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Evaluation of Microleakage of Bioactive Glass, Bioactive Glass-Hydroxyapatite and Mineral Trioxide Aggregate-An in Vitro Study

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Abstract: Introduction: The aim of this study was to evaluate the sealing ability of Bioactive glass (BG) and Bioactive glass-Hydroxyapatite (BG-HA) in comparison to Mineral Trioxide Aggregate (MTA). Methods: The sealing ability of Bioactive glass and Bioactive glass-hydroxyapatite mixed with glass ionomer cement and Mineral Trioxide Aggregate used as retrograde filling material was assessed by bacterial leakage test. The biocompatibility was statistically analyzed using one way ANOVA followed by Tukey HSD Post hoc test and micro leakage was statistically analyzed using Pearson chi-square test. Results revealed that in The sealing ability of bioactive glass and bioactive glass-hydroxyapatite mixed with glass ionomer cement were similar to MTA and that all three study groups were not significantly different from each other. In Conclusion: Bioactive glass-hydroxyapatite showed similar sealing ability compared to bioactive glass and Mineral Trioxide Aggregate.

Key words: Micro Leakage · Bioactive Glass · Bioactive Glass-Hydroxyapatite · Mineral Trioxide Aggregate

INTRODUCTION

New endodontic techniques, more effective materials and instruments have been developed in recent years, but, sometimes the resolution of periapical pathology is only achieved through surgical procedures with apical root-end filling that creates a hermetic seal.

The ideal root end filling material should have biocompatible characteristics, dimensional stability, adhesiveness, low solubility and the capacity to create a seal at the apical third of the canal to isolate the root canal system from the periapical region.

Amalgam, Super-EBA, IRM, Diaket, GIC and recently Mineral Trioxide Aggregate are being used as root-end filling materials, but each has its own drawbacks.

Mineral Trioxide Aggregate (MTA) is an accepted retro filling material with good physical, chemical and biologic properties but with the disadvantages of difficult manipulation and prolonged setting time.

Bioactive glass developed by Larry Hench *et al* is a type of bioceramic. It is used as a type of alloplastic synthetic bone graft, which has the property to promote adsorption and concentration of proteins utilized by osteoblasts to form a mineralized extra cellular matrix and thus, promote osteogenesis by allowing rapid formation of bone [1].

Bioactive glass has been used to reduce the permeability of dentin by providing permanent occlusion of dentinal tubules [2] and also as a pulp capping agent [3].

The purpose of our study is to evaluate the sealing potential of bioactive glass, bioactive glass-Hydroxyapatite in combination with GIC when used as a retro filling material.

MATERIALS AND METHODS

Methodology:
Microleakage Test: Fifty freshly extracted maxillary central incisors were taken and stored in 10% Formalin for 2 weeks. The teeth were divided into five groups- (n=10)

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The Study Groups Considered Were:

Group I: Bioactive glass (BG)
Group II: Bioactive glass-Hydroxyapatite (BG-HA)
Group III: Mineral Trioxide aggregate (MTA)
Group IV: Positive control
Group V: Negative control.

BG and BG-HA were mixed in a ratio of 30wt% with the powder of type II glass ionomer cement by ball-milling to achieve a workable mass. The liquid of type II GIC was used for manipulation.

The crowns of maxillary central incisors were removed at the cemento-enamel junction. The roots were prepared by protaper rotary instruments up to size F5. During instrumentation, the canals were irrigated with 5.25% Sodium hypochlorite and alternated with EDTA. The canals were dried with sterile paper points and obturation was done using greater taper gutta-percha cones (F5). Zinc oxide eugenol was used as sealer.

The sealer was allowed to set for 24 hours. The root ends were cut off with a fissure bur for about 3 mm. The root-end cavity preparation was done with ultrasonic retro-tip (Satelec- S12-90D) to a depth of 3mm.

The retrograde cavities were filled with bioactive glass ionomer cement, bioactive glass ionomer-hydroxyapatite cement and MTA. The positive control includes teeth with obturation of the canal with gutta-percha but the retrograde cavities unfilled whereas the negative control group includes teeth completely covered with sticky wax. All the teeth were coated with nail varnish except in the apical portion.

The sealing ability of the retrograde filling materials were found out by bacterial leakage test using a culture of *Enterococcus faecalis* with a two-chamber model.

For this test, the tips of ependorf tubes were cut off and the teeth were fitted into the tubes with sticky wax. The ependorf tubes with the teeth projecting were inserted into glass vials which serve as the bottom chamber. The bottom chamber was filled with brain heart infusion broth such that 1-2 mm tips of the teeth were touching the broth.

The assembly was incubated at 37°C for 24 hours and this served as the sterility check. A culture of *Enterococcus faecalis* was added in the upper chamber onto the tooth surface. Then it was incubated for 5 days. At the end of the fifth day, turbidity of the broth in the samples was noted. Then the seal was broken and it was sub cultured in Nutrient agar plate by streak method.

After 24 hrs of incubation, colony growth on nutrient agar was noted followed by smear and biochemical tests to confirm the organism. Micro leakage was assessed in the study groups using the following criteria.

Scoring Criteria: 1-Leakage Present

0-No Leakage

Statistical Analysis: The micro leakage test was analyzed statistically using Pearson chi-square test.

RESULTS

The micro leakage study (figure 1) revealed that when comparing all the five groups, group IV was significantly different from other groups and group V was significantly different from other groups ($p<0.01$). When comparing groups I, II and III, there was no significant difference between the three groups ($p<0.05$)

DISCUSSION

A root end filling is commonly placed after resection and preparation of the root end during endodontic surgery. The most ideal healing outcome after filling of the resected root end would be formation of a normal attachment apparatus with healthy bone, periodontal ligament and cementum. The efficiency of the apical seal is also an important factor in retrograde filling.

In our study, we evaluated the sealing ability of bioactive materials-Bioactive glass, Bioactive glass-Hydroxyapatite and Mineral Trioxide Aggregate.

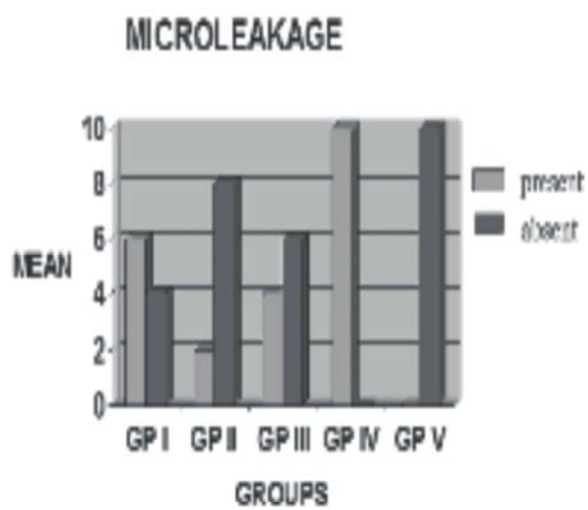

Fig 1:

By definition, a bioactive material is "one that elicits a specific biological response at the interface of the material that results in the formation of a bond between the tissues and the material".

The bioactive glass contains oxides of Calcium, Sodium, Phosphorus and Silicon. They are capable of generating a carbonated hydroxyapatite layer that is equivalent chemically and structurally to the biological mineral of bone, which forms the basis of biointegration.

Bioactive glass-hydroxyapatite is a less expensive, resorbable synthetic porous ceramic granular graft. The particle size is in the range of 150-500 μm and pore size in the range of 100-200 μm. The material is a composite of amorphous and crystalline phases such as hydroxyapatite, calcium silicate, tricalcium phosphate and amorphous silicon compounds.

The glassy part (BG) is 17% silicon (as SiO2), 53% calcium (as CaO) and 30% phosphorous (as P2O5). The glass is composite with an equal quantity (50%) of synthetic hydroxyapatite(HA).The particles are synthesized as porous granules and mixed to have improved bioactivity.

Bioactive glass-Hydroxyapatite contains calcium-phosphosilicate glass made through low-temperature sol-gel route and composited with hydroxyapatite (a calcium phosphate similar in composition to bone and dental mineral) and tricalcium phosphate phases. These granules act as substrates for osteoblast proliferation and biointegration.

The initial osteoconductive property leading to faster integration into bone is provided by synthetic hydroxyapatite, while the bioactive glass phase promotes rapid bone growth. Previous studies have shown that the composited form has improved mechanical property [4].

Microleakage: The single most important factor in determining the success of an apicectomy is the efficacy of the apical seal. Many invitro techniques have been used to assess the sealing potential of root-end filling materials.

Bacterial leakage tests are frequently used for evaluation of the sealing ability of endodontic materials. Bacterial leakage tests can be done by using any micro organism, whole human saliva or endotoxin leakage can also be evaluated.

In this study, the two chamber model was used [5]. *Enterococccus faecalis* was chosen as the test bacteria, as they are part of the normal flora in humans and are frequently isolated in failed endodontically treated teeth together with other facultative anaerobes [6].

30 wt% of Bioactive glass and Bioactive glass-Hydroxyapatite were mixed with GIC because of the known bioactivity of the GIC [7]. A study by Helena Yli-Urpo *et al*.[8, 9] suggested that mixing bioactive glass particles into conventional GIC powders weakened the compressive strength of GIC on average by 54%. They recommended that their clinical use ought to be restricted to applications where their bioactivity would be beneficial, such as root surface fillings and liners in dentistry and where high compressive strength is not necessarily needed. The studies by Helena *et al*, also showed that the surface hardness of conventional GIC based materials were higher than the resin-modified GIC. In this study, the bioactive glass and bioactive glass-hydroxyapatite were mixed with conventional type II GIC.

When all the five groups were compared in this study, group IV (positive control) and group V (negative control) were significantly different from the other groups. When groups I, II and III which are bioactive glass, bioactive glass-hydroxyapatite and Mineral Trioxide Aggregate, respectively were compared, there was no statistically significant difference between the three groups.

The inability of the microorganism to penetrate is due to the sealing potential of the materials. In addition, the materials were also found to have antibacterial activity. The antimicrobial activity of Bioactive glass were found to be due to incorporation of H_3O^+ protons into the corroding glass and release of Na^+ and Ca^{2+} ions and silica in closed systems which results in a high-pH environment that is destructive to the microorganisms [10].

MTA was found to have antibacterial activity against *Enterococcus faecalis*. The glass ionomer cements containing bioactive glass were found to have antibacterial activity against *Streptococcus mutans* and *Candida albicans* [11]. The antibacterial activity of glass ionomer cements containing bioactive glass against *Enterococcus faecalis* are yet to be studied.

Within the limitations of this study, when assessing the micro leakage, it could be concluded that the bioactive glass-hydroxyapatite group was better than the other two groups. But further studies should be done before recommending this as a better root-end filling material, when compared to other materials.

CONCLUSION

Bioactive glass and Bioactive glass-hydroxyapatite which were mixed with glass ionomer cement and Mineral Trioxide Aggregate were not significantly different from each other in the sealing potential.

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