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Use of a hydroxylapatite-based material and calcium sulfate as artificial floors to repair furcal perforations

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Objective. The purpose of this study was to evaluate a hydroxylapatite-based material and calcium sulfate when each was used under a resin-modified glass ionomer cement to repair furcation perforations.
Study design. Perforations of pulp chamber floors were made in 72 teeth of 9 dogs. Perforations were divided into 3 equal-sized groups and repaired with resin-modified glass ionomer either alone or over an artificial floor. The artificial floor was either a hydroxylapatite-based material or calcium sulfate. Three dogs were killed at each of 3 intervals (1, 3, and 6 months). The tissue response to the tested materials was evaluated clinically, radiographically, and histologically.
Results. The hydroxylapatite-based material showed the highest radiographic success; this was followed by calcium sulfate and glass ionomer. From histologic evaluation, the average success rate was found to be 67% for calcium sulfate, 62% for the hydroxylapatite-based material, and 59% for glass ionomer. However, there was no statistical significant difference with the resin-modified glass ionomer when it was used alone and when it was used over a barrier. There was also no significant difference between the hydroxylapatite-based material and the calcium sulfate when they were used as artificial floors.
Conclusion. The use of an artificial floor may not be necessary when flowable resin-modified glass ionomer cements are used.
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Although furcation perforations are not common, they represent a serious problem that significantly decreases prognosis. A nonsurgical approach to repairing furcation perforations is preferred; however, extrusion of repair material into the periodontal space may interfere with healing.[1] Periodontal reattachment will not occur when a material such as amalgam, gutta percha, or calcium hydroxide is used to repair perforation defects.[2,3] Materials such as bioceramic and bone fillers may initiate formation of new bone and/or periodontal attachment, but they cannot adequately seal the dentin defect; this could result in failure because of leakage of bacteria and their byproducts, particularly if the perforation communicates with gingival sulcus.[4]

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Furcation perforation is not a defect of homogenous structures; rather, it involves different interrelated tissue types. Treatment considerations of this defect require use of materials consistent with the characteristics of dentin and periodontium. Therefore, some biomaterials have been used as internal matrices under seal-providing materials to control their extension into the periodontal tissues. Dentin chips and calcium hydroxide have formed matrices under resin-based sealer for obturating the perforation defects[5]; periodontal defects have occurred apical to the perforations regardless of the technique or material used. Beavers et al[6] allowed root perforations to fill with blood and obturated the coronal orifices with either calcium hydroxide or polytetrafluoroethylene disks as internal matrices; they found that the 2 groups showed similar adverse periodontal tissue response. Hydroxylapatite was also described as a biological internal matrix to control the extrusion of the filling material into the periodontal area[7] and was found to be biocompatible, nontoxic, and osteoconductive.[8] However, Balla et al[4] reported no new tissue formation after repairing furcation perforations with hydroxylapatite or tricalcium phosphate.

Table I. Means and standard deviations of sulcus depths for experimental teeth before surgery and at time dogs were killed

Material	Before surgery	At time dogs were killed
Glass ionomer	2.3 ± 0.5	3.4 ± 1.5
Hapset	2.2 ± 0.4	3.1 ± 1.0
Calcium sulfate	2.2 ± 0.4	3.1 ± 1.3

There was no statistically significant difference between any groups at time dogs were killed.

Table II. Radiographic evaluation of tested materials

Judgment	Glass ionomer (%)	Hapset (%)	Calcium sulfate (%)
Success	41	53	33
Uncertain	23	14	25
Failure	36	33	41

There was no statistically significant difference between any groups at time dogs were killed.

Calcium sulfate (plaster of paris) has proved to be a good barrier against the extrusion of materials used to fill furcal perforations.[9] It was examined as a filler of experimental alveolar bone cavity in dogs.[10] Radiographs showed that resorption of the calcium sulfate began around the fifth day and continued until the second week. At the third week, the homogenous appearance changed to a trabecular pattern. The plaster sites were better organized at earlier stages, and the bone was at higher levels than the controls. Plaster of paris bone barrier guided bone regeneration and excluded epithelial tissue from the site of bone formation.

The purpose of this study was to evaluate tissue responses to a resin-modified glass ionomer cement used to repair dentin defects of furcation perforations with and without artificial floors. It was also aimed at comparing 2 artificial floor materials—a hydroxylapatite-based material (Hapset) and a medical-grade calcium sulfate—when they were used as barriers against periodontal wounds.

MATERIAL AND METHODS

Nine adult female dogs in good oral and systemic health were used. General anesthesia was induced by intravenous injection of thiamylal sodium in a 15 mg/kg dose. Anesthesia was maintained through inhalation of 1.0% to 1.5% isoflurane USP and oxygen mixture.

Fourth premolars and first molars were used; the total number of teeth was 72. Preoperative periapical radiographs were made, and periodontal probing gingival sulcus depths were recorded. After rubber dam isolation of the operative quadrant, sterile #171 burs reduced the cusps until pulp was exposed. After access, canals were prepared according to the step-back technique and obturated with warm lateral condensation of gutta percha. Sterile #2 round burs in a low-speed handpiece were used to create perforations in the center of the chamber floors in the furcations. A rubber stopper was used to limit the perforation length to 2 mm in the alveolar bone. The perforations were irrigated with normal saline solution, and bleeding was controlled with sterile cotton pellets. The 72 perforations were divided into 3 experimental groups of 24 perforations each.

Group 1

The perforations in group 1 were obturated with a resin-modified glass ionomer cement (Vitrebond, 3M, St Paul, Minn). After the material was mixed on a paper pad with a sterile cement spatula, it was carried into the perforation on the tip of an explorer and allowed to flow apically. When the glass ionomer cement had apparently obturated the apical end of the defect, it was light-cured for 60 seconds. Incremental layers were then applied and light-cured until the perforations were completely obturated.

Group 2

For the perforations in group 2, a hydroxylapatite-based material called Hapset (Lifecore Biomedical, Chaska, Minn) was mixed in a sterile plastic well. The material was carried through the chamber, placed into the perforation with a plastic instrument, and condensed into the bony portion of the defect with a finger condenser. After 10 minutes, the dentin surface of the perforation was refreshed with a #55 bur at low speed and the perforation cavity was rinsed with water spray and dried with oil-free air. The clean, dry cavity that had been obtained at this point was filled with glass ionomer in the same manner as the cavities in group 1.

Group 3

For the perforations in group 3, medical-grade calcium sulfate powder (MGCSH, USG, Chicago, Ill) was mixed with normal saline solution on a paper pad with a sterile cement spatula. The material was placed into the defects through the pulp chambers with a plastic instrument and condensed with a finger condenser. The dentin surface was prepared as in group 2, and glass ionomer cement was then applied over the calcium sulfate as in group 1.

The procedures were performed at 2.5× through use of magnification loops (Surgical Telescope, Designs For Vision Inc., Ronkonkoma, NY). All materials were manipulated according to manufacturer's instructions.

Table III. Percentages of periodontal ligament reorientation and epithelial proliferation for 1-month interval

Tissue reaction	*Glass ionomer (%)*	*Hapset (%)*	*Calcium sulfate (%)*
PDL reorientation	43	67	50
Presence of epithelium	43	33	25

There was no statistically significant difference between any groups.

Table IV. Percentages of periodontal ligament reorientation and epithelial proliferation for 3-month interval

Tissue reaction	*Glass ionomer (%)*	*Hapset (%)*	*Calcium sulfate (%)*
PDL reorientation	62	50	50
Presence of epithelium	25	12	25

There was no statistically significant difference between any groups.

Table V. Percentages of periodontal ligament reorientation and epithelial proliferation for 6-month interval

Tissue reaction	*Glass ionomer (%)*	*Hapset (%)*	*Calcium sulfate (%)*
PDL reorientation	57	57	75
Presence of epithelium	29	43	25

There was no statistically significant difference between any groups.

Table VI. Percentages of periodontal ligament reorientation and epithelial proliferation for combined intervals

Tissue reaction	*Glass ionomer (%)*	*Hapset (%)*	*Calcium sulfate (%)*
PDL reorientation	54	57	58
Presence of epithelium	32	29	25

There was no statistically significant difference between any groups.

Coronal preparations were filled with Copalite (Kerr, Romulus, Mich) and amalgam.

Group 4 (Control)

An additional 36 teeth were used as a control group. The second molar of each quadrant did not receive any preparation. Radiographs were made, the periodontal condition was evaluated and followed, and histologic examination was carried out.

After removal of the rubber dam, postoperative baseline radiographs were made. The dog was then given an intramuscular injection of butorphenol at 6 mg/kg for pain control, and a soft diet was provided for 3 to 4 days; this was followed by regular diet. Radiographs were made at monthly intervals through use of a specially designed standardizing tool. Periodontal evaluation was done at weekly intervals until the time when the dog was killed.

Clinical evaluation

Probing of the gingival sulcus was measured in millimeters, and the teeth were checked for periodontal pockets, mobility, draining sinus tracts, and any soft or hard tissue pathoses. At-sacrifice sulcus depths were analyzed by one-way analysis of variance at a level of significance of 95%.

Radiographic evaluation

Radiographs were evaluated blindly; those taken when the dogs were killed were compared with those taken preoperatively and at base-line (immediately after surgery). Radiographic manifestations of bone loss in the furcation, periapical periodontitis, widening of periodontal ligaments, or root resorption in at-sacrifice radiographs led to a judgment of failure; normal appearance of periodontium and radiographic manifestation of bone formation in the furcation areas in at-sacrifice radiographs led to a judgment of success. When it was not possible to judge the radiograph as success or failure, the classification was uncertain. Radiographic findings were statistically analyzed through use of the chi-squared test.

Histologic evaluation

Three dogs were killed at each of 3 intervals—1, 3, and 6 months—with a 150 mg/kg overdose of sodium pentobarbital (65 mg/mL). The jaws were removed, and the teeth with surrounding bone were block-sectioned and placed immediately in 10% neutral buffered formalin for tissue fixation. After 4 weeks, the blocks were rinsed for 12 hours under running water.

Specimens were placed in a solution of 20% sodium citrate and 50% formic acid for 6 weeks or until it was determined radiographically that they were adequately decalcified. Amalgam fillings were removed, and the sections were trimmed with a razor blade to eliminate extraneous tissues and then decalcified for 1 additional week. After dehydration and histologic processing, 6-μ–thick sections were cut in longitudinal sections parallel to the mesiodistal plane. Serial sections through the perforation were mounted on glass slides, deparaffinized, hydrated, and stained with hematoxylin and eosin. The stained histologic sections were blindly examined with light microscopy (10× and 40×) by a pathologist. Histologic scoring was based on the following criteria:

Bone apposition (scored from 0 to 3). 0 = no osteoblasts or osteoid; 1 = slight osteoblastic rimming with no osteoid; 2 = moderate osteoblastic rimming with some osteoid; 3 = heavy osteoblastic with abundant osteoid.

Bone resorption (scored from 0 to 3). 0 = no osteoclasts;

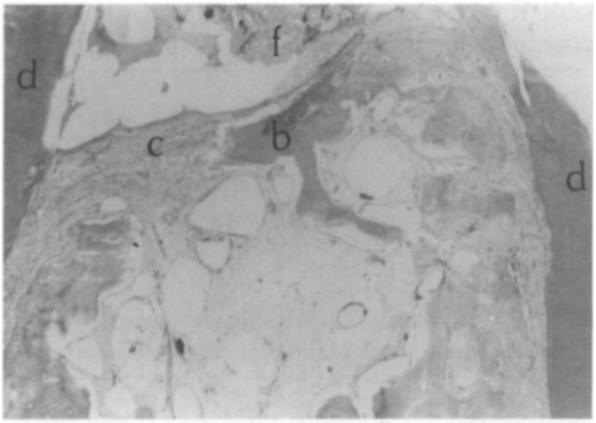

Fig 1. Features of healing as demonstrated by bone regeneration and reorientation of periodontal ligaments in furcation. There is dense connective tissue *(c)* in lesion above bone *(b)*; in dentin defect *(d)*, some filling materials *(f)* appeared (hematoxylin-eosin, original magnification ×10).

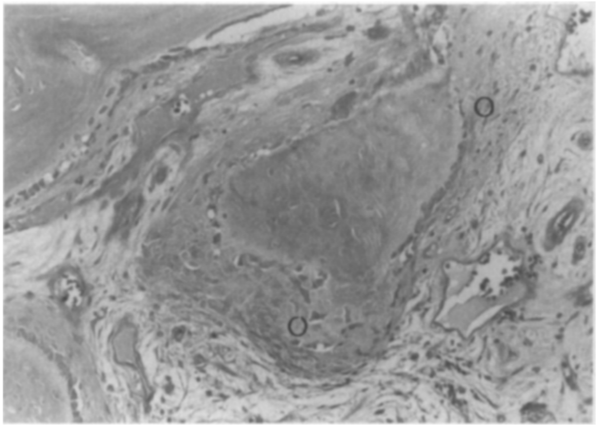

Fig 2. Osteogenesis with heavy osteoblastic rimming *(o)* is shown in perforation area (hematoxylin-eosin, original magnification ×10).

1 = few osteoclasts; 2 = moderate number of osteoclasts; 3 = many osteoclasts.

Inflammation (scored from 0 to 5). 0 = none; 1 = minimal; 2 = small focus, chiefly chronic, slightly intensive; 3 = acute or chronic infiltrated inflammatory cells of moderate intensity; 4 = acute or chronic inflammation of severe intensity; 5 = large area of acute or chronic inflammation, severe intensity or abscess formation.

Fibrosis (scored from 0 to 3). 0 = none; 1 = early granulation tissue with a small focus of delicate collagen fibers; 2 = mixture of delicate collagen fibers and dense collagen bundles; 3 = dense fibrosis with dense collagen bundles.

Regeneration of periodontal ligament. (+), Present; (–), absent.

Epithelium. (+), Present; (–), absent.

Four sections from each tooth were scored, and a mode score (most frequent) was obtained. Mode scores were tabulated and statistically analyzed. Scores for bone reaction, inflammation, and fibrosis were analyzed through use of Kruskal-Wallis analysis, whereas the chi-squared test was used to analyze data pertaining to epithelium and periodontal ligament. Treatment was considered histologically successful when healing reactions such as bone apposition and periodontal ligament reorientation occurred. Any section with epithelial proliferation, abscess formation, or advanced alveolar bone resorption was considered a failure.

RESULTS

The dogs suffered no ill effects from either anesthesia or the experimental procedures.

The control teeth (no treatment) showed no manifestation of gingivitis and no radiographic changes. Histologic examination revealed normal alveolar bone with evidence of osseous remodeling. Trabecular spaces were generally occupied with a normal fatty bone marrow and blood vessels. No inflammatory changes were seen within the periodontium, and periodontal ligament fibers were obliquely oriented.

The experimental groups clinically showed no manifestations of tooth mobility, draining sinus tracts, or swelling. However, there were various degrees of chronic gingivitis, as evidenced by red, inflamed gingival tissues and bleeding on probing. The increases in gingival sulci ranged from 1 to 4 mm, with only 1 tooth (lower premolar, calcium sulfate, 1 month) showing a 5-mm increase. Table I shows the means and standard deviations of sulcus depth for the experimental groups. Statistical analysis revealed no significant differences between the experimental groups with respect to sulcus depth at any time interval.

Radiographic evaluation at the 1-month interval revealed success in 8 teeth and failure in 13; results in 3 teeth were uncertain. Glass ionomer alone showed the highest success rate; this was followed by hydroxylapatite and plaster of paris. At the 3-month interval, radiographs of 5 teeth were excluded from the study because of damage to the films. Eight of 19 teeth manifested successful repair, whereas 7 failed and 4 were uncertain. Hydroxylapatite revealed the highest rate of success; this was followed by plaster of paris and glass

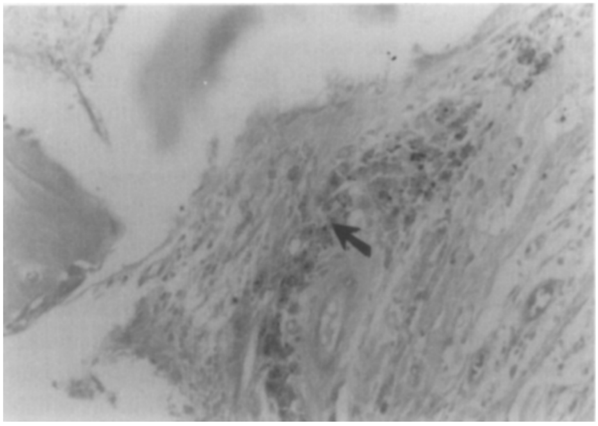

Fig 3. Macrophages *(arrow)* pigmented with plaster of paris are shown in perforation area after 1 month (hematoxylin-eosin, original magnification ×10).

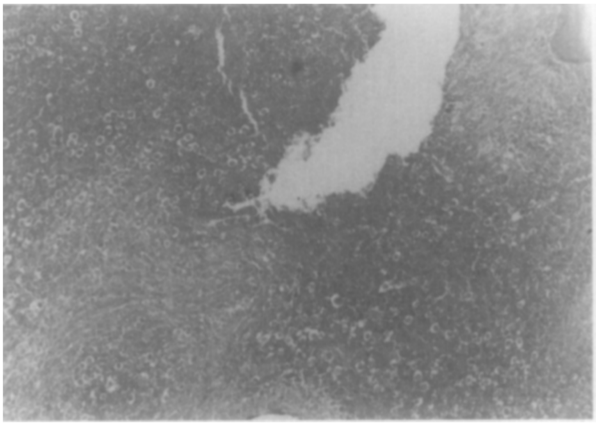

Fig 4. Abscess in furcation that appears as dense infiltration of chronic inflammatory cells into lesion is shown in perforation area (hematoxylin-eosin, original magnification ×10).

ionomer. At the 6-month interval, 13 teeth showed success manifestations, 4 were judged as failures, and 7 were uncertain. Table II presents the radiographic evaluations of the 67 teeth after the exclusion of 5 teeth at the 3-month interval. Statistical analysis revealed no significant differences between the experimental groups at any time interval.

Histologic scores for the tested materials at the 3 intervals were similar. Bone apposition scoring for all materials increased with time. Glass ionomer revealed the highest score at the 1-month interval; this was followed by plaster of paris and hydroxylapatite. However, hydroxylapatite showed the highest score at 3 months, when plaster of paris and glass ionomer were equal. Plaster of paris gave the highest score at the 6-month interval; this was followed by hydroxylapatite and glass ionomer.

Bone resorption in the hydroxylapatite group decreased with time, whereas glass ionomer and plaster of paris showed the highest score at the 3-month interval. Hydroxylapatite showed the lowest score of bone resorption at all time intervals; this was followed by glass ionomer and plaster of paris. At 3 months, glass ionomer showed bone resorption almost equal to that of plaster of paris.

Inflammatory reaction in the hydroxylapatite group decreased with time. The 3-month interval had the highest score for inflammatory reaction in the glass ionomer group and the plaster of paris group. After 1 month, glass ionomer revealed the lowest score of inflammation; this was followed by plaster of paris and hydroxylapatite (the latter 2 were equal at 3 months). At 6 months, hydroxylapatite showed the lowest score for inflammation; this was followed by plaster of paris and glass ionomer.

Scoring for fibrosis in the hydroxylapatite group decreased with time. Comparison of the scores at 1 month and 6 months revealed a decrease in fibrotic reaction toward glass ionomer and plaster of paris. Hydroxylapatite showed the lowest score at the 1-month and 3-month intervals; this was followed by glass ionomer and plaster of paris. At 6 months, the score for glass ionomer was slightly higher than that for plaster of paris.

Percentages of periodontal ligament reorientation and epithelial proliferation are presented in Tables III through V. Scores of tissue reactions to repair materials are presented in Table VI. The average success rates of the tested materials were 59% for glass ionomer, 62% for Hapset, and 67% for calcium sulfate. Statistical analysis revealed no significant differences between the test materials. Examples of tissue reactions are shown in Figs 1-5.

DISCUSSION

Various evaluation methods (clinical, radiographic, histologic) were used in this study to overcome the limitations of each method. If only one means of evaluation were used, the treatment under investigation could appear successful but in reality be undergoing a process of failure. One example of this would be the sulcus that clinically appears with normal probing depth; histologically, there could be a continuing disease that has not yet destroyed the attachment apparatus. Radiographs also have their limitations inasmuch as they are black and white 2-dimensional pictures.

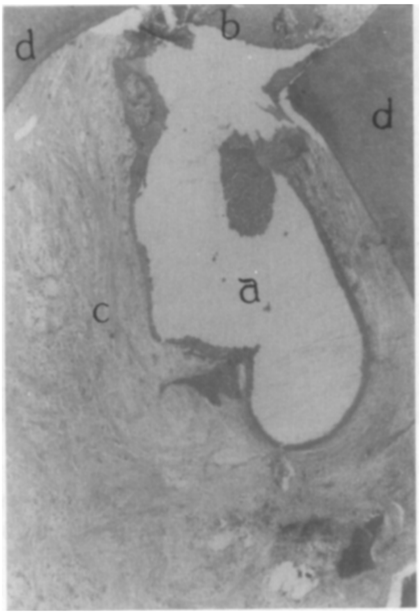

Fig 5. Cyst formation in wound site **(b)** that was surrounded by dense fibrous tissue. **a,** Cyst lumen; **b,** perforation; **c,** connective tissue; **d,** dentin (hematoxylin-eosin, original magnification ×10).

The radiolucency of furcation areas does not necessarily indicate a failure; it may be due to repair material being resorbed and replaced by mineralizing fibrous tissues. An example of a limitation of histologic evaluation is the scoring of bone reaction. In some cases, bone showed remodeling activity with an apparent balance between intensity of apposition and resorption. This balance could represent an ongoing pathologic process of degeneration (osteolysis) and regeneration (osteogenesis) or merely a physiologic process of remodeling. However, the overall scores for bone behavior might represent a trend of one process over the other.

Dogs were used for this study because their teeth have well-developed roots and suitable furcations that provide good accessibility and visibility. Their teeth are large enough to facilitate the study of tissue reaction and to allow ample room for perforating without hemisecting the tooth.[11] However, the morphologic character of the dog tooth is different from that of a human tooth. The posterior teeth of the dog are much narrower buccolingually than mesiodistally, have pointed long cusps, and lack a root trunk; this may allow the periodontal disease to progress faster in dogs than in humans.

In our study, standardized perforations were created at the middle of pulp chamber floors. These perforations were relatively larger in premolars than in molars in relation to tooth width. The study reflected the significance of this relative difference, as the molars showed a greater success rate. The perforation in the premolar may be closer to the gingival sulcus and increase the chance of communication with the oral cavity. This concept is in agreement with the findings of Himel et al,[12] who reported that the tendency toward furcation involvement varied inversely with tooth size when standard perforations were performed.

The timing of repair is an important variable in the treatment of furcation perforations.[13] To minimize this variable in our study, the defect was created and repaired at the same appointment. Accordingly, our results may not apply to the clinical situation in which treatment of the perforation is delayed.

Scattered foci of glass ionomer cement were seen within the periodontium of some teeth treated with glass ionomer alone. These foci showed no inflammatory reaction and seemed quite compatible with osteogenesis. This finding demonstrates the biocompatibility of glass ionomer and confirms the results of Callis and Santini.[14]

The bioceramic used in this study, Hapset, showed no infiltration into the periodontal lesion of any tooth after 1 month, which indicates that hydroxylapatite is a relatively nonbiodegradable material; this supports other findings.[15-17] However, Hapset is a composite of hydroxylapatite and calcium sulfate. This may explain the presence of small foci of the material in the periodontal lesion surrounded by macrophages at 3 and 6 months. This also agrees with the findings of Holmes,[18] who reported significant solubility in a bioceramic composite material with a high hydroxylapatite content.

All specimens repaired with calcium sulfate showed deposition of the material into the lesion at 1 month, and no plaster was seen within the lesions at 6 months. This indicates that calcium sulfate had a high degree of solubility and confirms the results of Radentz and Colling,[10] who found that plaster resorption began early after its implantation into the alveolar bone. Bell[19] reported that the average resorption rate for plaster of paris was 4.7 weeks after implantation. However, the present study revealed some granules of the filling material in the calcium sulfate group at 3 months. These granules might represent foci of the overlying glass ionomer degraded after complete resorption of the plaster.

There was no significant difference in tissue reaction to resin-modified glass ionomer whether it was used alone or placed over an artificial floor. This may be due to the flowing ability of resin-modified glass ionomer that allowed adequate seal of the perforation without extension into the periodontal space. The importance of the perforation seal has been emphasized by El Deeb et al[2] and Balla et al,[4] who concluded that failure of the furcation perforation repair was attributable to the inadequate sealing ability of the repair material. This also confirms the results of previous in vitro studies[9,20] that compared the sealing ability of light-cured glass ionomer with that of different materials.

The success rate obtained collectively in our study for different materials and techniques was 50% radiographically, 64% clinically, and 63% histologically, with an overall average of 59% for the 3 evaluation methods. This success rate indicates that perforation of the pulp chamber floor has an equivocal prognosis, and it supports the consensus concerning this type of perforation. However, Pitt Ford et al[21] reported a success rate of 83% using mineral trioxide aggregate for repair of furcal perforation; the authors related this high success rate to the good sealing ability of mineral trioxide aggregate, to a hard-set that provides a solid barrier against which tissue can organize, and to the bactericidal effect of the material that results from its high pH. Further studies of repair materials and techniques are recommended so that a more favorable prognosis for perforation of the pulp chamber floors may be achieved.

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