 2010/19136-1; 2010/13734-3; 2011/-09047-4 and CNPq 302575/2009-0.

**PR90**

**Micro-Computed Tomography Analysis of the Effect of Different Irrigation Solutions on Dentin Quality**

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Objective: To assess the effects of NaOCl, EDTA, chlorhexidine (CHX), as irrigation solutions, on the demineralization/remineralization of dentine using micro-computed tomography (micro-CT). Materials and Method: Thirty-five single-rooted teeth were collected and randomly allocated into seven groups, each group was treated with different irrigation solution. Teeth were scanned with a micro-CT scanner, before and after treatment. Volume measurements to assess the demineralization effect were carried out with micro-CT software. Results: The most pronounced effect was observed at the inner ROI of the coronal part of the root, while the least effect was observed in the outer ROI of the apical part. NaOCl (5.5%), EDTA and their combination showed a high demineralization effect in dentin, while CHX showed no demineralization effect. Conclusion: NaOCl and EDTA irrigation solutions have changed the quality of dentin, in a way that might alter carries susceptibility of endodontically treated teeth. Remineralizing products, such as fluoride, are highly recommended. This study was supported by King Abdulaziz University, Faculty of Dentistry Gothenburg University.

**PR91**

**Using the BigMouth Dental Data Repository as an Educational Resource in Endodontics**

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The purpose of this study was to use the BigMouth Dental Data Repository as an educational resource to evaluate the quality of care at a university setting. Data mining was performed to determine the types and numbers of endodontic procedures performed by dental students (DS) and residents (R) at the UT Health School of Dentistry between the years of 1996 and 2013. BigMouth contains data extracted from dental school electronic health records. For this research, only data from UT Health School of Dentistry was explored. A total of 52,365 endodontic procedures were identified for: pulp cap, pulpotomy, nonsurgical root canal treatment (NSRCT), nonsurgical retreatment, apicectomy and apicoectomy. NSRCT was the most frequent procedure [35,035 total, 11,974 by R, 23,061 by DS]. Pulpotomy was the second most frequent procedure [7,615 total, 1,301 by R, 6,314 by DS]. A total of 3,615 endodontic retreatments were identified [3,449 by R, 196 by DS], and 606 pulp caps [295 by R, 311 by DS]. Apicectomy and apicoectomies were performed by residents only [184 and 918, respectively]. Of note, only 3,868 recalls were identified [1,093 by R, 2,775 by DS]. The number/distribution of endodontic procedures among treatment providers appears to correlate with expected procedures across universities. Recall rates were much lower than expected, and prompted a re-evaluation of the methods currently in use. Additionally, these results highlight the need for entering diagnostic codes to determine if appropriate treatments were given. Finally, the repository can be used to potentially compare the characteristics of treatments across different institutions.

**PR92**

**Analysis of Apical Root Canal Morphology in Mandibular Incisor by Micro-focus X-ray CT**

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Mandibular incisor was a single root. However, it had a flattened root and also had root canal ramifications and apical delta. Therefore, it has been difficult to treat. The purpose of the present study was to scan by using a micro-focus X-ray CT and to investigate the apical root canal morphology of mandibular incisor. In this study, 50 permanent mandibular incisors extracted from Japanese subjects were used. Each specimen was scanned by using a micro-focus X-ray CT (ELE-SCAN, NS-ELEX, Japan) at 21.1 μm resolution with 80 kV and 70 μA, and was reconstructed three-dimensionally using image processing software (TRI/3D-BON, RATOC System Engineering, Japan). Apical root canal ramifications were evaluated on 3-D images. Then, directions of apical foramen were categorized into central, labial, lingual or mesiodistal on cross-sectional images. Apical root canal ramifications were presented in 22% of teeth: two ramifications were found in 14%, three ramifications in 6% and five ramifications in 2%. On labiolingual images, apical foramina were opened at apex center (30%), labial (58%) and lingual (12%). On mesiodistal images, they were opened at apex center (60%) and mesiodistal (40%). Further, 16% of apical foramina were opened at apex center on mesiodistal and labiolingual images. These results suggest that apical root canal morphology in mandibular incisors is rather complicated. To attain more comprehensive understanding on this problem, further investigations would be necessary in future studies. This study was supported by the Nippon Dental University Research Fund.
Cyclic Fatigue Resistance of Two Different Nickel-Titanium Rotary Instruments in Interrupted Rotation

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Objective: To investigate the influence of interrupted rotation on cyclic fatigue of two different nickel-titanium rotary instruments. Methods: Three hundred new ProTaper Next size X1; X2 and Mtwo size #10/0.04; #15/0.05; #20/0.06 and #25/0.06 were tested for cyclic fatigue resistance using rotation continuous or stopped. Fifty files of the same brand and size were randomly assigned to five different groups (n=10). Files of group 1 were tested in continuous rotation; group 2 and 3 in interrupted rotation for one second every 10 or 20 seconds, respectively; group 4 and group 5 interrupted rotation for five seconds every 10 or 20 seconds, respectively. Cyclic fatigue was determined by counting the numbers of cycles to failure (NCF) obtained from the real seconds of continuous rotation in an artificial canal with 60° angle and a 5 mm radius of curve. A two-way analysis of variance were used to evaluate the data. Results: Cyclic fatigue resistance of groups 2 and 4 of ProTaper Next X2 and Mtwo #25/0.06 was lower than group 1 of the same instruments (p<0.01). ProTaper Next X2 showed a reduced cyclic fatigue also in groups 3 and 5 (p<0.05). No differences were found by interrupting the rotation for one or five seconds in all instruments (p>0.05). Fatigue of all other instruments were not affected by interrupted rotation (p>0.05). Conclusions: The interrupted rotation reduced cyclic fatigue resistance of instruments with a greater size especially when a major number of interruptions were performed.

Smear Layer Removal With Different Intracanal Irrigation Regimes: An In Vivo Study Analyzed With Scanning Electron Microscopy

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To evaluate the smear layer removal capacity of the QMix™ 2-in-1 compared to the conventional irrigating solutions. This study was approved by the Institutional Review Board and written informed consents were signed by the patients. Sixty human root canals with curvatures were used in this study. Cleaning and shaping of the canals was performed using PathFiles and WaveOne Systems with 1 ml of 6% Sodium Hypochlorite solution. The specimens were randomly assigned to the following different irrigation solutions: QMix™ 2-in-1 with or without activation, EDTA with activation. Final irrigation with 1 ml of 6% Sodium Hypochlorite was applied. Instrumentation was performed with no final irrigation in the negative control group and no cleaning and shaping was done in the positive control group. All teeth were extracted after the treatment due to the orthodontic reasons. The roots were bisected longitudinally and the root halves were observed under a low vacuum condition with the SEM. The amount of smear layer present on the root canal surfaces at the coronal, middle and apical portion was scored according to Nova Grid System. Statistical analyses were made with multinomial logistic regression with robust standard errors. There was a significant difference between QMix™ 2-in-1 and the other groups on the evaluation of smear layer, debris removal and tubule visibility (p<0.05). QMix™ 2-in-1 with activation was shown to be effective in the apical thirds of the curved root canals.

Prevalence of Root Canal Treatment and Periapical Radiolucency in Elders: A Systematic Review

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Objective: As we age, disease and treatment may accumulate, but teeth may also be extracted, and access to care may be problematic. For elders, the prevalences of root canal treatment (RCT) and periapical radiolucency (PARL), a surrogate for disease, have not been subjected to systematic review, the highest level of clinical evidence. The purpose was to conduct a systematic review and meta-analysis of the prevalences of RCT and PARL in patients aged 60+ years. Methods: Inclusion/exclusion criteria were used for defined searches in MEDLINE and Cochrane CENTRAL. Titles were scanned and abstracts read to determine utility. Articles meeting the criteria were analyzed. Mean percentages and standard deviations were calculated for the included teeth. Results: Defined searching produced 3,576 titles; 24 articles were included. The prevalences and (standard deviations) of RCT in age groups 60-69, 70-79 and 80+ were: 19 (5%), 22 (7)% and 25 (9)%, respectively. For all 60+ patients the prevalences of all RCTs, RCTs with PARLs, PARLs without RCTs, and all PARLs were: 18 (15%), 30 (15%), 4 (2)% and 6 (3)%, respectively. Incidence was rarely reported; it varied from 1% to 2% of teeth in elders per year. The technical quality of RCT was infrequently reported, but described as poor in 69 (7)% of teeth. Conclusions: The prevalences of RCT and PARL were high in elders; however, cross-sectional studies cannot distinguish between healing and worsening PARLs. The prevalence of RCT rose with age, despite increasing tooth loss, indicating that RCT was related to tooth survival.
Physico-Chemical Properties and Cytotoxicity of RetroMTA®

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RetroMTA® (BioMTA, Seoul, Korea) is a new hydraulic bioceramic indicated for pulp capping, perforations or root resorption repair, apexification and apical surgery. The aim of this study was to compare the radiopacity, pH variation and cytotoxicity of this material to ProRoot MTA®. Cements were mixed according to the manufacturers’ recommendation. For radiopacity evaluation, cements were placed into stainless steel rings and exposed to a digital X-ray along with an aluminium stepwedge. The grey pixel values were measured and converted into millimeters of Al. For pH evaluation, plastic tubes were filled with the cements, immersed in distilled water and incubated for three, 24, 48, 72 and 168 hours. pH values were verified after each time period. For cytotoxicity evaluation, human periodontal ligament fibroblasts were exposed to 24- and 48-hour extract media of each material. Culture medium was used as negative control and SDS as positive control. The cytotoxicity was evaluated with a multiparametric assay (XTT-NR-CVDE). Data were analyzed with ANOVA and Tukey’s multiple comparison test. Results showed higher radiopacity for ProRoot MTA® when compared to RetroMTA® (p<0.001). There were no significant differences for pH levels of the tested materials in all time periods (p>0.05). Both ProRoot MTA® and RetroMTA® allowed for significantly higher cell viability when compared with positive control (p<0.001). No significant differences were found between ProRoot MTA® and RetroMTA®, except for the 48-hour extract media in the neutral red (NR) assay (p<0.05). Overall, RetroMTA® had similar results to ProRoot MTA®. This study was supported by Scholarship CAPES (Brazil) BEX 1099/12-4.

Revascularization Using Dental Epithelial-Mesenchymal Reciprocal Interaction


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Introduction: This study evaluated the in vivo response of the immature permanent teeth with apical periodontitis after revascularization treatment with different tissue engineering therapies. Methods: Premolars of canine model dogs aged approximately five to six months were used for this study, and randomly assigned to treatment groups as follows: group 1: intracanal bleeding, group 2: intracanal bleeding + scaffold + experimental signaling molecule A, group 3: intracanal bleeding + scaffold + experimental signaling molecule B, positive control: left infected, negative control: untreated. Teeth were periodically monitored with radiograph until 12 weeks after treatment. The animals were sacrificed, and sections from the teeth were histologically evaluated. Results: The positive control teeth (infected and left with no treatments) showed no signs of newly formed tissues. All of the teeth in negative controls showed normal development and maturation of roots. The teeth of the experimental treatment groups had significantly different treatment outcomes. There were different types of newly formed mineralized tissues observed in the canal space. The dentin-like mineralized tissue formation along the inner wall of root canal with dentinal tubules was occasionally found from the teeth in group 2. Conclusion: The findings in this study marked the possible change in treatment strategy for immature permanent teeth with pulp necrosis and apical periodontitis.