Guided tissue regeneration (GTR) versus cementum-impregnated gelatine membrane (CGM) techniques: A histologic comparison of relative effectiveness in promoting periodontal attachment

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Some prior reports have suggested that guided tissue regeneration (GTR) procedures achieve only partial regeneration and induces the ankylosis rather than true attachment. Accordingly, others have developed an alternative procedure employing gelatine membrane compounded with bovine cementum particles (CGM) which has proven effective in stimulating a more physiologic form of attachment. This study was undertaken to perform a direct comparison of histological results when CGM and GTR membrane were used at comparable sites in the same monkey. Three monkeys with no periodontal disease were used. Following flap surgery, recession type defects were created on the buccal side of the maxillary lateral incisors and second premolars, and the cementum was removed from the root surface at an area corresponding to the bone crest. The right and left lateral incisors and second premolars were covered with CGM and GTR membrane, respectively. The GTR membranes were removed after 4 weeks. At 6 wks, the animals were sacrificed, and specimens were prepared for histological examination. More coronally placed true new attachment was observed following application of CGM to the planed root surfaces. Application of the GTR membrane resulted in formation of bone-like cementum and ankylosis, whereas CGM established true periodontal regeneration. (J Osaka Dent Univ 1997; 31:11-17)

Key words: cementum-impregnated gelatine membrane; GTR membrane; new attachment formation

INTRODUCTION

The ultimate goal of periodontal therapy is complete regeneration of periodontal tissue lost due to periodontitis. Unfortunately, conventional periodontal surgery does not have the capability to achieve this result. Recent research, however, yields reason for optimism in that "progenitor cells" responsible for the formation of new cementum containing insertion fibers, have been demonstrating within the remaining apical portion of the healthy periodontal ligament (PDL). This hypothesis is supported by animal studies in which periodontal wound healing has been evaluated histologically in the presence of a physical barrier which favors population of the instrumented root surfaces by cells from the PDL.

The latter concept of "guided tissue regeneration (GTR)", aimed at formation of new attachment, has recently been called into question for two main reasons: both the bone and PDL tissue compartments act as a source of cells to the periodontal wound, and bone cells likely migrate to the root surface more rapidly than cells of PDL. Moreover, the cells populated on the root surface are basically pluripotent. That is, their ultimate cell type is regulated by local modulating factors rather than the membrane barrier itself. In fact, some reports suggest that the GTR procedure provides only partial regeneration with induction of ankylosis rather than true attachment.
Recently, a new method has been developed featuring cementum particles-impregnated gelatine membrane (CGM) which effectively inducing true periodontal attachment on previously denuded root surfaces. \(^1\) Clearly, cementum or components such play a crucial role in cell migration, cell attachment, and cell differentiation into cementoblasts in that application of CGM to curetted root surface results in formation of a distinct layer of cementum exhibiting insertion fibers along the entire root surface. This study represents an extension of Nishimura’s work \(^1\) and was undertaken to histologically examine the effectiveness of CGM in comparison with GTR membrane at comparable sites using the same experimental model.

**MATERIALS AND METHODS**

*Preparation of cementum-impregnated gelatine membrane and guided tissue regeneration membrane*

Gelatine membranes (GM) were prepared as follows: Commercial gelatine [viscosity 28 mP and jelly strength 96 g (W. Bloom) in 6.66% aqueous solution] (Nippi Inc., Tokyo, Japan) was purchased, and 2 ml of aqueous gelatine solution (5%) containing 0.15% of glycerol polyglycidyl ether (Denacol Ex-313; Nagase Chemical, Ltd., Tokyo, Japan) was cast in a 64 cm\(^2\) area of 2 mm thickness polymethyl methacrylate mold (Sumitomo Chemical Co., Ltd., Tokyo, Japan). Subsequently, aqueous gelatine was cooled to form a gel, frozen on a cold plate at -70°C and lyophilized.

The lyophilized product was heat-treated for 2 h at 110°C to accelerate formation of crosslinks and relyophilized after washing with 50°C water. Prepared GM consisting of a thin film of porous, bilayer sheets with an exposed outer side and a smooth inner surface. Numerous pores approximately 40 nm in size were placed on the exterior membrane surface to facilitate cell migration following surgery. The inner surface was left smooth to permit close adaptation to the roots.

Cementum particles were prepared as follows: root surfaces of bovine teeth were curetted carefully (approximately 20 strokes) to obtain samples of superficial cementum. Resultant fragments were subsequently reduced to small particles by mortar and pestle. Ground particles of bovine cementum were then compounded into the gel on the open pore side by pouring on an aqueous suspension followed by freeze drying (Fig. 1). Membranes were sterilized by ultraviolet radiation for 2 h immediately before experimental use.

Expanded polytetrafluoroethylene (e-PTFE) membrane, GTR membranes were prepared by W. L. Gore, Inc. Briefly, PTFE-fine powder of extremely fine grains (including formation assistance liquid)
were passed between high compression dies. At that time, fibrils were caught between fine powder and grains because of cutting force. The formation assistance liquid was then removed, and pure PTFE was heated and extended in one or many axis directions.

**Surgical procedure**

Three healthy adult monkeys of both sexes (*Macaca Fuscata*), having full dentitions and weighing approximately 12 kg, were used in this study. Scaling was performed 1 month prior to the experiment as a component of preoperative treatment. All scaling procedures were performed under anesthesia induced by an i.m. injection of Ketalar 50® for animal use (Warner-Lambert Co., Ltd., USA) at a dose of 0.1-0.2 ml/kg. Afterwards, under the same anesthetic conditions, the teeth were brushed with topical application of 0.1% chlorhexidine gluconate solution three times a week. All monkeys exhibited a healthy gingival condition at the end of this 1-month period.

The maxillary lateral incisors and second premolars were selected as the experimental teeth. Under general anesthesia (induced by i.m. injection of Ketalar 50® at a dose of 0.1-0.2 ml/kg and i.p. injection of Somnopentyl®[Pitman-Moore Co., Ltd., USA] at a dose of 0.5 ml/kg), buccal gingival flaps were elevated, and recession type defects were created on the buccal sides of experimental teeth. The exposed root surface was curetted and planed in order to remove cementum corresponding to the bone crest. CGM and GTR membranes were applied on the right and left maxillary lateral incisors and second premolars, respectively (Fig. 2). Flaps were repositioned and sutured with 3-0 silk suture. GTR membranes were removed after 4 wks. Post-operative plaque control was carried out using topical application of 0.2% chlorhexidine in addition to tooth brushing daily. After 6 wks, the monkeys were sacrificed.

**Histologic procedure**

Each experimental specimen consisted of an block section of the tooth, gingiva and bone. Excision was carried out following perfusion-fixation with 2.5% glutaraldehyde-2.0% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). Blocks were then refixed in 2.5% glutaraldehyde-2.0% paraformaldehyde in the same buffer for 48 h. Each block was subsequently trimmed to as small as possible and then decalcified in 5% formic acid. Decalcification was followed by dehydration in a series of alcohols, paraffin embedding and cutting of serial sections 6 μm in thickness in the buccal-palatal direction. The sections were then stained with hematoxylin and eosin for examination with a light microscope.

**RESULTS**

Healing following surgery was clinically uneventful. There were no visible signs of soft tissue inflammation associated with the presence of membranes of either type. Gingival tissues to which membranes of both types were applied appeared firm and pink and showed no recession.

Both types of experimental membranes were effective in completely preventing epithelial down-growth. Root surface application of CGM produced new connective tissue attachment. The formation of
new cementum was seen over the entire planed root surface. In addition, considerable regeneration of alveolar bone occurred (Fig. 3), and new cementum appeared to closely resemble original cementum. New collagen fibers, functionally oriented to the new cementum, were apparent. New cementum was uniformly thin and predominantly acellular. However, occasional embedded cementocyte-like cells were noted to be present although somewhat smaller than the cells observed in the new cementum in the GTR specimens (Fig. 3-1, 3-2 and 3-3). An apparently physiologic periodontal ligament space was maintained between new cementum and new bone (Fig. 3-3).

In the majority of specimens receiving the GTR membrane, predominant finding was that of post-

Fig. 3 Formation of new cementum is observed over the entire planed root surface following CGM apposition (×22). Note that apparently physiologic periodontal ligament space is maintained between the new cementum and new bone (1, 2, 3, ×88).

Fig. 4 Following application of GTR membrane, new cementum and new bone formation is noted similar to that seen in the CGM specimen. However, the new "cementum" bears a strong resemblance to bone (×22). Furthermore, ankylosis is observed in the apical third of the lesion, and surface resorption is also noted along the entire root surface. (1, 2, 3, ×88).
surgical bone formation (Fig. 4). However, this active bone formation apparently represented ankylosis fusion between native bone and the root surface. Bone was seen outlining the denuded root surfaces with bony bridges connecting tooth roots to the adjacent alveolar bone in the central portions of the defects (Fig. 4-2). Surface resorption was also observed along the entire root surface. New cementum on root surfaces were very thick and contained numerous osteocyte-like cells. Newly elaborated tissue did not show characteristic morphology of normal cementum but rather exhibited structural features strongly resembling those of bone. Connective tissue fibers adjacent to the bone-like cementum appeared to be oriented parallel to the root surfaces (Fig. 4-1 and 4-3).

In one of the monkeys, the CGM-treated specimens appeared to induce formation of new cemen-

![Fig. 5](image1)  
*Fig. 5* CGM specimens in another monkey show the formation of thin layer of new cementum over the entire planed root surfaces (1, 2, 3, ×88), but there is scant new bone formation (×22).

![Fig. 6](image2)  
*Fig. 6* Specimens to which GTR membrane is applied in another monkey show no new cementum on the planed root surface (×22). However, there is slightly more new bone formation than in the specimen to which CGM is apposed. Root surface resorption is observed (1, 2, 3, ×88).
turn over the entire planed root surface, but there was scant new bone formation (Fig. 5-1, 5-2 and 5-3).

In another monkey, a specimen treated with GTR membrane appeared to stimulate connective tissue healing along the planed root surface, but no new cementum was observed. In addition, there was only a small amount of new bone formation. Root surface resorption was also noted (Fig. 6-1, 6-2 and 6-3).

**DISCUSSION**

In this experiment we evaluated histologically whether or not the procedure utilizing CGM is comparable to application of GTR membrane in terms of promoting periodontal re-attachment. Findings indicate that the apposition of CGM on the curetted root surface induces new attachment with formation of true cementum. Thus, present findings are in accord with those of Nishimura et al.13 and support the theoretical concept of using the CGM modified composite membrane to induce differentiation of root surface mesenchymal cells into cementoblasts.

In the present study, findings suggest that is true cementum, not bone-like cementum, that forms on the curetted root surface when the CGM was used. Clearly, cementum contains several potent biologic substances important to cell migration, cell attachment and cell differentiation.14-21 In fact, a number of cementum-specific proteins have recently identified.21,22 It is probable that these proteins are capable of sending a cementum-specific signal to uncommitted cells that stimulate them to differentiate into functional cementoblasts.

Another noteworthy result of this study was that cementum components, including cementum proteins and cementum collagen, are likely to modulate cementoblasts, osteoblasts, and other periodontal cells simultaneously to orchestrate the formation of new cementum, new periodontal ligament and new bone. These substances have the property of inhibiting bone cell migration towards the root surface. As a result, a periodontal ligament with normal width was formed between new cementum and new bone during the healing phase. Periodontal fibers, inserted into new cementum, passed through the ligament space and attached into the bone. Thus, cementum proteins and associated factors seem to control not only attachment but also differentiation of appropriate cells at a given site, both temporally and spatially.

Previous reports13 have indicated that application of GTR membrane results in formation of new cementum on the instrumented root surface in concert with the formation of new periodontal ligament and new bone. Results here strongly support the concept underlying GTR application. That is, periodontal ligament cells have the capacity to form new attachment when epithelial cells, gingival connective tissue cells, and bone cells are prevented from occupying the wound area adjacent to the root during the initial phase of healing. However, the present study also revealed ankylosis between the alveolar bone and the root surface. Furthermore, new cementum on the instrumented root surface appeared not to be loosely attached to the root surface and did not exhibit a normal cementum morphology. Rather, it had an appearance resembling alveolar bone. The connective tissue fibers adjacent to the bone-like cementum were not functionally oriented but rather were aligned parallel to the root surface.

Recent studies6 have brought into question the fundamental concept that periodontal ligament cells migrate to the instrumented root surface prior to bone cell attachment. Ighuat et al.6 have suggested that the bone, as well as periodontal ligament, is a potential source of cells to the instrumented root surface during healing. Further, bone cells reported have the capacity for more rapid growth than periodontal ligament cells, and only bone cells produce bone matrix in multilayers in vitro.6 In addition, Melcher et al.23 have shown that only cells derived from bone produce a cementum-like tissue on the root. Further yet, others have performed histochemical analysis on both original cementum and the new cementum formed under the GTR membrane using bone-specific vanadate-resistant alkaline phosphatase.24 The latter results plus those of Melcher et al.23 strongly suggest that the new cementum is formed by the cells derived
from alveolar bone.

In summary, I conclude that the apposition of CGM onto the curetted root surface following flap surgery results in formation of new attachment, new cementum, and new bone. In contrast, the application of GTR membrane, at least in some instance, produces complications such as root surface resorption and ankylosis in addition to the formation of bone-like new cementum and new bone. Results therefore indicate that the CGM technique induces regeneration of periodontal tissues following flap surgery in monkeys whereas the GTR procedure is not likely to establish true attachment.

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REFERENCES