Evaluation of a new biodegradable membrane to prevent gingival ingrowth into mandibular bone defects in minipigs

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Abstract
Objective: The aim of this study was to test whether a synthetic, biodegradable membrane made of polyethylene glycol (PEG) can prevent soft-tissue ingrowth into alveolar defects.

Material and methods: In each of 16 minipigs, three mandibular premolars were bilaterally extracted. Three months later, acute standardized defects (diameter 8 mm; depth 8 mm) were prepared. Four treatment modalities were randomly allocated to the defects: (1) PEG membrane plus collagen sponge, (2) polylactide (PLA) membrane plus collagen sponge, (3) collagen sponge alone, and (4) empty defect. Animals were sacrificed at 10 days (n = 5), 21 days (n = 5), or 2 months (n = 6) after treatment. Qualitative and quantitative histological evaluations of soft-tissue ingrowth and bone regeneration were performed on non-decalcified ground sections. For statistical analysis, the Mann–Whitney–Wilcoxon test, the Kruskal–Wallis, and the paired t-test were applied. P-values were adjusted using the Dunnett–Hsu adjustment.

Results: At 10 days, the PEG membrane group showed the least soft-tissue ingrowth (mean value = 0.75 mm; range = 1.35 to 0.10), followed by the PLA membrane group (0.18 mm (0.80 to 0.44)), the collagen group (0.04 mm (0.65 to 0.73)), and the empty defects (0.60 mm (0.08 to 1.29). Statistically significant differences were observed between the PEG membrane group and the empty defects (P < 0.05). At 21 days, the highest percentage of newly formed bone was found in the PEG membrane group (mean 28.4%; range 21.6–35.2) compared with 23.7% (16.9–30.5; PLA membrane), 15.2% (8.2–22.2; collagen group), and 21.6% (14.5–28.8; empty defects). Statistically significant differences were only found between the PEG membrane group and the collagen group (P < 0.05). At 2 months, the tested parameters revealed no statistically significant differences between the groups.

Conclusion: The experimental PEG membrane applied in the present study successfully prevented collapse of the covering soft tissues to a degree similar to the PLA membrane. The combination of a collagen sponge and the PEG membrane showed the least soft-tissue ingrowth at 10 days and promoted more bone formation at 21 days.

Numerous preclinical and clinical studies have described the principle of guided bone regeneration (GBR) and a wide range of resorbable and nonresorbable membranes have been investigated over the last two decades (Dahlin et al. 1988; Hammerle et al. 1992; Linde et al. 1993; Sandberg et al. 1993; Schenk et al. 1994; Hammerle & Jung 2003). The principle of excluding the connective tissue from a bone area has been used for bone augmentation procedures in combination with or without...
simultaneous implantation (Buser et al. 1990; Dahlén et al. 1991; Lang et al. 1994). However, several problems are associated with the use of membranes. Nonresorbable membranes tend to get exposed, need a second-stage surgery for removal, and can induce severe cellular reactions [Simion et al. 1994; Aaboe et al. 1998]. On the other hand, resorbable membranes have become a reliable alternative, as they are not associated with these difficulties [Mayfield et al. 1997; Zitzmann et al. 1997]. However, when they were compared with nonresorbable membranes, the nonresorbable membranes better preserved the marginal bone levels in peri-implant bone regeneration [Zitzmann et al. 2001]. Several animal and clinical studies showed gain in marginal bone, using resorbable membranes in combination with an underlying osteoconductive membrane-supporting material, which avoided the collapse of the barriers [Hockers et al. 1999; Brunel et al. 2001; Hämmere & Lang 2001; Zitzmann et al. 2001; Strietzel et al. 2006]. According to these investigations, two main parameters are desirable: the barrier function and the space maintenance.

Collagen membranes, as the most often used type of resorbable membranes, lack enough stiffness for space maintenance and tend to collapse [Strietzel et al. 2006]. Additionally, collagen membranes are prefabricated in standard sizes and need to be adapted to the morphology of the surgical site.

Hence, this individualization of form and size is a time-consuming and tricky procedure, which limits the use of membranes in everyday clinical practice [Pihonen et al. 2006].

Synthetic resorbable membranes consisting of poly(1-lactide) (PLA) and lactide/glycolide copolymers have been developed in the late 1980s and have been investigated in several animal and clinical studies [Fleisher et al. 1988; Magnusson et al. 1988; Gottlow et al. 1994]. One of these membranes, made of PLA dissolved in N-methyl-2-pyrrolidone (NMP) (Atrisorb®), was tested by several authors [Polson et al. 1995; Coonts et al. 1998; Hou et al. 2004]. This barrier membrane is applied customized in a liquid manner, therefore eliminating the cutting, trimming, or handling of preformed barriers. This PLA membrane was shown to have good tissue response, biocompatibility, and to be an effective barrier in promoting bone regeneration in periodontal defects [Coonts et al. 1998].

Recently, the function of a synthetic hydrogel made of polyethylene glycol (PEG) as a barrier membrane for GBR has been evaluated [Jung et al. 2006]. In a preclinical study in the rabbit calvaria, an in situ formed PEG was compared with an ePTFE membrane. It was concluded that the PEG membrane could be successfully used as a biodegradable barrier membrane in the treatment of noncritical-size defects in the rabbit skull, and that it led to similar amounts of bone regeneration as an ePTFE membrane [Jung et al. 2006]. Based on the finding that the membrane can successfully be used to regenerate bone, the question arises regarding its stiffness and its ability to prevent collapsing.

The aim of this study was to test the barrier function of a synthetic, biodegradable hydrogel membrane (PEG membrane) in acute standardized mandibular bone defects in minipigs. The hypothesis was that the PEG membrane (test) would prevent soft-tissue proliferation into a standardized bone defect. This hypothesis was assumed to be validated if the soft-tissue proliferation into the defects filled with a collagen sponge and protected by the PEG membrane was lower than the soft-tissue proliferation in the defects that were left empty and not covered with a membrane (negative control) at 10 and 21 days.

Material and methods

Animals

This study was designed as a randomized experimental study employing 16 female minipigs (Göttingen MiniPig, Ellegaards, Denmark). At the beginning of the study, the animals were about 18 months old and weighed between 25 and 40 kg. The study was performed at the Experimental Department at the University Hospital of Malmö, Sweden, according to the guidelines of the Swedish law of animal keeping. The minipigs were kept under humid diet. The protocol was approved by the Malmö/Lunds Djurförsöksnämnden (M262-03).

Surgery

All the surgical procedures were performed under general anesthesia in an operating room. For premedication, the following agents were used: prophylactic antibiotic treatment with Streptocillin vet., 250 i 200 mg/ml [Boehringer Ingelheim, Copenhagen, Denmark], was started the day before the tooth extraction procedure and was continued for 7 consecutive days. Each animal received an injection of Temgesic 0.3 mg/ml [Scherings-Plough, Brussels, Belgium] for analgesia on the day of the surgery 1 and on the next 3 days. Subsequently, the minipigs were anesthetized according to a standard procedure using Ketalar 50 mg/ml [Pfizer, Sollentuna, Sweden], and Midazolam Hameln 5 mg/ml [Pharmaceuticals GmbH, Hameln, Germany]. After disinfection of the surgical site with 0.2% chlorhexidine solution [Corsodyl, GlaxoSmithKline, Brentford, Middlesex, UK], local anesthetic [Lidocaine HCl 2% with epinephrine 1:100,000; Henry Schein Inc., Port Washington, NY, USA] was administered by infiltration at the respective buccal and lingual sites. All the premolars (p2, p3, and p4) were bilaterally extracted from the lower jaw during the first surgery.

Three months later, defect preparation and regeneration surgery (surgery 2) was performed on both sides of the mandible in all minipigs. Following a mid-crestal incision from the m1 to the canine, a full-thickness mucoperiosteal flap was prepared to the buccal and lingual alveolar plate, approximately 1-2 mm below the mucogingival junction. All granulation tissues were carefully removed. The edentulous osseous ridge was flattened in order to obtain a width of at least 10 mm. Two defects [at a distance of 12 mm between them and 7 mm to m1] were drilled on each side of the mandible to a depth and width of 8 mm (Fig. 1). Because of insufficient ridge height, complications sometimes arose by perforation into the top of the mandibular canal. The following four treatment modalities were randomly allocated to the four surgically created defects:

Test: a collagen-cone [TissuCone E, Baxter, Deerfield, IL, USA] and a PEG membrane [Institut Straumann AG, Basel, Switzerland]

Control 1: a collagen-cone [TissuCone E, Baxter] and a polylactide
membrane (PLA, Atrisorb®, Collagenex Pharmaceuticals Inc., Newtown, PA, USA)  
Control 2: a collagen-cone (collagen, TissueCone E, Baxter)  
Control 3: no filler, no membrane  (empty defect).

At the test sites and the control 1 and 2 sites, collagen-cones were placed in the defects 1 mm below the alveolar crest [Fig. 1]. No further treatment was applied for the control 2 sites. The control 3 sites were left empty and without a membrane. Bleeding was allowed to form a blood clot [Fig. 2]. Two titanium pins were placed 1 mm below the alveolar crest on the buccal and lingual side of each defect. At the test sites, the PEG membrane was applied by a syringe on the collagen-cones and the surrounding alveolar ridge in order to completely cover the defect and to extend 2 mm beyond the defect margins. Within less than 1 min, the degradable synthetic membrane gelled and stabilized [Fig. 3]. Similarly, the PLA membrane in control 1 sites was applied on the collagen-cone and on the alveolar crest. After placement, sterile saline solution was used for approximately 30 s to start the precipitation of the membrane. This was determined by the surface beginning to opacify [Fig. 4]. Following irrigation, meticulous removal of the periosteum was performed in the soft-tissue area coming in contact with the standardized defect. Closure of the flaps was then accomplished by placing horizontal mattress sutures (Gore-Tex® sutures, W.L. Gore & Assoc., Flagstaff, AZ, USA). The sutures were removed 10 days after surgery 2.

Sacrifice
Ten days [five animals], 21 days [five animals], or 2 months [six animals] following surgery 2, the animals were painlessly sacrificed by lethal doses of injected Pentothal® (Pentobarbital Veterinary, Apoteksbolaget, Sweden).

Macroscopic observations
At the day of sacrifice, a macroscopic observation of the implanted sites was performed. Any local inflammation, necrosis, hemorrhage, or any other lesion was recorded. Immediately after dissection, the two hemi-mandibles were block resected and fixed by immersion in 10% formaldehyde in phosphate buffer at pH 7.

Histology and histomorphometry
Histological preparation
X-rays recordings were performed for each site in order to accurately determine the cutting planes. The 64 sites [four per animal] were dehydrated in a series of graded alcohol solutions and embedded in polymethylmethacrylate (PMMA, Merck AG, Darmstadt, Germany). From each specimen, two central orfacial sections through the defect were prepared for histological assessment. The longitudinal sections through the defect of 50–60 μm thickness were obtained by a microcutting and grinding technique adapted by Donath & Breuner [1982]. Thereafter, the sections were stained with McNeals tetrarchrome solution and toluidine blue [Schenk et al. 1984].
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Criteria for histological interpretation

A qualitative analysis was performed with a stereocope [Nikon Eclipse 90i, Nikon, Egg, Switzerland], evaluating the different components (mineralized bone, nonmineralized tissue, pins, membranes, and teeth) according to the standard nomenclature of the International Society for Stereology (Exner 1987).

Histomorphometrical analysis

Computer-assisted histomorphometric measurements were obtained using an automated image analysis system [Visiopharm Integrator System®, Visiopharm A/S, Hansholm, Denmark], coupled with a video camera [Nikon Digital Sight DS-iMc] mounted on a light microscope [Nikon Eclipse 90i]. The borders of the newly formed bone area including bone marrow within the defects were manually marked on the computer screen using a digital pen. Within this region of interest (ROI), the tissue was digitally classified into mineralized bone, nonmineralized tissue, pins, membranes, and teeth. Subsequently, the total number of pixels and the number of pixels allocated to bone and nonmineralized tissue within the ROI were counted by the software. All the measurements were done by two blinded examiners and thereafter compared and discussed to aim for congruence. The following parameters were calculated (Fig. 5):

- the average soft-tissue depth [mm] (calculated as an average of five vertical measurements taken between the level of the original bone crest and the most apical limit of soft-tissue ingrowth at equal intervals between the buccal and lingual borders of the initial bone defect);
- the soft-tissue ingrowth [%] with respect to the initial bone defect area;
- the newly formed bone [%] with respect to the initial bone defect area;
- the position of the most apical point of the soft-tissue ingrowth [mm].

Statistical analysis

Data analyses were performed with SAS software [SAS Institute, Cary, NC, USA]. Measured parameters were summarized in terms of mean values and standard deviations. The one-to-one relationship between the measured parameters and each of the factors (individual, side, position, and treatment) was examined using the Mann–Whitney–Ullcoxon test (for factors with two levels) or the Kruskal–Wallis test (for factors with more than two levels). Unadjusted comparisons of the means of the measured parameters between the PEG membrane treatment and each of the other treatments were explored using the paired t-test. Generalized linear regression models were used to calculate the adjusted means of the different measurements for the comparison between treatments. The means were adjusted for the effect of the individual and the side within the mandible. The factor position was not significant in the univariate or multivariate comparisons and therefore not included in the final model. P-values were adjusted using the Dunnett–Hsu adjustment for multiple comparisons. Statistical significance was set at the 2 level of 0.05 and all tests were two sided.

Results

Clinical findings

The pigs were healthy and no systemic complications occurred during the entire study. Generally speaking, the postoperative wound healing was good. At the time of suture removal, minor soft-tissue dehiscences (defined as incomplete soft-tissue closure) were observed in all groups. Subsequently, the dehiscences healed over with new soft tissue and further healing was uneventful.

Descriptive histology

Ten-day observations

All the five PEG membranes successfully prevented soft-tissue ingrowth. The apical
extension of the soft tissue was consistently above [coronal] the original defect level (Fig. 6). Four membranes were almost intact. In one specimen, because of the histological processing, the membrane had almost completely disappeared and only minor remnants of the membrane could be seen. At three of the five PLA membrane sites, the level of the covering soft tissue was at the original bone crest. The remaining two sites revealed minor collapse of the membranes with concomitant invagination of the covering soft tissue. In both groups, the defects were almost completely filled with a honeycomb-like structure representing the collagen sponge. In two control sites, the collagen-cones prevented soft-tissue ingrowth in the absence of membranes. Whereas in the other three defects, only parts of the original collagen sponge remained and, hence, more pronounced soft-tissue ingrowth had taken place. Marked soft-tissue ingrowth was detected in all the five untreated defects. New bone apposition was observed along the walls and the bottom of the original defects in all groups. Additionally, new bone was formed adjacent to the defects on the alveolar crest, sometimes covering the pins.

Twenty-one-day observations
At this time, complete wound closure by a structured gingival tissue was observed in all groups. Almost complete bone fill was found in the two membranes groups [PEG, PLA] (Fig. 7). Parts of the PEG and PLA membranes were still detectable. The soft-tissue ingrowth was, therefore, minimal in these groups. In one site with a PLA membrane, most of the original defect was filled with nonmineralized tissue. At this site no old bone was present between the bottom of the prepared defect and the roof of the mandibular canal. Here a perforation into the mandibular canal and proximity with the root of a canine were observed. In the two nonmembrane groups, new bone apposition was detected in the apical and lateral parts of the original defect. Soft-tissue ingrowth into the original defect took place in all sites in these two groups.

Two-month observations
The specimens looked similar for all the four treatment modalities (Fig. 8). Complete bone fill of the original defect was a consistent finding. Most original defects showed bone apposition above the level of the original bone crest. Small differences were detected between the animals. In some of the defects, some bone marrow had already developed in the central portion.

Histomorphometry
Ten-day observations
Regarding the soft-tissue ingrowth, the PEG membrane group showed the lowest values: adjusted mean $= -0.75$ mm ($95\%$ confidence interval $= -1.15$ to $-0.10$), compared with $-0.18$ mm ($-0.80$ to $0.44$) for the PLA membrane group, $0.04$ mm ($-0.65$ to $0.73$) for the collagen group, and $0.60$ mm ($-0.08$ to $1.29$) for the empty defects (Table 1, Fig. 9). Generally, more soft-tissue ingrowth was observed at the two nonmembrane groups. Statistically significant differences were found between the PEG membrane group and the untreated defects. The ratio of soft-tissue area ingrowth to original defect area was also lowest for the PEG membrane group with a mean value of $0.1\%$ ($-10.9$ to $11.1$), compared with $0.8\%$

Fig. 6. Histology at 10 days. From left to right: polyethylene glycol [PEG] membrane, polylactide [PLA] membrane, collagen sponge, empty defect.

Fig. 7. Histology at 11 days. From left to right: polyethylene glycol [PEG] membrane, polylactide [PLA] membrane, collagen sponge, empty defect.
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Fig. 8. Histology at 3 months. From left to right: polyethylene glycol (PEG) membrane, polylactide (PLA) membrane, collagen sponge, empty defect.

Table 1. Descriptive statistics: histomorphometric parameters in the 10 days group (n = 5)

![Table Image]

Fig. 9. Multivariate analysis: association between soft-tissue ingrowth and treatment adjusted for the effect of individual and side factors. Whiskers indicate 95% confidence intervals. Polyethylene glycol (PEG) membrane to empty comparison at 10 days. P-value = 0.0283 (Dunnett-Hsu adjusted).

(-10.7 to 11.8) [PLA membrane], 8.5% (-3.6 to 20.7) (collagen), and 16.9% (4.7-29.0) [empty defects] (Fig. 10). The most apical point of soft-tissue ingrowth was always outside of the original defect in the PEG membrane group with a mean value of -0.90 mm (-2.97 to 1.12), compared with 0.19 mm (-1.86 to 2.23) for the sites treated with a PLA membrane, 0.19 mm (-1.86 to 2.23) for sites treated with a collagen sponge, and 2.82 mm (0.55-5.09) for empty defects. No statistically significant differences between the test group and the other treatment modalities were found for the last two described variables.

Twenty-one-day observations
The lowest values for soft-tissue ingrowth were observed for the PEG membrane group, adjusted mean = 0.23 mm [95% confidence interval = -0.38 to 0.84], compared with 0.63 mm (0.01-1.24) for the PLA membrane group, 1.04 mm (0.41-1.68) for the collagen group, and 0.14 mm (-0.31 to 0.78) for empty defects (Table 2, Fig. 9). The ratio of soft-tissue area ingrowth to original defect area was also lowest for the PEG membrane group with a mean value of 7% (-3.4 to 17.4), compared with 13.8% (3.4-24.3) for the PLA membrane group, 23.3% (12.7-34.1)
for the collagen group, and 9.3% [−3.6 to 18.3] for the empty defects (Fig. 10). The most apical point of soft-tissue ingrowth measured for the PEG membrane group was 1.64 mm [0.2–3.3], compared with 2.24 mm [0.62–3.87] for the PLA membrane group, 4.94 mm [3.27–6.61] for the collagen group, and 2.77 mm [1.07–4.50] for the empty defects. There were no statistically significant differences regarding the parameters cited above. Among the evaluated treatment options, the highest percentage of newly formed bone was found for the PEG membrane group (28.4%; 21.6–35.2) compared with the PLA membrane group (23.7%; 16.9–30.5), with the collagen group (15.2%; 8.2–22.2), and with the sites left empty (21.6%; 14.5–28.8) (Fig. 11). Statistically significant difference was only found between the PEG membrane group and the collagen group.

Two-month observations
The adjusted mean values for the soft-tissue area (gingival ingrowth) were negative for all the four treatment modalities. This indicates that the newly developed bone was coronal to the original bone crest (Table 3, Fig. 8).

The area of newly formed bone varied between 55.6% [47.2–63.9] for the PEG membrane group, 54.9% [46.5–63.2] for the PLA membrane group, 60% [51.2–68.8] for the collagen group, and with lowest bone regeneration for empty defects (48.4%; 39.8–57.1) (Fig. 11). All the tested parameters

**Fig. 10.** Multivariate analysis: association between the ratio of soft-tissue area to original defect area and treatment adjusted for the effect of individual and side factors. Whiskers indicate 95% confidence intervals. PEG membrane to empty comparison at 10 days: P-value = 0.1207 (Dunnett–Hsu adjusted).

**Table 2.** Descriptive statistics: histomorphometric parameters in the 21 days group (n = 5)

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**Fig. 11.** Multivariate analysis: association between the ratio of bone regeneration area to original defect area and treatment adjusted for the effect of individual and side factors. Polyethylene glycol (PEG) membrane to collagen comparison at 31 days: P-value = 0.0535 (Dunnett–Hsu adjusted).
Table 3. Descriptive statistics: histomorphometric parameters in the 2 months group (n = 6)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soft-tissue ingrowth into bone defect area (%)</th>
<th>Ratio of soft-tissue area ingrowth to original defect area (%)</th>
<th>Bone area Pn (% of soft tissue)</th>
<th>Ratio of area of new formed bone to original defect area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty</td>
<td>0.22</td>
<td>1.05</td>
<td>0.72</td>
<td>0.67</td>
</tr>
<tr>
<td>PLA</td>
<td>0.17</td>
<td>1.01</td>
<td>0.74</td>
<td>0.65</td>
</tr>
<tr>
<td>Collagen</td>
<td>0.24</td>
<td>1.05</td>
<td>0.77</td>
<td>0.71</td>
</tr>
<tr>
<td>PLA-Coll</td>
<td>0.32</td>
<td>1.10</td>
<td>0.81</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Discussion

The results of the present experiment demonstrated a tendency for the PEG membrane to better perform in terms of prevention of soft-tissue ingrowth and promotion of bone regeneration. No statistically significant differences to the other membrane were found.

The most effective prevention of soft-tissue ingrowth was achieved by the PEG membrane. Statistically significant differences were obtained between the PEG membrane group and the untreated defects regarding soft-tissue ingrowth at 10 days. The ratio of soft-tissue area ingrowth to original defect area was also lowest for the PEG membrane group, but without statistically significant differences relative to the other groups. No significant differences were found between the PLA membrane group, the collagen sponge group, and the empty group regarding soft-tissue ingrowth and soft-tissue area.

Theoretically, membranes need to fulfill two criteria in order to successfully prevent soft-tissue ingrowth: [i] prevent cells derived from the covering soft-tissue flap from migrating into the area intended for bone formation underneath the membrane (cell occlusive function), and [ii] provide sufficient mechanical stability in order to maintain the space underneath the membrane.

The cell-occlusive function of a PEG membrane was already evaluated in a preclinical study in the rabbit calvaria [Jung et al. 2006]. Three treatment modalities [HA/TCP plus PEG membrane, HA/TCP plus ePTFE membrane, and HA/TCP alone] were applied. The two membrane groups showed significantly more bone formation compared with the negative control [HA/TCP alone]. The results demonstrated that the PEG membrane could be used as a biodegradable barrier membrane in the treatment of noncritical-size defects in the rabbit skull, leading to similar amounts of bone regeneration as an ePTFE membrane. This has been confirmed in a rat experiment [Wechsel et al. 2007]. Using a subcutaneous implant model in rats, the barrier function of the PEG-based hydrogel was evaluated over time. The PEG membranes were implanted in dorsal subcutaneous pockets. After healing periods of up to 7 months, explants were collected. Histological analysis revealed that for at least 4 months cellular infiltration into the membrane explants was lower than 1% of that of the positive controls (fibrin sponges). These results indicated that the PEG membrane has the potential to be used as a GBR membrane [Wechsel et al. 2007]. In an earlier study, a PLA membrane was tested in buccal dehiscence defects in dogs [Coonts et al. 1998]. The PLA membrane barrier function was compared with sham-operated control sites in a split-mouth design. It was concluded that the PLA membrane was effective in promoting bone and cementum regeneration in periodontal defects and therefore proved its barrier function.

Previous studies have investigated the ability of membranes to withstand the mechanical pressure exerted by the covering flap during healing following GBR surgery [Hämmerle et al. 1992; Schmid et al. 1997; Lundgren et al. 1998; Slotte & Lundgren 1999]. In the study mentioned above [Jung et al. 2006], the use of the PEG membrane was leading to similar amounts of bone regeneration as an ePTFE membrane. Hence, the PEG membrane demonstrated sufficient mechanical stability in combination with HA/TCP.

In a sheep model, round cranial defects were prepared and six different treatment modalities tested [Schmidmaier et al. 2006]. The results demonstrated that the two PLA membranes successfully prevented soft-tissue prolapose and allowed new bone to form inside the defect.

The collagen sponges used in the present study were almost completely intact at 10 days, exhibiting a honeycomb-like structure. Up to this time point, the collagen sponges stabilized the soft tissue on its original level at the bone crest. At 21 days, however, no remnants of the collagen sponges were detected and collapse of the soft tissue into the defect had occurred. This collapse was more pronounced in the collagen group than in the other treatment groups. These results are in agreement with previous clinical studies, which revealed that the collagen was not able to withstand the soft-tissue pressure and to sufficiently support membranes [Buser et al. 1990, 1993].

In the untreated defects, remnants of the blood clot were observed at 10 days. The most pronounced soft-tissue ingrowth was observed in these sites at 10 and 21 days [with statistically significant differences to the PEG membrane]. The influence on GBR of barriers with varying occlusiveness was tested in a rat experiment [Lundgren et al. 1998]. Special devices were fixed on the top of the skull. These devices were open towards the skull. Towards the soft tissues of the covering skin flap, they were either left completely open, or completely closed, or were perforated to varying degrees. It was found that devices, which were open towards the soft tissue, led to pronounced soft-tissue collapse into the space within the devices.

In the present study, at 21 days, the highest percentage of newly formed bone was found in the PEG membrane group. Statistically significant differences were only found between the PEG membrane...
group and the collagen group. Several reasons may explain the small differences between the groups and the relatively good results obtained by the collagen sponge without a membrane and by the untreated defects at 10 and 21 days. Acute and not chronic defects were prepared. Acute defects have a high potential to form bone as a biologic reaction to the trauma [Schenk 1992]. All the five surrounding bone walls consisted of cancellous bone with exposed bone marrow, which are the primary source for angiogenic and osteogenic cells [Frost 1963; Schenk et al. 1994]. The dimensions of the defects in the present study were, on the one hand, large enough to allow soft-tissue collapse during early healing. On the other hand, during later phases the defects healed well with new bone formation even in the control groups. These rather favorable conditions regarding bone healing could explain the minor differences observed between the tested treatment modalities.

Interestingly, bone formation on top of the alveolar crest was observed around and in the vicinity of the pins. This new bone formation was not associated with the experimental defects but located on the buccal and lingual aspects of the ridge. Bone had formed between the external cortical bone plate and the periosteum, which had been elevated and repositioned in these areas. Several other studies have demonstrated bone formation underneath the periosteum following flap elevation (Cohen & Lacroix 1955; Elbersir 1990; Lemperle et al. 1998; Weng et al. 2000). It is generally assumed that the periosteum itself may have worked as a barrier membrane in these areas.

At 2 months, the tested parameters (soft-tissue ingrowth, bone regeneration) revealed no statistically significant differences between the test and the control groups. Complete bone fill was observed for all treatment modalities. Bone marrow was visible within the original defects. Moreover, bone apposition over the original level of the bone crest was observed in all sites. These results are in agreement with the results from previous studies, where different filling materials and barrier membranes were evaluated in bone defects in minipigs [Buser et al. 1998]. At 24 weeks of healing, all the five filling materials showed similar values. The sites with collagen sponge and with blood clots demonstrated the same healing at 12 weeks, with the defects almost completely filled with new bone. Although animal study was conducted in the mandibular angle and not on the mandibular ridge as in the present study, the anatomical situations are comparable. In both studies, the surrounding bone walls consisted of cancellous bone and the elevated flaps were redraped for complete closure of the prepared bone defects. Obviously, both models exhibit similar potential for bone and soft-tissue healing. Similar findings were also demonstrated in a recent preclinical study, where different membrane types were investigated for use in GBR procedures [Strietzel et al. 2006]. In standardized defects in the pig mandible, the barrier function of resorbable and nonresorbable membranes was tested. The defects revealed well-vascularized cancellous bone at 8 weeks, and bone healing was completed in all groups at 12 weeks [Strietzel et al. 2006].

Conclusions

The experimental PEG membrane applied in the present study successfully prevented collapse of the covering soft tissues to a degree similar to the PLA membrane. The combination of a collagen sponge and the PEG membrane showed the least soft-tissue ingrowth at 10 days and promoted more bone formation at 21 days. Within the limits of this study, the experimental PEG membrane can successfully be applied to prevent soft-tissue ingrowth and to promote bone formation. More research is demanded for the use of this experimental membrane in more challenging defects.

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