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Comparison of microleakage of bioactive bone cement, mineral trioxide aggregate, and glass ionomer cement as root end filling materials using dye extraction analysis

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Abstract
Introduction: Inadequate apical seal is a major cause of surgical endodontic failure. The root end filling material used after apicectomy should adhere or bond to tooth tissue and seal the root-end three-dimensionally. Aim of this study was to evaluate the sealing ability of modified (bioactive) bone cement, Mineral trioxide aggregate and glass ionomer cement when used as root end filing materials.
Materials and Methods: Thirty human maxillary central incisor teeth were prepared and obturated using gutta percha and AH Plus sealer. After 24 hours, apical 3mm was removed from each tooth and root end cavities made with ultrasonic tips. Samples were randomly divided into three groups of 10 samples each based on root end filling material - Group I: MTA, Group II: Bioactive Bone Cement and Group III: Glass Ionomer Cement. After one week of storage in PBS solution, specimens were subjected to dye extraction analysis and amount of dye absorbed was calculated in terms of absorption units using UV-Visible light spectrophotometer.
Results: Results showed that samples back filled with Bioactive bone cement had absorbed lowest dye (0.69±0.078 AU) followed by samples filled with MTA (0.72±0.108 AU). Highest dye absorption values were shown by samples filled with glass ionomer cement (1.53±0.200 AU). Statistical analysis showed no significant difference between Bioactive Bone cement group and MTA group (P =0.872) while as significant difference existed between these two and Glass ionomer group (P<0.001).
Conclusion: Bioactive bone cement is a viable alternative to currently used materials with ease of use and cost effectiveness as shown by low microleakage values when used as root end filling material.

Keywords: Bone cement, dye extraction, MTA, root end filling

Introduction
Success of endodontic treatment is achieved by elimination of the microorganisms from the root canal system and development of fluid tight seal using a material of adequate compatibility [1]. Despite of the new endodontic techniques, effective materials & instruments, the resolution of periapical pathosis is not achieved in certain cases [2] where surgical endodontic intervention is needed [3]. This procedure includes exposure of involved apex, resection of the apical end of the root, preparation of root end cavity & insertion of root end filling material. The rationale for removing the most apical three millimeters of the root is that it removes 98% of apical ramifications, and 93% of lateral canals.[4] Studies have shown that the best outcome of surgical retreatment is achieved by apical cavity preparation and placing an apical filling material [5]. The function of the root-end filling is to provide an ideally hermetic seal of the root canal, to inhibit coronal leakage of pathogens and their products into the periradicular tissues, and thus promote healing and formation of cementum on the cut dentinal surface [6].
Amalgam, IRM, Glass ionomer cements, Resins, MTA and many other dental materials have been tried and tested as root-end endodontic filling materials. At present, MTA is widely used material for this procedure and it has shown good sealing ability and biocompatibility in previous in-vitro and in-vivo studies [7]. However, MTA has its disadvantages: It contains toxic arsenic, the material is expensive, it is difficult to handle and it has a long setting time.

No solvent exists, and hence removal of MTA is difficult [8]. Glass-ionomer cements (GIC) have been retained as viable alternatives to MTA because of their ability to bond with dentin, biocompatibility, antimicrobial activity, and favorable cost-benefit analysis [9]. However, Sealing ability of GIC is adversely affected when the root end cavities are contaminated with moisture at the time of placement of cement [10].

Bone cement is a potentially new repair material that has been investigated in dentistry recently, although it has been used in orthopedic surgery for more than four decades. However, it lacks ability to directly bond with living body and hence needs to be modified to incorporate bioactivity in bone cement. Bioactive Bone cement has many characteristics that make it well suited as a repair material for variety of endodontic treatments [11]. The purpose of this study was to evaluate the sealing ability of MTA, bioactive bone cement and glass ionomer as root end filling materials using dye extraction leakage model.

Material & Methods

Thirty maxillary central incisors with mature apices and free of cracks and curvatures extracted for periodontal reasons were selected for the study. Access cavity preparation was made using a #2 round diamond point (NSK, Japan) and coronal preflaring was done using Gates-Glidden drills (MANI, Inc, Japan). Size #10 K-file (Mani, Inc, Japan) was introduced into the root canal until it was visible at the apex and then 1mm was subtracted from that point to establish the working length. Biomechanical preparation was done using step-back technique with apical enlargement up to #60 size K-file. Copious irrigation with 3% sodium hypochlorite (Prime Dental, Jammu, India) was done all through the procedure. Final irrigation was done with 17% EDTA (Prime Dental Products, India) followed by 3% sodium hypochlorite for 1 minute each and rinsing with saline. The canals were dried using absorbent points and obturation was done with 2% gutta percha points and AH Plus sealer, using the lateral compaction technique. Kerr Sticky Wax (Kerr Corporation, Orange, CA, USA) was melted in a cauldron and the specimens were dipped in it to achieve a noticeable wax-covering of the apical and lateral portions of the root. During the wax covering procedure, the root segments were held using an endodontic spreader. After 24 hours of obturation, the root ends were resected 3mm from the apex using a No.1557 fissure bur; retrograde cavity was prepared to a depth of 3mm coaxially using surgical ultrasonic retro-preparation tips (Satelec AS6D, France). Then the teeth were randomly divided into 3 groups according to the root end filling material used, with each group containing 10 teeth.

Group 1- MTA (ProRoot MTA, Dentsply- Tulsa Dental, Tulsa, OK, USA)

Group 2- Bone Cement (Surgical Simplex P, Stryker)

Group 3- Glass ionomer cement (Type II, GC Corporation - Japan)

All the materials were mixed according to the manufacturers recommendations and placed incrementally, following which radiographs were taken labio-palatally and mesio-distally to confirm proper filling of the material. To incorporate bioactivity in bone cement both powder and liquid of bone cement were modified with MTA powder and silane coupling agent (Ultradent Products Inc., USA) respectively to make bone cement bioactive so as to be used as root end filling material. 0.4mg of MTA powder was mixed with 0.6mg of bone cement powder and thoroughly mixed until all MTA particles were uniformly distributed in polymer powder of bone cement. 1 drop of silane coupling agent was added with 1ml of monomer liquid of bone cement and mixed till two liquids were completely miscible. The modified powder and liquid were mixed together in the ratio of 2:1 under ambient conditions at room temperature. After root end filling, a moist cotton pellet was placed on MTA for setting of the material and all the samples were stored at 37 °C for one week in such a way that all specimens were immersed in phosphate buffered saline up to cemento-enamel junction.

The specimens were then transferred into disposable sample collection containing 2% methylene blue held with vinyl-polysiloxane material so that only apical 3mm of root was immersed in dye. All samples were stored similarly for 48 hours. After removal from the dye, teeth were rinsed under tap water for 30 min and sticky wax removed. Apical 3mm of each tooth that had root end filling material was sectioned with the help of diamond disk and stored in a vial containing 1000µl of concentrated (65 weight%) nitric acid for 3 days. The solutions thus obtained were centrifuged at 3500 rpm for 5 min. 100µl of the supernatant was then analyzed in ultraviolet-visible spectrophotometer (UV-1800, Shimadzu Scientific Instruments, Japan) at 550 nm wavelength with concentrated nitric acid as the blank and readings were recorded as absorbance units (AU). The obtained readings were statistically analyzed.

Results

SPSS (Version 20.0) and Microsoft Excel software were used to carry out the statistical analysis of data. Data was analyzed with the help of descriptive statistics viz. mean and standard deviation. Analysis of variance (ANOVA) was used to determine whether significant differences existed between various groups. Tukey-Kramer multiple comparison test was applied to identify which pair of the groups were different. A P-value of less than 0.05 was considered statistically significant.

The mean absorbance values of experimental groups showed that the dye absorption was least for bioactive bone cement (0.69±0.078AU) followed by Mineral Trioxide aggregate (0.72±0.108AU) and highest dye absorption was found in glass ionomer cement group (1.53±0.200AU) (Table 1). Statistical analysis showed that there was no significant difference between dye absorption values of bioactive Bone cement and Mineral Trioxide Aggregate (P=0.872) with both of them having significantly lower dye absorption ($P<0.001$) than glass ionomer cement group (Table 2).

Table 1: Mean, Standard Deviation (SD) Values for Absorbance Units (AU) of three materials

Group	Mean	SD	95% Confidence Interval	Range	P-value
Group 1 MTA	0.72	0.108	0.65-0.79	0.56-0.86	<0.001*
Group 2 BBC	0.69	0.078	0.64-0.74	0.56-0.79	
Group 3 GIC	1.53	0.200	1.40-1.66	1.29-1.85	

Table 2: Comparison between individual groups

Comparison Groups	Mean Difference	Q value	P value
MTA vs BBC	0.03	0.78	0.872
MTA vs GIC	-0.81	20.33	<0.001*
BBC vs GIC	-0.84	21.11	<0.001*

Discussion

The goal of a periradicular surgery is to gain access to the affected area, evaluate the root circumference and root canal anatomy and place a biocompatible seal in the form of root end filling that stimulates the regeneration of periodontium. Numerous substances have been used as root end filling materials. The choice of a root-end filling material could be governed by handling properties, biocompatibility, apical seal and long term clinical success [12]. Most *in vitro* studies evaluate leakage of the apical seal, but the correlation between dye leakage around root-end filling materials and their clinical performance is uncertain. The clinical significance of microleakage in apical surgery has not been elucidated. However it seems logical that lesser leakage would prevent migration of bacteria and toxins into the periradicular tissue. Dye-extraction provides more reliable results than dye penetration study because of its ability to measures all of the dye taken up in the root. The dye-extraction technique is as good as fluid filtration technique because it also takes into account all of the porosities of the interfaces between the filling material and the root. In addition, both techniques are based on quantitative measurements of the passage of a liquid within these interfaces [13].

MTA has been investigated and used as a root end filling material since its introduction. Despite its good physical, biological properties and it being hydrophilic in nature [7]. MTA has some disadvantages such as long setting time, high cost and difficult manipulation [8]. Sensitivity to excessive or deficient moisture affects its setting and properties in detrimental way [14]. The search for alternative materials is aimed to reduce costs and to increase the feasibility to both professional and patient. Glass ionomer cements bond physico-chemically to dentine. Biocompatibility studies have shown evidence of initial cytotoxicity with freshly prepared samples, with decreasing toxicity as setting occurs [15]. Sealing ability of GIC is adversely affected when the root end cavities are contaminated with moisture at the time of placement of cement [10] although marginal adaptation and adhesion of glass ionomer cements to dentin has been shown to improve with the use of acid conditioners and varnishes [16]. However, clinically, the plasticity and stickiness of glass-ionomer cement impede condensation into the root-end cavity.

Bone cement consists of polymethylmethacrylate (PMMA) and methylmethacrylate (MMA) and is widely used for fixation in orthopedic fields but due to lack of bioactivity a number of modifications have been used [17, 18]. The essential requirement for an artificial material to show bioactivity is the formation of a biologically active bone-like apatite layer on its surface in the body environment [19]. Miyazaki et al have shown that apatite formation can be induced by release of calcium ion (Ca^{2+}) from the modified bone cement into the body fluid and by a catalytic effect of Si-OH groups formed on the surface of material [11]. Addition of MTA powder to bone cement acts as a source of calcium ions whereas addition of silane to liquid component provides Si-OH group due to hydrolysis of alkyloxysilane after exposure to the body environment that induces heterogeneous nucleation of hydroxyapatite. Addition of silane also improves the mechanical properties of bone cement because chemical bonding can be formed with polymerized MMA. Bioactive bone cement showed better sealing ability than MTA. This can be attributed to the fact that on exposure to simulated tissue fluid, it gets covered with layer of apatite crystals which nucleate and grow, filling the microscopic space between bone cement and the dentinal wall.[11] Being osteoinductive in nature, it acts as a medium for crystal growth and nucleation. The exothermic reaction of polymethy lmethacrylate (PMMA) bone cement during its setting does not seem to have any negative effects due to very small amount needed in root end fillings [20]. Also the slight expansion of MTA during setting [21] counteracts any polymerization shrinkage of PMMA resulting in improved sealing of modified bone cement. The cement also tolerates a moist environment very well and is not affected by blood contamination. It was also observed that the cement had excellent handling properties, could be easily manipulated in to a dough form and placed to adapt to the root end cavity area readily [22].

Conclusion

Within the limitations of this study it can be concluded that bioactive bone cement is viable alternative for root end fillings due to its ease of manipulation and cost effectiveness associated with good sealing ability.

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