Effects of Fibrin Adhesive Material (Tissucol) Application on Furcation Defects in Dogs

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Abstract

A histological study was conducted to evaluate the effects of fibrin adhesive material (FAM) application on periodontal healing in seven experimental dogs. Bilateral class III furcation defects were surgically created at the second and third premolars, then orthodontic wires were placed around the teeth to induce periodontitis. Six weeks later, the wires were removed and the defects were treated by either surgery alone or surgery plus FAM application. A total of 21 specimens were obtained for histological and histometrical analysis on days 7, 21 and 42.

The Mann-Whitney U test showed significantly more new attachment and bone regeneration in the FAM-treated group compared to the control (p<0.05).

Surgery plus FAM application in the treatment of class III furcation defects seemed to be effective in promoting connective tissue attachment and bone regeneration.

Introduction

Traditional periodontal therapy results in only minimal regeneration of the periodontium in the most apical portion of the defect. The downgrowth of epithelial cells into the healing wound forms a long junctional epithelium preventing regeneration. The results of some experimental studies suggest that apical migration of the epithelium may not occur or may be limited under certain conditions¹⁻⁴.

The first requirement for successful regeneration may rest with clot adhesion to the root surface, possibly by virtue of a fibrin linkage⁵. The rapid adhesion of blood clots to the root surface may thus form a sufficient barrier to epithelial apical migration⁵⁻⁶.

Application of a commercially available adhesive protein concentrate to a planed root surface may facilitate early adhesion of a fibrin clot⁷⁻⁸. Moreover, regeneration of fiber attachment and alveolar bone in fenestration defects appears to be enhanced by this material, which contains various plasma factors and highly

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concentrated attachment glycoproteins.[9]

The purpose of this study was to evaluate the effect of fibrin adhesive material (FAM)(TISSUCOL KIT 0.5 ml, IMMUNO AG, VIENNA, AUSTRIA) on periodontal wound healing in furcation defects.

**Materials and Methods**

Seven adult experimental dogs in clinically healthy condition were used. During the operative procedures, the dogs were sedated with intravenously injected pentothal sodium. After clinical and radiographical examinations, mucoperiosteal flaps were raised on the buccal and lingual surfaces of the 2nd and 3rd mandibular premolar teeth on both sides. Furcation defects were surgically created using a slow-speed round burr and continuous irrigation with saline. Then orthodontic wires were placed around the teeth to induce periodontal tissue breakdown around the furcation areas during a period of six weeks. At the end of this period the orthodontic wires were removed and radiographs of the related teeth were taken.

After planing of the root surfaces to remove remnants of periodontal ligament and cementum, reference notches were prepared at the level of the bone margin. A total of twenty-one defects were treated by either periodontal surgery alone or application of commercially available fibrin adhesive material to the curetted root surfaces and mucoperiosteal flaps following surgery. The flaps were then returned to their original position and sutured interproximally.

Plaque control was accomplished by topical application of 0.2% chlorhexidine gluconate solution until the end of the experiment. The dogs were sacrificed on days 7, 21 and 42 with an intravenous overdose of pentothal sodium. Block biopsies were taken and fixed in formalin for seven days.

Following decalcification in formic acid-sodium citrate solution, the biopsy samples were dehydrated and embedded in paraffin.

Step-serial mesio-distal sections were cut at 6 μm thickness, parallel to the long axis of each root. The sections were stained with hematoxylin and eosin and Crossmon’s modified connective tissue stain.

Histometrical analysis was performed on each specimen block using a calibrated grid mounted in a light microscope (OLYMPUS) at x10 magnification, and the following linear distances were measured: a) from the apical border of the notch to the apical extent of the junctional epithelium, b) from the apical border of the notch to the crest of the alveolar bone.

The Mann-Whitney U test was used for histometrical analysis, and differences were considered significant at p < 0.05.

**Results**

**Histological Observations**

*Seven days after surgery.* The granulation tissue adjacent to the experimental root surface appeared to consist of mononuclear cells with occasional polymorphonuclear leucocytes, as well as numerous capillaries and strands of fibrin. Some localized root resorption activity in the notch areas and newly deposited fine
collagen fibers and osteoclastic activity in the periodontal space were also observed. In addition to these common findings, clot organization and maturation of newly deposited collagen fibers were relatively pronounced in the FAM-treated group (group F) (Fig. 1a) than that in the control group (group C) (Fig. 1b). The young connective tissue filling the defect area was in contact with the root surface. Epithelization was newly initiated in some of the specimens.

Twenty-one days after surgery. In the specimens of group F, more new attachment formation was observed in the supracrestal area, where a new layer of cementum extending coronally showed cellular connective tissue with insertion of fibers to the root surface (p<0.05)(Table 1)(Fig. 2a,b). Although a substantial amount of connective tissue attachment had formed in the notch, coronal to this region apical migration of the epithelium was commonly seen in group C (Fig. 3a,b). In many of the specimens in both groups, infiltration of mononuclear cells and reduced edema were still present. A small amount of bone regeneration was also observed in both groups, remaining around the notch region.

Forty-two days after surgery. The sections from both groups displayed further connective tissue maturation. The attachment of connective tissue to the root surface and formation of new alveolar bone were enhanced in group F (Fig. 4a, b) compared with group C (Fig.5a,b)(p<0.05)(Table 1). Formation of new alveolar bone in both groups had increased along the root surfaces rather than in the mid-portion of the furcation defects.

New cementum of varying thickness was clearly observed with artifactual splitting from the dentin surface. Collagen fibers were functionally oriented and inserted into the cementum. Forty-two-day specimens from group F demonstrated significantly more connective tissue and bone repair compared with 21-day specimens(p<0.05).

Table 1 Results of histometrical measurement and levels of significance

<table>
<thead>
<tr>
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<th>21 DAYS</th>
<th>42 DAYS</th>
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<tr>
<td></td>
<td>FAM</td>
<td>CONTROL</td>
</tr>
<tr>
<td></td>
<td>(x, Median)</td>
<td>(x, Median)</td>
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<tr>
<td>Attachment Bone</td>
<td>18.6</td>
<td>12.5</td>
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<td>Bone</td>
<td>6</td>
<td>4.9</td>
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Discussion
The present study demonstrated significant histometrical differences in the regeneration of connective tissue attachment and bone in periodontal furcation defects following periodontal flap surgery including topical application of fibrin adhesive material. For the purpose of the study, furcation defects were surgically created in the mandibular premolars of dogs and exposed to the oral environment...
for six weeks without plaque control. Healing was evaluated at 7, 21 and 42 days after surgery. A significantly increased amount of connective tissue attachment to the root surface treated with FAM compared with roots treated by surgery alone may have been due to the early adhesion of protein concentrate to the root surface. Thus, FAM might prevent the apical migration of epithelium at the root-soft tissue interface and maintain a tractional field for migrating fibroblasts\cite{10,11}. The present results are in agreement with the data obtained from previous studies\cite{7,9,12,13}.

However, it has also been reported that the use of biologically active materials and attachment glycoproteins did not enhance connective tissue attachment formation and might have exerted an inhibitory rather than an enhancing effect on fibroblast migration and connective tissue attachment\cite{14}. In the present study, limited root resorption areas were observed in day-21 specimens, although the root resorption areas were replaced with new cementum in day-42 specimens.

The treatment of bone defects with FAM is usually followed by enhanced regrowth of new bone\cite{15-17}. This was consistent with our study. Recently, it was reported that the use of FAM in a flap model with reduced alveolar bone and fenestration defects effectively induced bone regeneration\cite{7,9}. The biological properties of fibrinogen, fibronectin, thrombin and factor XIII may have contributed to the regenerative process, facilitating the new attachment and bone formation. It is postulated that any of the components present in FAM acting as alone or in combination may account for the favorable results\cite{7,9,12,13}.

Further experimentation is needed to improve the adhesion and strength of FAM on the root surface and to clarify the effects of individual components of FAM.

References


Fig. 1  a. Photomicrograph of a fibrin adhesive material (FAM)-treated specimen at 7 days
    b. Photomicrograph of a control specimen at 7 days D, dentin; C, cementum; B, bone;
       GT, granulation tissue; A, artifactual space (connective tissue stain-CTS x10)

Fig. 2  a. Photomicrograph showing a 21-day specimen treated with FAM (CTS x4)
    b. Higher-magnification view of Fig. 2, a. (CTS x20) D, dentin; C, new cementum; B, bone;
       E, epithelium; CT, connective tissue; A, artifactual space
Fig. 3  a. Photomicrograph of control specimen at 21 days (CTS x4)
b. Higher-magnification view of Fig. 3, a (CTS x10) D, dentin; C, cementum; B, bone; CT, connective tissue; E, epithelium; A, artifactual space; N, apical border of the notch

Fig. 4  a. Photomicrograph showing 42-day specimen treated with FAM (CTS x4)
b. Higher-magnification view of the notch area (CTS x20) D, dentin; C, new cementum; B, bone; E, epithelium; CT, connective tissue; A, artifactual space; N, apical border of the notch
Fig. 5  
a. Photomicrograph of control specimen at 42 days (CTS x4)  
b. Higher-magnification view of the notch area (CTS x10)  
D, dentin; C, new cementum; B, bone; E, epithelium; CT, connective tissue; A, artifactual space; N, apical border of the notch