In vitro evaluation of bacterial leakage through different perforation repair materials of teeth

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ABSTRACT: Purpose: This study was conducted to evaluate micro-leakage through different furcation repair dental materials which are used to repair iatrogenic perforations in teeth. Six commercially available dental materials high copper amalgam, glass ionomer cements (GIC) Fuji II and Fuji IX, intermediate restorative material (IRM), mineral trioxide aggregate (MTA) and fully injectable calcium phosphate cement (Chitra-CPC) were evaluated.

Methods: Eighty extracted human molar teeth were prepared chemomechanically and allocated to six experimental and two control groups, each comprising of 10 teeth. Microleakage was evaluated using the Enterococcus fecalis bacterial penetration test and confirmed with a confirmatory broth. On a daily basis broth was evaluated for visual turbidity for 45 days and leakage was confirmed using Mac Conkey’s medium.

Results: Statistical analysis using the Chi-Square test has revealed a significant difference among the materials tested, with MTA and Chitra-CPC showing minimal leakage when compared to the other repair materials within this period.

Conclusions: MTA and Chitra-CPC showed a similar micro leakage patterns and had a better sealing ability when compared to other materials in this study. (Journal of Biomaterials & Biomechanics 2009; 7: 179-84)

Key words: Micro-leakage, Enterococcus fecalis, Mineral trioxide aggregate, Fully injectable calcium phosphate cement, Intermediate restorative material

INTRODUCTION

Perforations are a relatively common cause of endodontic failure. When left untreated, perforations on the pulpal floor result in an inflammatory response in supporting tissues, with epithelial proliferation and eventual periodontal pocket formation (1, 2).

Furcation perforation may be treated either using an internal or external (surgical) approach. Regardless of the approach used the success rate is low (3). Factors affecting the prognosis include location (4), adequacy of seal (5), degree of contamination (6) and the material used to seal the perforation (7).

Another important factor appears to be the ability of the material to seal the defect. Various restorative materials have been tried and tested to seal the furcation perforations in the literature. But no material which satisfies all the requirements of an ideal furcation repair agent is available yet. Unintentional extrusion of the sealing material into the alveolar bone may preclude success regardless of the material used. This produces additional inflammatory and foreign body reactions. Attempts have been made to control this extrusion using biocompatible matrices, and it has been suggested that hydroxyapatite would be a highly biocompatible choice (8). However, hydroxyapatite particles tend to migrate from the site before bony growth secures them (9).

If the material were combined with a binder, this may prevent the migration of particles (10). The Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, India, has developed novel fully injectable calcium phosphate cement (ISO-7405), which is viscous and cohesive by incorporating a binder to overcome this problem. This patented (Indian IPR) formulation, Chitra-CPC, has enhanced viscous and cohesive properties compared to conventional CPC. Chitra-CPC could be mixed in varying consistencies, from moldable putty to injectable paste. This flexibility provides an immense advantage in clinical application as a bone and dentin substitute (11).

Chitra-CPC powder contains tetracalcium phosphate (TTCP) and dicalcium phosphate dihydrate (DCPD), particle size in the range of 100 micron mixed in an equimolar ratio, and salts of alginic acids in a dry powder form in a ratio of 2% w/w as the gelling agent. The cement mass undergoes isothermal setting and is converted to hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂). The moldability of CPC gives it an edge over hydroxyapatite ceramics used in skeletal repair. In the presence of moisture CPC sets into hydroxyapatite (12).

The aim of this study was to evaluate the furcation perforation sealing ability of MTA, IRM, Chitra-CPC, GIC Fuji II, GIC Fuji IX and high copper amalgam on extracted human molar teeth. Sealing ability was assessed using the Enterococcus fecalis microbial leakage test.
In vitro evaluation of bacterial leakage through different perforation repair materials of teeth

MATERIALS AND METHODS

Eighty multi rooted human molars free of caries with well diverged roots were selected. Apical 5 mm of roots were resected with a diamond disc to eliminate the lateral canals (Fig. 1). A standardized 5 x 5 mm endodontic access opening was prepared using a No 4 round bur and all canals were located. Cleaning and shaping all teeth was done chemo-mechanically using endodontic k-files sequentially nos 15, 20 and 25. Root ends were sealed with cyanoacrylate cement. An iatrogenic furcal perforation was created in 70 teeth using a no 2 round bur centered between the roots. The width of the perforation was standardized to the diameter of a no 2 round bur and the depth of the perforation to 3 mm from the dentin-cemental junction with a marked straight probe.

The entire tooth except at furcal perforation was coated with two layers of nail varnish to avoid leakage through the accessory canals, open dentinal tubules, apical foramen-cement interface and lateral canals. All teeth were sterilized in autoclave at 121 °C with 15 lb pressure.

Preparation of specimens for microbial leakage

Eighty molars were divided into eight groups, each group comprising of 10 teeth. The first six experimental groups were repaired with MTA, IRM, Chitra CPC, GIC Fuji II, GIC Fuji IX and high copper amalgam, respectively. Ten teeth in which the perforation was not repaired served as a positive control group. The negative control group comprised of the last 10 teeth in which iatrogenic perforation was not prepared. All the materials were manipulated according to the manufacturer’s instructions and restored aseptically in a luminary flow chamber to maintain sterilization throughout the study.

In test tubes filled with Enterococcus falcis confirmatory broth (HI-MEDIA), all the experimental groups were suspended in the broth with an orthodontic suspension wire until the furcal repair portion of the tooth was dipped into the medium. Then the specimens were placed into an incubator maintained at a constant temperature of 35°+/−2 °C at 100% humidity.

The bacterial strain used in this study was Enterococcus falcis (ATCC 29212) grown in brain heart infusion medium.
for 24 hr at 37 °C. Using a sterile disposable micropipette tip, 100 µL of the culture suspension (10⁶ cells per mL) was placed in the pulp chamber of each tooth every 48 hr (Fig. 2). Before fresh inoculum was placed, the existing inoculum was aspirated and randomly plated on Mac Conkey’s medium to assess the viability of the bacteria and to check for contamination. On a daily basis, the Enterococcus fecalis confirmatory broth in each tube was checked visually for turbidity, color change (from blue to light green) and compared with the negative control. A loop full of turbid broth (10 µL) from each tube was inoculated into Mac Conkey’s medium and incubated at 37 °C over night. The next day, the culture plates were checked for growth (Fig. 3) and observed under a microscope (Fig. 4). The tubes in which growth occurred were noted and the day of leakage was recorded for each sample. All the samples were monitored for a period of 45 days to evaluate the presence or absence of bacterial leakage. Micro-leakage between various groups (repair materials) was compared by statistically analyzing the data using the Chi-Square test (χ²). SPSS (Statistical Package for Social Scientists) version 11.5 was used for the analysis.

RESULTS

The positive control (group 7) showed complete leakage within 24 hr whereas the negative control (group 8) did not demonstrate any leakage. When compared against the experimental groups, all the materials revealed a statistically significant difference in the degree of micro-leakage with values p≤0.001 (Tab. I, Fig. 5).

Groups 1 and 2

Teeth repaired with MTA and Chitra-CPC showed the least amount of micro-leakage when compared to the other experimental groups. Four samples leaked (40%) between 30 and 45 days.

Group 3

Perforations repaired with IRM showed leakage in all samples (100% leakage) within 45 days. Of these 80% leaked within the first 15 days and 20% leaked between 16 and 30 days.

Group 4

GIC FUJI IX showed similar leakage to GIC FUJI II; 10% of samples leaked between 15 and 30 days and 60% between 30 and 45 days. In total 70% leakage was noted.

Group 5

Perforations repaired with GIC FUJI II showed leakage in 70% of samples within 45 days. Of these 70% leaked between 31 and 45 days.

Group 6

Perforations repaired with amalgam showed higher leakage. All samples (100%) leaked within 15 days which is statistically very highly significant.

Results are significant since the p value is <0.005.

DISCUSSION AND CONCLUSION

The success and long-term prognosis of furcation perforations in teeth depend on the size of the defect and the related damage to the periodontal ligament and bone, the time interval between perforation and its repair (immediate repair, the better the prognosis) and the ability to hermetically seal the perforation.

Of the several studies found in the literature for evaluating the sealing ability of various repair materials, the dye penetration method is the most widely used, because of its simplicity and reproducibility. Despite its popularity, dye or isotope penetration studies have several disadvantages as follows:

1. The molecular size of most dye particles is smaller than bacteria.
2. Most dye leakage studies have measured the degree of leakage in one plane, making it impossible to evaluate the total leakage.
3. Compared with clinical conditions, in vitro dye leakage studies are static and do not reflect the dynamic interaction between the root canals and peri radicular tissues.

Hence, microbiological studies are more clinically oriented tests for assessing leakage, as they provide more realistic data. The bacteria used in this study were Enterococcus fecalis (ATCC-29212). It was chosen as a test or-
ganism in this study because it is one of the most resistant microorganisms found in the infected root canals. This bacterium is a gram-positive (1-3 µm in diameter) facultative anaerobic motile bacillus, with smaller size and faster rate of migration (13).

In this study the sealing ability of MTA and Chitra-CPC was comparable and better than other materials tested. IRM did not show leakage initially up to 13 days, which can be attributed to the antibacterial property of the eugenol in it. Free eugenol may diffuse from the set cement resulting in the antibacterial property of the material providing only an intermediary seal. Eugenol can competitively inhibit cyclooxygenase enzyme, decreasing prostaglandin synthesis. Release of eugenol by IRM discourages its use as a material to be placed in contact with vital tissues and bone as it inhibits sensory nerve activity, mitochondrial respiration and can be an allergen. A marginal gap of 11+/−7.9 µm was observed in some scanning electron microscopy (SEM) studies when restored with IRM (14).

Delayed leakage in the majority of the GIC samples (GC Fuji II & GC Fuji IX) can be attributed to its adhesion to dentin chemically, its insolubility and flow properties. Better flow properties of the glass ionomer (GIC samples) has allowed better adaptation to the walls of the defect. Low viscosity of this material with better flow properties allows easy placement in the furcation areas, but both an under filling and an over filling of materials was noticed (15).

Biocompatibility studies have shown the evidence of initial cytotoxicity with freshly prepared samples of GIC, with decreasing toxicity as setting occurs. It was reported to cause inflammation when placed in contact with bone. The sealing ability of GIC was adversely affected when the perforations were contaminated with moisture at the time of cement placement. Micro-leakage through the GIC could be explained by higher volumetric shrinkage leading to adhesive failure at the dentin interface during setting (16). Both GICs in this study (FUJI II and FUJI IX) had shown almost similar results.

All the samples that were sealed with amalgam showed complete leakage within the first 15 days. This poor sealing ability of the amalgam could be attributed to its

Table I - Comparison of bacterial leakage among different materials

<table>
<thead>
<tr>
<th>Micro-leakage</th>
<th>Groups</th>
<th>Total</th>
<th>χ² test</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTA</td>
<td>CHITRA-CPC</td>
<td>IRM</td>
<td>FUJI IX</td>
</tr>
<tr>
<td>Micro-leakage in 1-15 days</td>
<td>0  0 8</td>
<td>0  0 0</td>
<td>10 100%</td>
<td>10 100%</td>
</tr>
<tr>
<td>Micro-leakage from 16-30 days</td>
<td>0  0 2</td>
<td>1  0 0</td>
<td>0  0 3</td>
<td>3 01</td>
</tr>
<tr>
<td>Micro-leakage from 31-45 days</td>
<td>4  4 0</td>
<td>6  7 0</td>
<td>21 35%</td>
<td></td>
</tr>
<tr>
<td>No micro-leakage in 45 days</td>
<td>6  6 0</td>
<td>3  3 0</td>
<td>18 30%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10 10 10</td>
<td>10 10 10</td>
<td>10 10 10</td>
<td>10 10 10</td>
</tr>
</tbody>
</table>

MTA  Mineral trioxide aggregate
Fuji II  Glass ionomer cements Fuji II
Fuji IX  Glass ionomer cements Fuji IX
IRM  Intermediate restorative material
CHITRA-CPC  Chitra-calcium phosphate cement
χ² test  Chi-Square test
P  Statistical significance
sensitivity to moisture, contraction and expansion reactions during setting and lack of chemical adhesion to the tooth structure. Furcation perforations act as a bottomless pit, so the proper compaction of amalgam is impossible, resulting in a poor adaptation of the material to the walls of the defect. Contraction of the alloy and the lack of a chemical bond to the tooth structure may have contributed to the leakage of bacteria in this study (13).

The least amount of bacterial penetration was observed through MTA when used as furcation repair material. The improved sealing ability of MTA is due to its hydrophilic nature and slight setting expansion (17, 18). In the presence of water MTA partially dissolves producing hydroxyapatite crystals, which provides a mechanical seal by filling the microscopic spaces between MTA and the dentinal wall. With time, a diffusion controlled reaction between the apatite layer and dentin leads to their chemical bonding. The favorable interaction of MTA with osteoblasts and its ability to induce cytokine production make it a biologically active substrate for bone cells. Even though MTA claims to seal well with dentine, SEM analysis of marginal adaptation to teeth in the literature showed a gap size of 2.68 +/- 1.35 µm (19). This gap is large enough to allow the passage of small microorganisms like \textit{E. coli} and \textit{Staphylococcus aureus}, which indicates MTA is not completely leak free. These findings concerning MTA were parallel to other studies in the literature (18).

Furcation sealing ability of indigenously developed fully injectable Chitra-CPC was comparable with MTA in this study. CPC gets converted into hydroxyapatite upon setting. It has osteoconductive and osteotransductive properties (i.e. active resorption at bony sites, facilitating bone remodeling) (20).

The favorable biocompatibility results from cytotoxicity studies and the non-hemolytic potential of this material makes it suitable for in vivo use. Inadvertent extrusion of the material into furcation is well tolerated by the periodontal tissues. Chitra-CPC has submicron-sized hydroxyapatite particles, inter-grown to form a homogeneous mass during cement setting (12). The inter-particle boundaries are weak enough to give way to newly growing bone and the particulate structure offers an enormously large surface area for osteoblasts to act upon. A disadvantage is it tends to degrade if exposed to an aqueous environment and is radiolucent. Chitra-CPC is more cost effective than compared with MTA.

The bacterial model system used in this study may be more clinically relevant than dyes or isotopes, but it too has limitations. In this study, a single bacterial species was used that is commonly found in the oral cavity, compared with the mixed flora found in vivo, also there was no interaction with body fluids such as blood, lymph, saliva and pus. Other variables to be considered include the physical changes that take place in the dentin of extracted teeth, which has not been taken into account in this in vitro study. In the in vivo state, the amount of bacteria as well as host response is highly significant factors that cannot be measured in this in vitro model.

Based on the above findings, it can be concluded that all the furcation repair materials to date show some amount of leakage. As MTA and Chitra-CPC showed significantly less leakage, they offer a significant scope for use as furcation perforation repair materials.

Conflict of interest statement: None.

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REFERENCES