Title: An *in vitro* comparison of apically extruded debris and instrumentation times with ProTaper Universal, Protaper Next, Twisted File Adaptive, and HyFlex instruments

Author: Capar, I.D. et al

Journal: JOE Vol 40, No 10;1638

Reviewer: Raj Shenoy, DDS

Purpose: This study compared the *in vitro* amount of apically extruded debris and instrumentation time with the ProTaper Universal, Protaper Next, Twisted File Adaptive, and HyFlex rotary systems.

Materials and Methods: Sixty freshly extracted mandibular premolars with a single canal, no calcifications, no resorption, no fractures, no open apices, and no curvature more than 10 degrees were included. The teeth were accessed and a 10 file was moved down the canal until just visible and a working length (WL) was established. The apical preparations were completed with a size 25 rotary instrument by using the instrument order specified by manufacturer. All files were used with low torque motor except twisted files which used the elements motor. Teeth were irrigated with 2.0ml of saline in between each instrument attached to a Surgic XT plus device. Each instrument was used in three canals. Preparations were completed by one operator and time was recorded. Debris was collected using Eppendorf tubes and weighed using and electronic scale.

Results: The Twisted file adaptive and ProTaper Next systems extruded significantly less debris than the ProTaper Universal HyFlex files. The instrumentation time with the ProTaper Universal was significantly longer than with all the other instruments. There was no significant difference (in time) between the other 3 file systems.

Conclusion: All the tested systems extruded debris however ProTaper Next and Twisted File adaptive instrumentation systems were associated with less debris extrusion compared with the ProTaper Universal and Hyflex systems

LOE: 5
Title: 10-Year follow-up of calcifying odontogenic cyst in the periapical region of vital maxillary central incisor

Author: Guedes O et al.

Journal: JOE Vol 40, No 10;1695

Reviewer: Raj Shenoy, DDS

Purpose: Calcifying odontogenic cysts (COCs) are rare intraosseous or extra osseous odontogenic lesions. Their frequency in the maxilla and mandible is the same and they are commonly found between incisors and canines. It may mimic periapical lesions of endodontic origin. This study describes a clinical case of a 10 year follow-up of COC’s in the periapical region of a vital maxillary central incisor.

Case Report: A white, 9 year old boy with history of swelling in the region near #9 presented to private clinic. The patient reported no history of trauma or pain. A clinical exam showed no mobility. On palpation a mild discomfort was felt when horizontal pressure was applied. Pulp vitality was confirmed in all anterior teeth. A radiographic showed an oval radiolucent lesion in the periapical region of a vital maxillary central incisor. Progression was unknown. Discomfort was recent. The treatment performed was surgical removal of the lesion and microscopic evaluation of specimen. At the 10 year recall after surgery panoramic and PA radiographs showed new bone formation. The patient was asymptomatic. Pulp vitality was present in all maxillary anterior teeth.

Discussion: The COC’s measure was 2-4cm in its greatest diameter. Radiographs usually show unilocular lesion but the cyst may also be circumscribed multilocular lesion containing diffuse radiopaque material. In this case surgery was completed due to vitality of tooth #9. The most histopathologic feature of a COC is the variable number of ghost cells within the epithelial component. Definitive diagnosis of COC may only be made by microscopic examination of surgical specimen. The recommended conservative treatment is enucleation of the lesion and the rate of reoccurrence is low. An important point is that great care was taken during the surgical removal of the lesion to decrease the chance of devitalization of tooth #9.

Conclusion: Not all lesions are of endodontic origin. Good clinical and radiographical findings are important when making a differential diagnosis. Follow up is important to confirm the survival of the pulp and to make sure there is no reoccurrence of COC.

LOE: 5
Title: Sealer penetration into dentinal tubules in the presence or absence of smear layer: A confocal laser scanning microscopic study

Author: Kuci, A. and et. al.

Journal: JOE, Vol 40, No. 10; 1627

Reviewer: Christopher Adams, DMD

Purpose: The smear layer is the organic and inorganic debris that forms after cavity preparation or root canal instrumentation and coats the dentin and clogs the orifice of the dentinal tubules. The smear layer is assumed to prevent the penetration of disinfectants and root canal sealers into the dentinal tubules. Its removal by using agents such as EDTA allows for better adaptation of sealers. The aim of this study was to test the dentinal tubule penetration of AH26 and MTA Fillapex in instrumented root canals. Canals were obturated by using cold lateral compaction or warm vertical compaction techniques in either the presence or absence of the smear layer.

Materials and Methods: Forty-five extracted single-rooted human mandibular premolar teeth were used. The crowns were removed, and the root canals were instrumented by using the Self-Adjusting File with continuous sodium hypochlorite (2.6%) irrigation. Final irrigation was either with 5% EDTA or with sodium hypochlorite. The canals were dried and obturated by using rhodamine B–labeled AH26 or MTA Fillapex in combination with the cold lateral compaction or the warm vertical compaction technique. After setting, the roots were sectioned horizontally at 4-, 8-, and 12-mm distances from the apical tip. On each section, sealer penetration in the dentinal tubules was measured by using confocal laser scanning microscopy.

Results: Regardless of the usage of EDTA, MTA Fillapex was associated with greater sealer penetration when used with the cold lateral compaction technique and AH26 was associated with greater sealer penetration when used with the warm vertical compaction technique. Removal of the smear layer increased the penetration depth of MTA Fillapex used with the cold lateral compaction technique however; it had no significant effect on the penetration depth of AH26. The flow of a sealer determines how effectively it obturates accessory canals, irregularities on the dentinal wall, and spaces between the core filling materials.

Conclusions: Greater sealer penetration could be achieved with either the MTA Fillapex–cold lateral compaction combination or with the AH26–warm vertical compaction combination. Smear layer removal was critical for the penetration of MTA Fillapex.

LOE: 5
Title: Ion Release, Porosity, Solubility, and Bioactivity of MTA Plus Tricalcium Silicate

Authors: Gandolfi M et al.

Journal: JOE, Volume 40, Number 10; p 1632-7

Reviewer: Hao Tran, DMD

Purpose: Mineral trioxide aggregate (MTA) is Portland cement, a hydraulic powders composed of tri- and di-calcium silicates combined with bismuth oxide powder. This study evaluated calcium release, the pH change, solubility, water sorption, porosity, surface morphology, and apatite-forming ability of MTA Plus (Avalon Biomed) immersed in simulated body fluid compared to ProRoot MTA and Dycal.

Materials and Methods: Three groups were made:

- MTA Plus [mixed with (a) gel & (b) water]
- ProRoot MTA
- Dycal

The materials were mixed then stored at 37°C water. Samples were collected and water was renewed after 3 hours at 1, 7, 14, and 28 days. The pH measurements were performed on the collected water with a pH probe. Calcium release measurements were made using a calcium ion selective probe. For water absorbance, sample disks were set at 37°C and 99% relative humidity for 70% of their setting time (55 minutes for MTA Plus and 250 minutes for ProRoot MTA). Each disk was immersed vertically in 20 mL distilled water at 37°C. After 24 hours, excess water was removed from each disk, and the saturated mass (M) was recorded. Each weight measurement was repeated 3 times to the nearest 0.001 g using an analytical balance. The exterior volume, the volume of open pores, the volume of impervious portion and the apparent porosity were calculated following Archimedes’ principle.

Bioactivity Test evaluated layers precipitated on the materials soaked in SBF solution. Prepared samples (approximately 5 minutes after mixing) and samples aged in HBSS for 1, 7, or 28 days were examined ‘wet’ using an environmental scanning electron microscope connected to a secondary electron detector for energy dispersive x-ray analysis.

Results: All three materials created an elevated pH (alkaline) after three hours of soaking, with the highest value for MTA Plus (pH 12.0). Over 28 days, all materials gradually decreased in their rate of release of hydroxyl ions, and the pH diminished. After 28 days, the pH was the highest for Dycal solutions (9.8) and the lowest for ProRoot MTA eluate (7.1). The calcium ion release after 3 hours was highest from MTA Plus, mixed with water (43 ppm) or gel (119 ppm), and lowest for Dycal (25 ppm) and ProRoot MTA (24 ppm). Porosity varied from 9% for Dycal to 40% for MTA Plus mixed with water or gel (39%). Water sorption was only 5% for Dycal and highest for MTA Plus with gel (26.5%). The solubility was lowest for Dycal and highest for MTA Plus powder with water (18.5%). The SEM results for freshly mixed MTA Plus with gel revealed compounds of calcium, silicon, bismuth, aluminum, and carbon on the surface. The freshly mixed and unexposed MTA Plus mixed with water had major EDX peaks for calcium, silicon, and bismuth and lesser peaks for sodium, sulfur, aluminum, and potassium, whose origins can be attributed to minor constituents of cement but may have been obscured in the gel sample. ProRoot MTA had a fine, granular surface with EDX peaks for major phases with calcium and silicon and lesser peaks for bismuth (10% wt) and aluminum (0.6% wt). Fresh Dycal exhibited a uniform surface with prominent calcium, phosphorous, tungsten, titanium, zinc, sulfur, and carbon.

Conclusion: These in vitro tests of the 2 MTA products and Dycal highlight the similarities of the MTA products and the contrast with Dycal, a Ca(OH)2-based product. All 3 materials released calcium and hydroxide ions. The MTA products are known to form Ca(OH)2 in a calcium silicate hydroxide matrix. Release of Ca(OH)2 diminished for all materials over 1 month. The precipitated calcium phosphate layer reduced the diffusion of calcium or hydroxide ions. MTA Plus with gel had high calcium release and pH. MTA Plus mixed with gel slightly reduced the porosity, sorption, and solubility. MTA Plus had a prolonged capability to release calcium and increase the local pH in comparison with ProRoot MTA. These ion releasing properties are interlinked with its noticeable porosity, water sorption, and solubility and with the formation of a calcium phosphate layer.

LOE: 5
In vitro biocompatibility, inflammatory response, and osteogenic potential of 4 root canal sealers: Sealapex, Sankin apatite root sealer, MTA Fillapex, and iRoot SP root canals sealer.

Author: Chang, S et al.

Journal: JOE Vol40, 10; 1642-1648

Reviewed by: Saehee Kim, DMD

Purpose: Sealers are essential to promote sealing ability of gutta-percha in root canals. The sealers are popular despite well documented toxicity and mutagenicity. Calcium hydroxide sealer, Sealapex, can decrease osteoclastogenesis and shows leakage with time. Apatite root sealer (ARS) has less leakage but osteogenic effects have not been reported. Newer sealer such as MTA has cytotoxic effect. iRoot Sp is novel premixed bioceramic sealer with excellent sealing properties, antimicrobial activities and without cytotoxicity to osteoblastlike cells. This study compared the cytotoxicity, inflammatory response, osteogenic effect and signaling mechanism of the four root canal sealers.

Material and Methods: Chemicals and reagents were obtained. Samples of Sealapex, ARS, MTA and iRoot were prepared (mixed, set, placed in culture plates, washed and dried then sterilized. Cell cultured (human PDLC) was obtained. Test materials were placed on 24 cell culture plates with PDLC cells. Culture media was changed every 2 days. Cells were collected on day 3, 7 and 14 and analyzed for biocompatibility, inflammatory response and osteoblastic potential. Signal transduction levels were determined by Western blot analysis. Negative controls were the culture medium without OS while positive control cultures use hydrogen peroxide for oxygen species production and inflammatory response.

- Cytotoxicity of the materials to hPDLC cells were examined using MTT assay. MTT solution added, culture incubated and viability of cells examined at day 3, 7, and 14. Reduced MTT measure spectrophotometrically.
- Early differentiation of cells to osteoblasts evaluated as a function of ALP activity. Amount of enzyme released quantified.
- Effects of materials on mRNA expression of genes involved in differentiation, cytokines and integrins examined by reverse transcription PCR.
- Mineralization effect assessed by staining with alizarin red S.
- Assess production of nitric oxide (NO) from hPDLC by measuring extracellular release of nitrite (NO₂).
- Analysis of PGE2 concentrations.
- Detect ROS production in hPDLC cells by CM-H2DCFDA.
- Western blot used to access iNOS, COX-2 and signal pathway.

Results: Cell viability as determined by MTT assay shows that none of the sealers had cytotoxic effects. ALP activity, alizarin red S staining and mRNA expression were used to investigate the effects of sealers on osteoblastic differentiation. ALP and mineralized nodules increased by MTA, ARS, and iRoot but not Sealapex. mRNA levels of osteonectin, osteocalcin and osteopontin were up-regulated by MTA, ARS, and iRoot at 7 days compared with Sealapex (mRNA levels of Runx2 and osterix unaffected). At 14 days, levels of Runx2 and osterix were up-regulated by MTA, ARS, iRoot compared with Sealapex. The effects of sealers on inflammatory response were determined by measuring the levels of pro-inflammatory mediators and cytokine (iNOS and COX-2 proteins as well as nitrite and PGE2 concentration). Sealapex induced higher production of NO, PGE2, iNOS and COX-2 then MTA, ARS, or iRoot. TNF-alpha up-regulated in Sealapex relative to other groups. No difference in expression of interleukin (IL 1B, 6, 8 mRNA) between the entire group. Signaling Pathways was evaluated by integrin. PDLC treated with MTA, ARS, and iRoot expressed higher levels A1, A2, and B1 integrin than Sealapex. Phosphorylation increased in MTA, ARS, iRoot.

Discussion: None of the tested sealers had a cytotoxic effect on PDLCs after 2 weeks of treatment. This data agrees with the study by Silva et al but not with the study by Guven et al (MTA has higher cytotoxicity than iRoot) and Scelza et al (Sealapex and MTA shows cytotoxicity). The author points the discrepancy between the studies from cell types used, experimental conditions and media. MTA, ARS and iRoot promoted osteoblastic differentiation on PDLCs cells to a greater extent than Sealapex (induction of ALP activity, up-regulation of osteoblastic markers and transcription factors Runx2 and Osterix, and enhanced deposition of mineralized nodules). The results agree with previous study by Zhang et al and Guven et al. MTA and iRoot induce osteogenesis. None of the sealers evoked a severe inflammatory response. However, MTA, ARS, and iRoot resulted in lower production of ROS and Proinflammatory mediators than Sealapex (by levels of iNOS, COX2 derived PGE2, IL1B, TNF a, IL6, IL8). These results are consistent with previous study by Silva et al. MTA, ARS and iRoot activated intracellular signaling cascade (Integrins) more so than Sealapex.

Conclusion: This is the first study to show that MTA, ARS and iRoot induce superior osteoblastic differentiation and less inflammatory response than Sealapex in PDLC.

LOE: 5