Periodontal repair in dogs: gingival tissue occlusion, a critical requirement for GTR?


Abstract
Background: Design criteria for guided tissue regeneration (GTR) devices include biocompatibility, cell occlusion, space maintenance, tissue integration, and ease of use. Previous studies have established the importance of wound stabilization and space provision during the early healing sequel for successful GTR outcomes as well as evaluated biocompatibility, tissue integration, and clinical manageability of various biomaterials. The importance of cell or tissue occlusion has yet to be established. The objective of this study was to evaluate the role of tissue occlusion as a critical determinant for GTR outcomes.

Methods: Routine, critical size, 5–6 mm, supra-alveolar, periodontal defects were created around the mandibular premolar teeth in six young adult Beagle dogs. Space-providing expanded polytetrafluoroethylene (ePTFE) membranes, with (macroporous) or without (occlusive) 300-μm laser-drilled pores, 0.8 mm apart, were implanted to provide for GTR. Treatments were randomly assigned to left and right jaw quadrants in subsequent animals. The gingival flaps were advanced to cover the membranes and sutured. The animals were euthanized at 8 weeks postsurgery for histologic and histometric analysis.

Results: Three animals experienced wound failure within 2–3 weeks postsurgery resulting in exposure and removal of the occlusive ePTFE membranes. All defect sites, irrespective of membrane configuration or history of membrane exposure and removal, exhibited substantial evidence of periodontal regeneration including a functionally oriented periodontal ligament. To evaluate the biologic potential of GTR devices, only animals without wound failure and membrane removal were included. Alveolar bone regeneration for animals receiving occlusive and macroporous ePTFE membranes averaged (± SD) 3.2 ± 1.1 versus 2.0 ± 0.4 mm (p = 0.3113). Cementum regeneration was enhanced in defect sites receiving the occlusive ePTFE membrane compared to the macroporous membrane (4.7 ± 0.4 versus 2.3 ± 0.2 mm; p = 0.0167). Ankylosis was observed in one animal. Limited root resorption was observed in a second animal.

Conclusion: Tissue occlusion does not appear to be a critical determinant for GTR. However, tissue occlusion may be a requirement for optimal GTR. Moreover, macroporous space-providing devices may increase the predictability of clinical GTR therapy.

Design criteria for guided tissue regeneration (GTR) devices include biocompatibility, cell occlusion, space maintenance, tissue integration, and ease of use (Scantlebury 1993, Hardwick et al. 1995). Since the formulation of the principles of GTR (for a review, see Karring et al. 1993), studies have shown that the formation of a connective tissue attachment rather than a junctional epithelium onto the root surface following periodontal reconstructive surgery is critically dependent on the stability of the wound (Wikesjö & Nilvéus 1990, Wikesjö et al. 1991, Haney et al. 1993). If the gingival flap remains stabilized during the early healing period, the epithelium will not migrate onto the root surface. The use of a purpose-designed expanded polytetrafluoroethylene (ePTFE) device has been demonstrated to contribute significantly to wound stability (Haney et al. 1993). Thus, it has been suggested that a junctional epithelium commonly observed following
periodontal reconstructive surgery is a sequel to wound failure (Wikesjö et al. 1992, Wikesjö & Selvig 1999). The gingival flap becomes detached from the root surface during the early healing events by mechanical means and/or due to compromised adsorption/adhesion/maturation of the fibrin clot at the tooth–gingival flap interface, thereby permitting migration of the epithelium into the interface.

Studies have further shown that alveolar bone and cementum regeneration is critically dependent on space provision by the GTR device (Haney et al. 1993, Sigurdsson et al. 1994, 1995, Trombelli et al. 1999, Wikesjö et al. 2002a). Limited or no regeneration of alveolar bone and cementum has been observed in supra-alveolar periodontal defects following gingival flap surgery alone without space-providing GTR devices, when the GTR device inadvertently has collapsed or been compressed onto the root surface, and when the space underneath the membrane has been filled with a space-consuming slowly or nonresorbable biomaterial. Thus, also limited space provision does not appear conducive to regeneration of alveolar bone and cementum. In the presence of more extensive space provision, cortical-appearing alveolar bone has been observed filling the wound space underneath an ePTFE device within a 4-week observation interval (Haney et al. 1993). In the presence of “abundant” space provision, the alveolar bone adopts a “physiologic” form along the tooth surface (averaging 75% of 5-mm supra-alveolar periodontal defects), leaving the remainder of the wound space underneath the membrane filled with dense fibrous connective tissue (Sigurdsson et al. 1994, 1995). Cementum regeneration, as determined by light microscopy, appears limited within a 4–week healing interval amounting to fractions of the defect height, if at all appreciable (Haney et al. 1993, Wikesjö et al. 2002a). Cementum regeneration following an 8-week healing interval has been observed to average 40% of 5-mm supra-alveolar periodontal defects in the presence of what appears to be adequate space provision (Sigurdsson et al. 1994, 1995, Wikesjö et al. 2002b, c).

A recent study has evaluated the influence of ePTFE characteristics on wound healing, i.e., the influence of ePTFE porosity on osteogenesis (Zellin & Linde 1996). In a rat calvaria model, ePTFE devices with a porosity of 20–25 and 100 µm increased the rate of osteogenesis compared to less porous devices (<8 µm). Increased permeability allowing transposition of extracellular matrix and cellular elements may have favored osteogenesis. A concept of alveolar bone regeneration utilizing space provision without connective tissue occlusion emerges from this observation. This concept is also supported by observations in a previous study evaluating the influence of space provision without connective tissue occlusion on osteogenesis (Karaki et al. 1984). Contralateral horizontal periodontal defects were surgically created between the mandibular premolar teeth in dogs. A tissue-expanding gold mesh was applied on one side, while the contralateral side served as surgical control. Callus formation was enhanced in defects treated with the gold mesh compared to that in the surgical control. Evidently, osteogenesis in a periodontal environment may proceed in the presence of space provision without strict occlusion of the gingival connective tissue.

The evidence presented has contributed to a paradigm shift for GTR. First, wound stability is critical to maintain wound integrity for successful periodontal regeneration (in part, wound stability may be provided by purpose-designed ePTFE devices). Second, space provision is critical for alveolar bone regeneration and for cementum regeneration. The importance of occlusion of cells from the gingival connective tissue for alveolar bone and cementum regeneration has yet to be established. The objective of this preclinical study was to evaluate tissue occlusion as a critical design criterion for GTR devices.

Material and Methods

Animals

Six male Beagle dogs (age 18–24 months, approximate weight 15 kg) exhibiting intact mandibular premolar dentition, without crowding or evidence of periodontal disease, obtained from a USDA approved dealer, were used. Animal selection and management, surgery protocol, and periodontal defect preparation followed routines approved by the Animal Care and Use Committee, W.L. Gore & Associates Inc., Flagstaff, AZ, USA. The animals had access to standard laboratory diet and water until the beginning of the study. Oral prophylaxis was performed within 2 weeks prior to the experimental surgeries.

GTR devices

Space-providing polypropylene-reinforced ePTFE barrier membranes (reinforced Gore-TEX ePTFE, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) were used (Fig. 1). The occlusive membranes, custom-made and pre-shaped for the supra-alveolar, critical-sized, periodontal defect model (Wikesjö et al. 1994), had a 15–25 µm nominal pore size and were reinforced with a laminated polypropylene mesh. These membrane characteristics have been shown to support alveolar bone and cementum regeneration in the supra-alveolar periodontal defect model (Sigurdsson et al. 1994). Membranes without provisions for tissue occlusion exhibited the same characteristics except for laser-etched 300-µm pores at 0.8-mm (center to center) intervals allowing for penetration of the gingival connective tissue. GORE-TEX ePTFE materials are considered to be inert biocompatible materials as supported by over 1 million clinical implants since 1975.

Surgical protocol

Food was withheld the night before surgical procedures. The animals were premedicated with atropine (0.02 mg/kg i.m.), buprenorphine (0.04 mg/kg i.m.), and flunixin meglumine (0.1 mg/kg i.v.). A prophylactic antibiotic (cefazolin; 22 mg/kg i.v.) was administered. General anesthesia was induced with diazepam (0.2 mg/kg i.v.) and ketamine (6 mg/kg i.v.). An endotracheal tube was placed and the animals were maintained on isoflurane gas (1–2%) in 100% oxygen using positive pressure ventilation. An i.v. line was placed and the animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h) to maintain hydration while anesthetized. Routine dental infiltration anesthesia with epinephrine was used at the surgical sites.

The maxillary first, second and third premolar teeth were surgically extracted, and the maxillary fourth premolars were reduced in height and exposed pulp tissues sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). This was done to alleviate the potential of trauma from the maxillary teeth to
the experimental mandibular sites postsurgery.

Supra-alveolar, critical size periodontal defects were created around the third and fourth mandibular premolar teeth in the right and left jaw quadrants in each animal (Fig. 1) (Wikesjö et al. 1994). Briefly, buccal and lingual mucoperiosteal flaps were reflected following buccal and lingual sulcular incisions from the canine tooth to the second molar. The first and second premolar teeth, and the first molar were extracted. Alveolar bone was removed around the circumference of the remaining premolar teeth using chisels and water-cooled rotating burs. The root surfaces were instrumented with curettes, chisels, and water-cooled rotating diamonds to remove the cementum. The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento-enamel junction (CEJ) and the cut surfaces smoothed. Exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). The clinical defect height, from the CEJ to the reduced alveolar crest, was set to 6 mm as measured with a periodontal probe.

**Wound management**

Occlusive and macroporous ePTFE devices were alternated between the left and right jaw quadrants in a split-mouth design in subsequent animals. To ensure an adequate blood clot underneath the membranes, 3 ml of autogenous venous blood was infused under the membrane. The blood was drawn using an i.v. catheter in aseptic manner and aspirated blood was expelled underneath the membrane. The membranes were fixed to the reduced alveolar bone with medical-grade stainless-steel tacks (FRIOS® Augmentation System, Friadent, Mannheim, Germany) designed for these applications. A sham-surgery control was not used since it has been established that gingival flap surgery alone will not provide conditions for periodontal regeneration in the critical-sized supra-alveolar periodontal defect model (Sigurdsson et al. 1994, 1995).

Following membrane placement and clot positioning, the periosteum was fenestrated at the base of the gingival flaps to allow tension-free flap apposition. The flaps were advanced, the flap margins being adapted 3–4 mm coronal to the ePTFE membranes and sutured (GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA). Intrasurgery photographs were taken prior to and immediately after placement of the barrier membrane, and following wound closure.

**Postsurgery protocol**

Animals were fed a canned soft dog food diet the first 14 days postsurgery. Thereafter, the animals received standard laboratory diet soaked in warm water until thoroughly soft. The animals received buprenorphine (0.04 mg/kg i.v., i.m., or s.q.) every 5 h for analgesia for the first few days postsurgery. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg, i.m., b.i.d.) was used for infection control for 14 days. Plaque control was maintained by twice daily topical application of chlorhexidine (chlorhexidine gluconate 20%, Xtrium Laboratories, Inc., Chicago, IL, USA; 40 ml of a 2% solution) until gingival suture removal and, thereafter, once daily (Monday through Friday) until the completion of study.
Radiographs were obtained immediately postsurgery, and at 4 and 8 weeks. Gingival sutures were removed under sedation at approximately 10 days. Observations of experimental sites with regard to gingival health, maintenance of suture line closure, edema, and evidence of tissue necrosis or infection were made daily until suture removal, and at least twice weekly thereafter for the duration of the study. The animals were anesthetized and euthanized at 8 weeks postsurgery. Following euthanasia, teeth with surrounding soft and hard tissues and membranes were removed en bloc.

**Histological processing and evaluation**

The tissue blocks were fixed in 10% buffered formalin for 3–5 days, decalcified in 5% formic acid for 8–10 weeks, trimmed, dehydrated, and embedded in paraffin. Serial sections (7 μm) were produced in a buccal–lingual plane throughout the mesial–distal extension of the teeth. Every 14th section was stained with hematoxylin for observations at 100 μm intervals.

The most central stained section of each root of the third and fourth premolar tooth was identified by the size of the root canal. This section and the adjacent stained step-serial section on either side were subjected to histometric analysis. Thus, three subsequent step-serial sections, encompassing 0.2 mm of the mid-portion of the mesial and the distal root for each premolar tooth, were used for analysis. Analysis was performed using incandescent and polarized light microscopy (BX 60, Olympus America, Inc., Melville, NY, USA), a microscope digital camera system (DP10, Olympus America, Inc., Melville, NY, USA), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetics, Silver Springs, MD, USA) by one experienced investigator masked to the specific experimental conditions. The following measurements were recorded for the buccal and the lingual tooth surfaces for each section:

- **Defect height**: distance between apical extension of the root planing and the CEJ.
- **Connective tissue repair**: distance between apical extension of the root planing and the apical extension of the junctional epithelium.
- **Cementum regeneration (height)**: distance between apical extension of the root planing and the coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.
- **Bone regeneration (height)**: distance between the apical extension of root planing and the coronal extension of regenerated alveolar bone along the planed root.
- **Root resorption**: combined linear heights of distinct resorption lacunae on the planed root.
- **Ankylosis**: combined linear heights of ankylosic union between the regenerated alveolar bone and the planed root.

**Data analysis**

Summary statistics (means ± SD) based on animal means for the experimental conditions were calculated using the selected step-serial sections. Differences

---

*Fig. 2.* Photomicrographs of supra-alveolar periodontal defect implanted with the occlusive ePTFE membrane without wound failure, membrane exposure and removal. Overview (a & b; original magnification 2.5 ×, 4 ×, hematoxylin) and higher magnification (c, d; 8 ×) of the buccal aspect of the defect show bone formation paralleled by regeneration of cementum and a functionally oriented periodontal ligament (e, f; 8 ×, polarized light). Animal #93.
between experimental conditions were analyzed using appropriate t-tests (N = 6).

Results
Clinical observations

Three jaw quadrants receiving the occlusive ePTFE membrane experienced membrane exposure within 1–2 weeks postsurgery. These membranes were removed within 2, 3, or 5 days following the observation of membrane exposure. Thus, the membranes were removed as early as 9, 11, and 21 days postsurgery. One macroporous ePTFE membrane showed a small exposure at 39 days postsurgery. This membrane exhibited a minimal local inflammatory reaction and was maintained in situ for the 8-week healing interval. Healing was uneventful in the remaining animals.

Radiographic observations

Radiographic evaluation at 8 weeks postsurgery showed significant new bone formation in jaw quadrants receiving the occlusive ePTFE membrane without exposure. Newly formed bone filled the furcation area and approached the CEJ. Jaw quadrants with membrane exposure exhibited bone fill approximating 30% of the defect height. Bone formation in jaw quadrants receiving the macroporous ePTFE membrane approximated 50–70% of the defect height almost obliterating the furcation area in three animals. The newly formed bone did not appear ankylosic but commonly exhibited a periodontal ligma-ment space with lamina dura formation.

Histologic observations

Regeneration of periodontal structures included newly formed bone approximating 50–100% of the defect height in sites receiving the occlusive ePTFE membrane without wound failure (Fig. 2). The newly formed bone consisted of woven and lamellar bone with fibrovascular tissue in the marrow spaces. Cementum formation extended above the newly formed bone in all animals approximating 86–100% of the defect height. A functionally oriented periodontal ligament with inserting fibers was observed at all sites. Ankylosis was observed in one site. Healing characteristics at defect sites with a history of wound failure and early membrane removal were similar to that observed in defects without such healing aberrations (Fig. 3).

Regeneration of alveolar bone and periodontal attachment in defects receiving the macroporous ePTFE membrane did not remarkably differ from that observed in defects receiving the occlusive ePTFE membrane (Figs. 4 and 5). Newly formed bone consisting of woven and lamellar bone with fibrovascular marrow was observed. Cementum formation reached the level of newly formed bone. Importantly, a functionally oriented periodontal ligament with inserting fibers was observed at all sites. In sites exhibiting evidence of membrane compression to the root surface, bone and cementum formation appeared to be reduced. Undermining root resorption was observed in one site.

Histometric analysis

The histometric analysis is presented in Tables 1–7. Table 1 shows healing in all

Fig. 3. Photomicrographs of supra-alveolar periodontal defect implanted with the occlusive ePTFE membrane. This site experienced wound failure and membrane exposure at 9 days postsurgery. The membrane was immediately removed and the mucogingival flaps were adapted and sutured to cover the tissue formed under the membrane. Overview (a; original magnification 2.5 ×, hematoxylin) and higher magnification (b; 4 × ) of the lingual aspect of the defect show new bone formation paralleled by regeneration of cementum and a functionally oriented periodontal ligament (c; 4 × , polarized light ). Animal #96.
Fig. 4. Photomicrographs of supra-alveolar periodontal defect implanted with the macroporous ePTFE membrane. Overview (a; original magnification 2.5 ×, hematoxylin) and higher magnification (b; 8 ×) of the buccal aspect of the defect show new bone formation paralleled by regeneration of cementum and a functionally oriented periodontal ligament (c; 8 ×, polarized light). Animal #91.

Fig. 5. Photomicrographs of supra-alveolar periodontal defect implanted with the macroporous ePTFE membrane. Overview (a, b; original magnification 2.5 ×, 4 ×, hematoxylin) and higher magnification (c, d; 8 ×) of the buccal aspect of the defect show bone formation paralleled by regeneration of cementum and a functionally oriented periodontal ligament (e, f; 8 ×, polarized light). Animal #95.
Table 1. Summary statistics for jaw quadrants receiving the occlusive ePTFE membrane

<table>
<thead>
<tr>
<th>Animal</th>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>4.9 ± 0.1</td>
<td>4.1 ± 0.8</td>
<td>2.0 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>92</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>4.4 ± 0.0</td>
<td>2.5 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>93</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>5.2 ± 0.3</td>
<td>2.5 ± 0.6</td>
<td>1.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>94</td>
<td>4.9 ± 0.3</td>
<td>3.5 ± 0.8</td>
<td>3.2 ± 0.4</td>
<td>1.8 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>95</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>96</td>
<td>5.1 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>group</td>
<td>5.0 ± 0.3</td>
<td>4.3 ± 0.9</td>
<td>3.6 ± 1.4</td>
<td>2.4 ± 1.1</td>
<td>0.2 ± 0.4</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>in % of the defect height</td>
<td>86</td>
<td>72</td>
<td>48</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data for animals without wound failure and early membrane removal (means ± SD; mm).

Table 2. Summary statistics for jaw quadrants receiving the occlusive ePTFE membrane

<table>
<thead>
<tr>
<th>Animal</th>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>92</td>
<td>5.1 ± 0.3</td>
<td>5.1 ± 0.3</td>
<td>4.4 ± 0.0</td>
<td>2.5 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>93</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.5</td>
<td>5.2 ± 0.3</td>
<td>2.5 ± 0.6</td>
<td>1.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>95</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.1</td>
<td>4.5 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>group</td>
<td>5.0 ± 0.5</td>
<td>5.0 ± 0.5</td>
<td>4.7 ± 0.4</td>
<td>3.2 ± 1.1</td>
<td>0.4 ± 0.6</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>in % of the defect height</td>
<td>100</td>
<td>94</td>
<td>64</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data for animals without wound failure and early membrane removal (means ± SD; mm).

Table 3. Summary statistics for jaw quadrants receiving the occlusive ePTFE membrane

<table>
<thead>
<tr>
<th>Animal</th>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>4.9 ± 0.1</td>
<td>4.1 ± 0.8</td>
<td>2.0 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>94</td>
<td>4.9 ± 0.3</td>
<td>3.5 ± 0.8</td>
<td>3.2 ± 0.4</td>
<td>1.8 ± 0.8</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>96</td>
<td>5.1 ± 0.3</td>
<td>3.2 ± 0.3</td>
<td>2.0 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>group</td>
<td>5.0 ± 0.1</td>
<td>3.6 ± 0.5</td>
<td>2.4 ± 0.7</td>
<td>1.7 ± 0.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>in % of the defect height</td>
<td>72</td>
<td>48</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Data for animals without wound failure and early membrane removal (means ± SD; mm).

Table 4. Comparison between animals receiving the occlusive ePTFE membrane with and without wound failure and early membrane removal (means ± SD; mm)

<table>
<thead>
<tr>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal wound failure</td>
<td>5.0 ± 0.5</td>
<td>5.0 ± 0.5</td>
<td>4.7 ± 0.4</td>
<td>3.2 ± 1.1</td>
<td>0.4 ± 0.6</td>
</tr>
<tr>
<td>wound failure</td>
<td>5.0 ± 0.1</td>
<td>3.6 ± 0.5</td>
<td>2.4 ± 0.7</td>
<td>1.7 ± 0.2</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.9087</td>
<td>0.0201</td>
<td>0.0082</td>
<td>0.0920</td>
<td>0.3739</td>
</tr>
</tbody>
</table>

Table 5. Summary statistics (means ± SD; mm) for jaw quadrants receiving the macroporous ePTFE membrane

<table>
<thead>
<tr>
<th>Animal</th>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>91</td>
<td>5.1 ± 0.5</td>
<td>5.1 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>92</td>
<td>5.0 ± 0.5</td>
<td>5.1 ± 0.5</td>
<td>2.5 ± 0.1</td>
<td>2.1 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>93</td>
<td>4.8 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>2.2 ± 0.3</td>
<td>2.3 ± 0.6</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>94</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.6</td>
<td>2.9 ± 1.0</td>
<td>2.4 ± 1.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>95</td>
<td>4.9 ± 0.4</td>
<td>4.9 ± 0.4</td>
<td>2.1 ± 0.8</td>
<td>1.6 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>1.8 ± 0.0</td>
</tr>
<tr>
<td>96</td>
<td>4.9 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>2.5 ± 0.7</td>
<td>2.6 ± 0.4</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>group</td>
<td>5.0 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>2.5 ± 0.4</td>
<td>2.4 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.7</td>
</tr>
<tr>
<td>in % of the defect height</td>
<td>100</td>
<td>50</td>
<td>48</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

defect sites receiving the occlusive ePTFE membrane. Tables 2 and 3 show histometric observations in these sites separated by wound failure. Membrane exposure and wound failure reduced the potential for cementum regeneration (Table 4). Table 5 shows healing in defect sites receiving the macroporous ePTFE membrane.

Tables 6 and 7 compare observations in defect sites receiving the occlusive and macroporous ePTFE membranes. When all defect sites were considered, there were no significant differences between occlusive and macroporous ePTFE membranes (Table 6). For the analysis of the biologic potential for regeneration, however, sites exhibiting membrane exposure and wound failure must be excluded from the analysis. Table 7 compares defect sites receiving occlusive and macroporous ePTFE membranes without wound failure. Bone regeneration approximated 64% of the defect height in sites receiving the occlusive ePTFE membrane and 41% in sites receiving the macroporous ePTFE membrane ($p = 0.3113$). Cementum regeneration was significantly increased in sites receiving the occlusive ePTFE membrane (94% of the defect height) compared with that of the macroporous membrane (47% of the defect height; $p = 0.0167$). There were no other significant differences between the groups.

Discussion

The objective of this study was to evaluate the validity of a concept of cell occlusion for GTR devices. Macroporous and occlusive ePTFE membranes were surgically implanted in contralateral supra-alveolar periodontal defects in six Beagle dogs and healing was allowed to progress for 8 weeks prior to euthanasia and histologic and histometric evaluation. Three animals receiving occlusive ePTFE membranes experienced wound failure within 2–3 weeks postsurgery, resulting in exposure and removal of the membranes. To evaluate the biologic potential of the GTR devices, only animals without wound failure and membrane removal were included. Similar amounts of bone regeneration were observed for animals receiving occlusive and macroporous ePTFE membranes. Cementum regeneration was significantly enhanced in sites receiving the occlusive ePTFE membrane compared with sites receiving the macroporous membrane. These
membrane removal

macroporous (P) ePTFE membrane

by Wikesjö et al. (2002b) also evaluated surgical controls (no membrane) following the defect height compared to 1% in the coronal ligament approximating 41% of the functionally oriented periodontal regeneration including a re-established ePTFE membrane in the same animal model. After excluding animals experiencing membrane exposure, mean cementum regeneration in sites receiving the occlusive ePTFE membrane approached 94% of the defect height. This observation supports the hypothesis that tissue-occlusive GTR devices may optimize conditions for periodontal regeneration. Nevertheless, observations of substantial cementum regeneration including a re-established functionally oriented periodontal ligament in defect sites receiving the macroporous ePTFE membrane suggest that tissue and/or cell occlusion is not an absolute requirement for GTR. Sigurdsson et al. (1994, 1995) also using the occlusive ePTFE membrane in the same animal model, observed enhanced cementum regeneration including a re-established functionally oriented periodontal ligament approximating 41% of the defect height compared to 1% in the surgical controls (no membrane) following an 8-week healing interval. A study by Wikesjö et al. (2002b) also evaluating the macroporous ePTFE membrane in the supra-alveolar periodontal defect model showed cementum regeneration approximating 22% of defect height. Although differences may exist between studies, collectively the studies suggest that the use of space-providing, occlusive or macroporous GTR membranes may significantly enhance periodontal regeneration.

The newly formed cementum included functionally oriented fibers irrespective of whether the defect site had received an occlusive or a macroporous ePTFE membrane. This observation is somewhat remarkable since the teeth were sheltered under space-providing ePTFE membranes that usually did not contact the teeth. The membranes, in turn, were submerged under the gingival flaps, the animals were kept on a soft diet, and the teeth in the opposing maxillary jaw quadrants had been removed or reduced in height to avoid any contact with the healing defect sites. The functionally oriented periodontal attachment was observed in the absence and presence of newly formed alveolar bone. Moreover, the newly formed periodontal attachment had reached this maturity within an 8-week healing interval consistent with observations by Sigurdsson et al. (1994, 1995) and Wikesjö et al. (2002b, c). These observations also suggest that the formation and maturation of the periodontal attachment exceeds that of alveolar bone. Intriguingly, a functionally oriented periodontal attachment was routinely formed without obvious functional challenge from occlusion or masticatory activity.

There was no significant difference in coronal regrowth of alveolar bone between defect sites receiving occlusive or macroporous ePTFE membranes without wound failure (3.2 versus 2.4 mm, respectively). Bone regeneration in sites with wound failure and early membrane removal amounted to 1.7 mm. Similar observations of bone regeneration were reported by Sigurdsson et al. (1994, 1995). Bone regeneration following use of the occlusive ePTFE membrane averaged 2.9 mm following an 8-week healing interval. Mean alveolar bone regrowth in surgical controls without a GTR device amounted to 0.6 mm. No bone regeneration was observed in defects with wound failure and membrane exposure. Wikesjö et al. (2002b) reported a mean regeneration of alveolar bone amounting to 2.0 mm in sites receiving the macroporous ePTFE membrane. Collectively, the data suggest that space-providing GTR devices, in these studies exclusively based on a proprietary ePTFE technology, whether occlusive or macroporous, support substantial regeneration of alveolar bone. Wound failure, membrane exposure, and infection may severely compromise regeneration of alveolar bone. However, if the GTR device is removed in the immediate sequence of wound failure and membrane exposure, the regenerative potential appears minimally compromised.

Only one tooth (with an occlusive ePTFE membrane) exhibited ankylosis in this study. Sigurdsson et al. (1994, 1995) reported that three of nine and two of eight teeth exhibited ankylosis in animals receiving the occlusive ePTFE membrane or served as surgical controls, respectively. Wikesjö et al. (2002b) reported limited ankylosis in two of eight teeth in animals receiving the macroporous ePTFE membrane. Gottlow et al. (1984) did not observe ankylosis following GTR in a nonhuman primate study. Differences in regeneration rates between the tissues sequestered underneath the GTR devices, i.e., the alveolar bone and the periodontal ligament, may account for these observations. Gottlow et al. (1984) suggested that “the migration rate of periodontal ligament cells is at least as high as that of bone cells”. In fact, tissue resources from the periodontal ligament may command an even higher regeneration rate than that of the alveolar bone. Nevertheless, the data clearly suggest that ankylosis is a rare but biologic possibility also following GTR under optimal conditions for wound healing.

Limited root resorption of undermining character was observed in one

Table 6. Comparison between groups (means±SD; mm) receiving the occlusive and the macroporous (P) ePTFE membrane

<table>
<thead>
<tr>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>ePTFE 5.0±0.3</td>
<td>4.3±0.9</td>
<td>3.6±1.4</td>
<td>2.4±1.1</td>
<td>0.2±0.4</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>P-ePTFE 5.0±0.1</td>
<td>5.0±1.0</td>
<td>2.5±0.4</td>
<td>2.4±0.5</td>
<td>0.0±0.0</td>
<td>0.3±0.7</td>
</tr>
<tr>
<td>p-value</td>
<td>0.9114</td>
<td>0.1272</td>
<td>0.1902</td>
<td>0.9409</td>
<td>0.3632</td>
</tr>
</tbody>
</table>

Table 7. Comparison between groups (means±SD; mm) receiving the occlusive and the macroporous (P) ePTFE membrane excluding animals exhibiting wound failure and early membrane removal

<table>
<thead>
<tr>
<th>Defect height</th>
<th>Connective tissue repair</th>
<th>Cementum height</th>
<th>Bone height</th>
<th>Ankylosis</th>
<th>Root resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>ePTFE 5.0±0.5</td>
<td>5.0±0.5</td>
<td>4.7±0.4</td>
<td>3.2±1.1</td>
<td>0.4±0.6</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>P-ePTFE 4.9±0.1</td>
<td>4.9±0.1</td>
<td>2.3±0.2</td>
<td>2.0±0.4</td>
<td>0.0±0.0</td>
<td>0.6±1.0</td>
</tr>
<tr>
<td>p-value</td>
<td>0.8399</td>
<td>0.8399</td>
<td>0.0167</td>
<td>0.3113</td>
<td>0.4226</td>
</tr>
</tbody>
</table>


animal receiving the macroporous ePTFE membrane. Most other teeth exhibited minor resorption of surface erosion character. There were no apparent differences between teeth receiving the macroporous and occlusive ePTFE membrane. These observations are consistent with those of Sigurdsson et al. (1994, 1995) and Wikesjö et al. (2002a, b, c). In contrast, the teeth in the surgical controls in the study of Sigurdsson et al. (1994, 1995) not only exhibited limited cementum regeneration but also frequent and advanced root resorption. Collectively, these observations suggest that the migration and proliferation rate of the gingival connective tissues are slower than that of the periodontal ligament and that only when the gingival connective tissues are allowed to collapse onto and contact the root surface, more advanced root resorption may routinely be observed.

Wound failure and membrane exposure has been associated with a decreased regenerative potential following GTR. Selvig et al. (1992) showed that the extent of oral exposure and bacterial contamination of an ePTFE membrane at the time of removal may be an indicator of the success or failure of the regenerative procedure. Trombelli et al. (1997) showed that intrabony periodontal defect sites with exposed GTR devices at the 5-week removal exhibited minimal, if any, gain of clinical attachment. In this study, ePTFE membranes were exposed and removed as early as 9, 11, and 21 days postsurgery. The defect sites exhibited substantial regeneration of cementum and alveolar bone at the 8-week evaluation time-point in spite of the membrane exposure. Importantly, the membranes were removed within days of exposure. These observations suggest that periodontal regeneration has reached sufficient maturity to allow membrane removal within a few weeks of wound healing. Comparing the observations in this study with that of membrane exposure in other preclinical studies suggests that early membrane removal is critical to maintain any newly regenerated tissues (Haney et al. 1993, Sigurdsson et al. 1994, 1995).

Sites receiving the macroporous ePTFE membrane did not experience wound failure and membrane exposure, indicating that the macroporous structure better supported the healing gingival flap than the occlusive ePTFE membrane. The macroporous membrane readily allowed passage of fibrovascular elements with limited signs of inflammation. This tissue “ingrowth” may stabilize the wound and secure the nutritional base for the flap during the early healing sequel, thus preventing wound dehiscence and membrane exposure. Similar observations of wound healing have been made when this macroporous membrane was used with rhBMP-2 in supra-alveolar periodontal (Wikesjö et al. 2002b) and peri-implant (Wikesjö et al. 2002d) defects. Sixteen animals received the macroporous membrane with or without rhBMP-2. None of the defect sites experienced wound failure and membrane exposure over an 8-week healing interval. In this study, half of the animals receiving the occlusive ePTFE membrane showed wound failure during the early period of healing, and subsequently exhibited less bone and cementum regeneration than those not showing wound failure. These observations are consistent with those of Sigurdsson et al. (1994, 1995). Two of five animals experienced wound failure and membrane exposure, however, the membranes were maintained in situ for the 8-week healing interval. No regeneration of periodontal structures was observed in these sites. These observations may have general clinical implications. A macroporous membrane technology may be a more predictable device for GTR and be more conducive to uncomplicated clinical management than occlusive GTR devices.

Conclusions

- Tissue occlusion does not appear to be a critical determinant for GTR; substantial bone and cementum regeneration including a functionally oriented periodontal ligament occurs in the presence of space provision with or without tissue occlusion. However, tissue occlusion may be a requirement for optimal GTR.
- Periodontal regeneration appears to establish early (within 9 days).
- Ankylosis and root resorption are rare findings following GTR.
- Use of macroporous space-providing devices may increase the predictability of GTR therapy.

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Membranen zur gesteuerten Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR?

Hintergrund: Die Gestaltungskriterien für Geweberegenera-

Zusammenfassung

Parodontale Reparation bei Hunden: Gewebeokklusivität, eine kritische Bedingung für GTR? 

Hintergrund: Die Gestaltungskriterien für Geweberegenera-
l’intégration tissulaire et la facilité d’utilisation. Des études antérieures ont établi l’importance de la stabilisation de la plaie et de l’espace durant les premières séquences de la guérison afin de faciliter le processus de GTR et ont évalué la bioocompatibility, l’intégration tissulaire, la mainabilité clinique des différents biomatériaux. L’importance de l’occlusion cellulaire et tissulaire doit encore être établie. Le but de cette étude a été d’évaluer le rôle de l’occlusion tissulaire comme déterminant critique des résultats de la GTR. Des lésions parodontales sus-alvéolaires de 5-6 mm, ont été créées autour des prémolaires mandibulaires de six jeunes chiens Beagle. Deux types de membranes en teflon ont été utilisées pour la GTR, avec macro-pores (300µm réalisés au laser) ou sans (occlusives). Les traitements ont été répartis dans les quadrants gauche et droit de la mandibule. Les lames gingivales ont été mis en place pour couvrir les membranes et suturés. Les animaux ont été euthanasiés huit semaines après la chirurgie pour l’exposition des membranes en teflon occlusives. Toutes les lésions, quelque soit la configuration ou l’histoire d’exposition de membranes et d’enlèvement, ont montré une évidence de régénération parodontale incluant un ligament parodontal et tissulaire qui a encore été observée chez un autre. L’occlusion tissulaire ne semble pas être déterminante dans le processus de guérison. Une résorption radiculaire importante de 1,1 mm vs 2,4 mm: p = 0,0186). L’ankylose n’a été observée que dans les lésions qui ont reçu les membranes occlusives et macro-pores (3,2 ± 1,1 mm vs 2,4 ± 0,5 mm: p = 0,3029). La régénération cémentaire a été augmentée dans les lésions qui ont reçu les membranes occlusives comparée aux macro-pores (4,7 ± 0,4 mm vs 2,5 ± 0,3 mm: p = 0,0186). L’ankylose n’a été observée que chez unanimal. Une résorption radiculaire limitée a été observée chez un autre. L’occlusion tissulaire ne semble pas être un déterminant critique pour la GTR. Cependant, l’occlusion tissulaire peut être nécessaire pour une GTR optimale. De plus, les systèmes de garde d’espace macro-poreux peuvent augmenter la capacité d’anticipation du traitement GTR clinique.

References


Address:
Dr. Ulf M. E. Wikesjö
Laboratory for Applied Periodontal and Craniofacial Regeneration
Temple University School of Dentistry
Department of Periodontology
3223 North Broad Street
Philadelphia, PA 19140
USA
E-mail: ulf.wikesjo@temple.edu