Gengiflex, an Alkali-Cellulose Membrane for GTR: Histologic Observations

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Class II furcation lesions in dogs with naturally occurring periodontitis were treated using a new non-resorbable cellulose membrane (Gengiflex, Biofill Produtos Biotecnológicos, Curitiba, PR, Brazil) for the guided tissue regeneration technique. The animals were sacrificed either at the time of removal of the membrane (4 weeks) or after 8 weeks healing (4 weeks after the removal of the membrane). While the healing pattern on the control side at 4-8 weeks was connective tissue isolated from the roots by epithelium, there was the beginning of formation of new cementum on the experimental side with the insertion of new fibers (4 weeks) and filling of the furca with bone and formation of a new ligament (8 weeks). This report shows histologically that GTR with the cellulose membrane can promote successful regeneration of Class II furcations.

Key Words: guided tissue regeneration, cellulose membrane, Gengiflex.

Introduction

Regeneration of periodontal tissues destroyed by periodontal disease through guided tissue regeneration technique (GTR) is now undisputable (Nyman et al., 1982; Gottlow et al., 1984; Becker et al., 1987, 1988). Among the lesions predictably treated by GTR are Class II furcation lesions (Gottlow et al., 1984; Becker et al., 1987), two- to three-wall infrabony defects (Becker et al., 1988), extensive defects combined or not with other techniques (McClain and Schallhorn, 1993), bone dehiscence (McGuire, 1992) and lesions associated with implants (Dahlin et al., 1989; Lazara, 1989; Becker et al., 1990). The objective of this study is to evaluate the possibility of regeneration of Class II furcation lesions in dogs, with naturally occurring periodontal disease, with a new membrane (Gengiflex, BioFill Produtos Biotecnológicos, Curitiba, PR, Brazil). This membrane has
been extensively tested for biocompatibility (Novaes Jr. and Novaes, 1992, 1993; Novaes Jr. et al., 1992), and has been shown adequate for GTR in periodontal defects in humans (Novaes Jr. et al., 1990) as well as in GTR for bone formation in association with osseointegrated implants (Novaes Jr. and Novaes, 1992, 1993). In an in \textit{vitro} study (Salata et al., 1993), osteoblasts colonized the surface of the membrane producing collagenous matrix resembling bone tissue. This membrane could be a suitable alternative for GTR.

\textbf{Material and Methods}

The cellulose membrane Gengiflex used in this study is synthesized and extruded by bacteria through a low-cost biotechnological process. This membrane was developed from a product used as artificial skin for burns and tissue loss (Biofill, Produtos Biotecnológicos, Curitiba, PR, Brazil). Several modifications were made to create a membrane with physical qualities appropriate for use in periodontal GTR surgery. It is comprised of two layers, an internal layer which is a network of crystalline cellulose microfibrils randomly extruded by bacteria giving body and rigidity to the membrane and an external alkali-cellulose layer, which is, in reality, a chemical modification of the internal cellulose layer. It is non-resorbable, inert, biocompatible, and has the physical qualities necessary for GTR (Novaes Jr. et al., 1992). It has been tested by Federal Institutions and has been liberated for medical use, being commercially available. (see annex).

Two adult male mongrel dogs (approximately 10 kg) with naturally occurring periodontitis were selected from the Department of Experimental Surgery, Faculty of Medicine of Ribeirão Preto, University of São Paulo.

The animals had intact crowns without gross occlusal alterations, did not have oral lesions or fungus infection and were in good general health.

The animals were not fed the night before surgery and were anesthetized with sodium nembutal (30 mg/kg, 500 mg nembutal diluted in 20 ml NaCl, resulting in a 25% solution). This slow endovenous infusion was applied through the cephalic vein using a 25 x 8 cm needle. The animals were maintained with spontaneous respiration, intubated with a 7.5 or 8.65 endotracheal tube.

The third and fourth mandibular premolars from both sides were chosen because they presented natural Class II furcation lesions, which were identified clinically and radiographically. A loss of bony tissue at the region of the bifurcations was evident.

Supra- and subgingival scaling were performed on all of the teeth using Gracey curettes (5/6, 11/12, 13/14) and an ultrasonic instrument (Cavitron, Dentsply International). Polishing with rubber cups was then performed.

One week later, the animals were again anesthetized for surgery. To simulate usual clinical practice, local infiltrating anesthesia was also used. Surgery was performed according to protocol for GTR and scaling and root planing were repeated in association with root surface treatment with 12-sided finishing burs (Brasseler, USA).

The only difference between the control and experimental sides was the use of the cellulose membrane with wide or narrow furca configuration on the experimental side.
According to the protocol recommended by the manufacturer, the full-thickness flap was sutured leaving the coronal margin of the membrane exposed for a more secure isolation of the gingival epithelium from the concavities present in the root trunk, the importance of which was recently emphasized by Lu (1992). Penicillin (500,000 IU every 24 hours) was administered intramuscularly for 3 days. Flap sutures were removed after one week when superficial cleansing with hydrogen peroxide was performed.

Twenty-eight days post-surgically, the animals were anesthetized for membrane removal from the experimental sides. One of the dogs was sacrificed at this time. The other dog was allowed to heal for another 28 days and then sacrificed (8 weeks post-surgically).

The mandibles were dissected, fixed in 10% buffered formalin, and decalcified in multiple baths of 45% formic acid and an equal part of 20% sodium citrate. After decalcification, blocks containing the experimental teeth were dehydrated and embedded in paraffin. Six μm thick sections were obtained in mesio-distal planes to provide an integral view of the furcation and stained with hematoxylin and eosin.

Results

Four weeks, Control - A general view of the furcation area showed that it was filled with connective tissue infiltrated by inflammatory cells and presenting epithelial projections in the transverse sections. All of this tissue was covered by epithelium which was interposed between the connective tissue and the dental surface. Due to tissue contraction during histological processing, the epithelium separated from the dental surface, giving more emphasis to the fact that a connective tissue-牙 union did not exist, having been impeded by the previous formation of epithelial tissue (Figure 1.1). Bone formation was not observed.

Greater magnification showed a long junctional epithelium which is characteristic of the type of healing seen for the control side (Figure 1.2).

Four weeks, Experimental - Granulation tissue with newly formed collagen fibers filled the entire furcation area and was in contact with the dental surface (Figure 1.3). The alignment of young fibers, fibroblasts and/or cementoblasts along the root surface showed a healing pattern different from the control side and in accordance with that suggested by GTR (Figures 1.4 and 1.5). Despite being apparently in the same position as it was pre-operatively, the bone margin presented intense osteoblastic activity which suggests a probable evolution to bone formation during longer healing periods, a fact not observed in the control side.

Eight weeks, Control - The histological characteristics after 8 weeks of healing were not altered. The furcation area was filled with more dense, less inflamed connective tissue, but was still covered by epithelial tissue which was in contact with the root surface (Figure 1.6). Bone formation still could not be observed.

Eight weeks, Experimental - The difference between this healing period and the 4 week specimen was remarkable. The furcation area was completely filled by newly formed bone tissue (Figure 1.7), in contrast with the control side.
The bone formation was complemented by the formation of a new periodontal ligament, seen both at the top of the furcation and laterally to the roots (Figure 1.8). New cementum formation occurred along the root surface, not only over old cementum not removed by root planing but also over dentin. In some areas, artifacts showed a separation between the new and old cementum (Figures 1.8 and 1.9), which emphasizes the presence of new cementum even more clearly, a fundamental fact of true regeneration.

Discussion

Results of this study are in agreement with previous studies (Gottlow et al., 1984, 1986; Becker et al., 1988; Niederman et al., 1989; Lekovic et al., 1989; Pontoreiro et al., 1989) which showed histologically that periodontal lesions can be regenerated when the principles of guided tissue regeneration are correctly employed. This is achieved by the placement of a barrier membrane which impedes the gingival connective tissue and epithelial cells from reaching the biocompatible root surface and at the same time maintaining a space for the coronal migration of periodontal ligament and alveolar bone cells.

Dogs with naturally occurring Class II furcation lesions were used in this study. In one animal the Gengiflex membrane was removed at 4 weeks and the animal was sacrificed. In the other, the membrane was removed at 4 weeks and the dog was sacrificed 4 weeks later. In other studies cited above, the membranes were removed following periods that varied from 4 weeks to 12 weeks but the animals were sacrificed after at least 3 months of healing. This study allowed us to document the characteristics of the healing tissue at an earlier period.

Figure 1 - 1.1, Four-week control specimen. Furca occupied by epithelialized connective tissue (CT); space due to tissue contraction (S); epithelium isolates connective tissue from dental surface (arrows) (original magnification X 8; H&E). 1.2, High magnification of 1.1 identifying apical aspect of long junctional epithelium (arrows); dentin (D); connective tissue (CT) (original magnification X 32; H&E). 1.3, Four-week experimental specimen. Furcation area filled with granulation tissue, and young connective tissue (CT) which is in contact with the dental surface (D) up to the top of the furca (original magnification X 8; H&E). 1.4, Close-up view of 1.3 (at arrow); young fibers (f) organizing in vertical orientation to dental surface (D). 1.5, Close-up view of 1.3 (between open arrows); newly formed connective tissue with young fibers pulling pre-cementum out (arrows) from dental surface (D) (original magnification X 32; H&E). 1.6, Eight-week control specimen. Similar type of repair as Figure 1.1: epithelialized connective tissue (CT); bone in previous position (B); space due to tissue contraction (S) (original magnification X 8; H&E). 1.7, Eight-week experimental specimen. Regeneration of the furcation area (compare to Figure 1.1 and 1.6); newly formed bone (B) can be seen up to the top of the furca; accidental fold of the section (arrow) (original magnification X 8; H&E). 1.8, Newly formed periodontal ligament (L); newly formed bone (B); newly formed cementum (arrow) (original magnification X 32; H&E). 1.9, High magnification of newly formed periodontal ligament (L) where newly formed cementum (arrow) is emphasized by its separation from old cementum (C). Cementoblasts and cementocytes are shown (original magnification X 64; H&E).
In the 4-week specimen, in the furcation lesion that remained as control, a long junctional epithelium was observed and no significant regeneration was detected. These findings are also in agreement with previously cited studies (Gottlow et al., 1984; 1986; Becker et al., 1988; Niederman et al., 1989; Lekovic et al., 1989; Pontoreiro et al., 1989). The experimental lesion for the same time interval demonstrated a connective tissue adaptation to the scaled root surface (dentin or old cementum). Newly formed collagen fibers ran parallel to the root surface with a few fibers already showing a perpendicular orientation. No cementum or bone formation could be seen at this point although an increase in osteoblast number could be clearly seen. Caton et al. (1992) showed some cementum and bone formation especially close to the apical level of root planing. However, they used another animal model system, the defects were surgically created and they did not deal with furcation lesions. With so many differences in methodology, a comparison should be avoided.

After 8 weeks of healing, the control lesion showed no improvement; on the contrary, recession of the gingival tissue occurred and the furcation lesion was open to the oral environment. The type of healing continued to present long junctional epithelium. For the experimental side, complete regeneration could be seen. There was bone formation up to the root of the furcation lesion and a functional periodontal ligament existed between the new bone and the newly formed cementum. The collagen fibers were functionally oriented and inserted into the new cementum.

Measurement of the depth of the defects and histometric data with statistical analysis were beyond the scope of this study.

Within the limitations of this descriptive report, we can conclude that the Gengiflex membrane, left in place for 4 weeks, can lead to regeneration of Class II furcation lesions in naturally occurring periodontitis in dogs. A previous paper (Novaes Jr. et al., 1990) reported Class II furcation lesions in humans treated by GTR employing the cellulose membrane, which led to closure of the defect. This closure was shown through clinical examination of attachment levels, radiographs and a reentry procedure.

References


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Annex

The following tests have been performed: protein content 0.19%; nontoxic in implantation tests in rabbits; no animals affected in systemic infection tests in rats; no tissue reaction in intracutaneous test on rabbits; nonirritant in cutaneous irritability test in guinea pigs; nonirritant in ocular irritability test in rabbits; noncytotoxic in cytotoxicity test in vitro with red blood cells, IAL (fibroblast cell lines from rabbit kidney) and HeLa cells (Novaes Jr. et al., 1992).

The above tests were first carried out on the original membrane (BioFill). The results mentioned above refer to Gengiflex, which included a considerable reduction of proteins from 0.5% to 0.19%.

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