A feasibility study evaluating an in situ formed synthetic biodegradable membrane for guided bone regeneration in dogs

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Abstract
Purpose: The aim was (1) to evaluate the soft-tissue reaction of a synthetic polyethylene glycol (PEG) hydrogel used as a barrier membrane for guided bone regeneration (GBR) compared with a collagen membrane and (2) to test whether or not the application of this in situ formed membrane will result in a similar amount of bone regeneration as the use of a collagen membrane.

Material and methods: Tooth extraction and preparation of osseous defects were performed in the mandibles of 11 beagle dogs. After 3 months, 44 cylindrical implants were placed within healed dehiscence-type bone defects resulting in approximately 6 mm exposed implant surface. The following four treatment modalities were randomly allocated: PEG + autogenous bone chips, PEG + hydroxyapatite (HA)/tricalcium phosphate (TCP) granules, bioresorbable collagen membrane + autogenous bone chips and autogenous bone chips without a membrane. After 2 and 6 months, six and five dogs were sacrificed, respectively. A semi-quantitative evaluation of the local tolerance and a histomorphometric analysis were performed. For statistical analysis, repeated measures analysis of variance (ANOVA) and subsequent pairwise Student’s t-test were applied (P < 0.05).

Results: No local adverse effects in association with the PEG compared with the collagen membrane was observed clinically and histologically at any time-point. Healing was uneventful and all implants were histologically integrated. Four out of 22 PEG membrane sites revealed a soft-tissue dehiscence after 1–2 weeks that subsequently healed uneventful. Histomorphometric measurement of the vertical bone gain showed after 2 months values between 31% and 45% and after 6 months between 31% and 38%. Bone-to-implant contact (BIC) within the former defect area was similarly high in all groups ranging from 71% to 82% after 2 months and 49% to 91% after 6 months. However, with regard to all evaluated parameters, the PEG and the collagen membranes did not show any statistically significant difference compared with sites treated with autogenous bone without a membrane.

Conclusion: The in situ forming synthetic membrane made of PEG was safely used in the present study, revealing no biologically significant abnormal soft-tissue reaction and demonstrated similar amounts of newly formed bone for defects treated with the PEG membrane compared with defects treated with a standard collagen membrane.

Guided bone regeneration (GBR) is the most commonly used technique to treat jaw bone areas with insufficient bone volume [for review see Hämmerle & Jung 2003]. It is based on the concept of using a barrier membrane to create a space into which the bone can grow without interference of the soft tissues [Dahlin et al. 1989].
The initial and successful use of expanded polytetrafluoroethylene membranes (ePTFE) as barriers has made this material the standard for GBR [Dahlén et al. 1991; Buser et al. 1993]. An obvious disadvantage of ePTFE materials is that they are non-resorbable and therefore have to be removed during a second surgical procedure. With regard to patient morbidity, risk for tissue damage, and cost vs. benefits, the replacement of non-resorbable by resorbable membranes is highly desirable [Hämmerle & Karring 1998]. Hence, research activities were directed towards the evaluation of resorbable membranes. The most commonly used resorbable membranes are made from collagen. In recent clinical studies, it has been demonstrated that the application of collagen membranes and bone substitutes in conjunction with the placement of dental implants lead to successful coverage of the previously exposed implant surfaces [Zitzmann et al. 1997; Hämmerle et al. 1998; Jung et al. 2003; Moses et al. 2005]. Because the risk of transmission of infecting agents can never be completely excluded for products originating from animals, synthetic barrier membranes made of polylactic or polylactide acid have been developed. Different polymer combinations have been developed to meet the criteria for the use as barrier membranes in GBR procedures and have been tested in animal models [Kohal et al. 1999; Stavropoulos et al. 2004]. However, some studies have shown therapeutic problems using these traditional polymers because they acidify and can form proinflammatory fragments upon degradation [Meikle et al. 1994; Schliephake & Kracht 1997].

Different experimental studies have introduced a newly developed synthetic hydrogel made of polyethylene glycol (PEG) for the use in bone regeneration therapy [Lutolf et al. 2003; Jung et al. 2006]. PEG has been shown to be highly biocompatible [Pang 1993; Working et al. 1997]. It is presently approved for several pharmaceutical applications [Zalipsky & Harris 1997] and as medical devices, e.g. a sprayable adhesion barrier [Mettler et al. 2005]. In a recent animal study, this PEG material was used for the first time as an in situ forming biodegradable membrane for GBR [Jung et al. 2006]. It was demonstrated that the PEG membrane can successfully be used as biodegradable barrier membrane in the treatment of non-critical size defects in the rabbit skull and leads to similar amounts of bone regeneration as an ePTFE membrane. These results have driven further research to test the feasibility of this synthetic PEG material in a more clinically relevant model in dogs.

The aim of the present study was two-fold:

1. To evaluate the soft-tissue reaction of the synthetic PEG hydrogel when used as a barrier membrane for GBR compared with a standard resorbable collagen membrane.

2. To test whether or not the application of this in situ formed synthetic hydrogel will result in a similar amount of bone regeneration as a standard resorbable collagen membrane.

Material and methods

Animals

Eleven adult (14 months old) female beagle dogs (Harlan, France), weighing between 10 and 16 kg, were used in the present study. All animal experiments were performed at Biomatech NAMSA (Lyon, France). The animal facilities are accredited and registered at the French Department of Agriculture for animal housing, care and investigations. This study was conducted in accordance with the requirements of the FDA ‘Good Laboratory Practice’ (GLP) Regulations (21 CFR 58 of April 1, 2002) and the ‘Bonnes Pratiques de Laboratoire (BPL), arrêté du 14 Mars 2000’ described in the ’Journal Officiel du Ministère Français de l’Emploi et de la Solidarité du 23 mars 2000’. The protocol was submitted and accepted by the internal Biomatech NAMSA ethical committee.

Membranes and grafting materials

The synthetic test membrane investigated in the present study was a PEG-based hydrogel. Preparation of the PEG membranes was performed according to a previously published protocol [Jung et al. 2006]. In brief, the membranes were made by mixing a multi-arm PEG with thiol endgroups and a multi-arm PEG with acrylate endgroups in an aqueous buffer system (triethanolamine/HCl) at physiological pH [Institut Straumann AG, Basel, Switzerland]. In this system, the PEG termini connect through a highly self-selective addition reaction, forming a network with hydrolyzable ester linkages. The material was delivered in two syringes containing the PEGs dissolved in buffer. Subsequently, the two syringes were attached to a static mixer and were ready to use.

Collagen membranes [Bio-Gide®, Geistlich AG, Wolhusen, Switzerland] were used for positive controls. The collagen membranes were stabilized by using resorbable pins made of polyactic acid [Resorpin®, Geistlich AG].

As grafting material, either autogenous bone or synthetic hydroxyapatite [HA]/tricalcium phosphate [TCP] granules [Straumann Bone Ceramic®, Institut Straumann AG] were used. The autogenous bone chips were harvested with a bone scraper [Safescrap® Micros, Meta Company, Reggio Emilia, Italy] from the surrounding bone area.

Preoperative treatment

One week before each surgery an oral hygiene including dental calculus removal and disinfection was performed under drug sedation. Local disinfection with 0.2% chlorhexidine [CHX] swabs was performed once a day for 3 days before surgery. Each animal was anesthetized according to a standard procedure: tranquillization by atropine [Atropine, Aguetant, France], induction by tiletamine-zolazepam [Zoletil 100, Virbac, France] then thiopenthal sodique [Neslonid®, Merial, France] followed by inhalation of an O₂-N₂O halothane (1–4%) mixture [Halothane, Belamont, France].

Surgery 1 (tooth extraction and defect preparation)

Tooth extraction was performed after carefully elevating a full thickness flap and extracting bilaterally the lower premolars [P1, P2, P3, P4] and the first molar (M1). Subsequently, two bone defects were prepared on each side of the mandible in such a way that the buccal bone plate was surgically removed. The dimension of each defect measured 12 mm length, 8 mm height and 6 mm depth (Figs 1 and 3). The lingual cortical bone wall was left intact. Wound closure was achieved using non-absorbable sutures.
Surgery 2 (implantation and lateral ridge augmentation)

Three months after surgery 1, implantation and regenerative surgery were performed. Following a mid-crestal incision as well as buccal vertical-releasing incisions distal to the canine, a full thickness flap was carefully elevated. On each side of the mandible, two sites with clinically healed bone surfaces exhibited bone volume deficiencies. In each of the four defects, an experimental cylindrical implant with a large grit sandblasted and acid-etched surface (Institut Straumann AG) was placed. The implants had a diameter of 3.3 mm and a length of 8 mm. The implants were placed in such a way that the shoulder was located at the level of the alveolar bone crest resulting in a buccal dehiscence defect of approximately 6 mm in vertical direction. Implant positioning according to this protocol allowed having standardized healed dehiscence-type defects without the need for additional defect adjustments (Figs 3 and 4). At this time-point, the vertical defect extensions were measured from the top of the implant shoulder to the first bone-to-implant contact (BIC) at the mid-buccal aspect. Following implant placement, perforations of the buccal bone plate were made to enhance bleeding using a round bur. The four implants inserted in each dog were randomly allocated to following four treatment modalities:

- Group 1: (Test 1) PEG biodegradable synthetic membrane + autogenous bone chips
- Group 2: (Test 2) PEG biodegradable synthetic membrane + HA/TCP granules
- Group 3: (Control 1) bioresorbable collagen membrane + autogenous bone chips
- Group 4: (Control 2) autogenous bone chips without a membrane

At the test sites, the PEG hydrogel was applied in a viscous liquid form over the bone graft material and the adjacent ridge in order to completely cover the defect and the implant and to extend 2–3 mm beyond the defect margins (Figs 5 and 6). After approximately 1 min, the PEG membrane had set to its gelated status. At the control sites, the collagen membranes were trimmed and draped over the bone graft material and the adjacent ridge in order to overlap the defect margins 2–3 mm. Each membrane was secured at the buccal aspect with two resorbable pins and tuck well underneath the lingual flap (Fig. 7). To allow tension-free wound closure, the periosteum of the buccal flap was relieved along the entire base. Thereafter, primary wound closure was obtained with horizontal mattress and interrupted sutures.

Clinical follow-up and observation

Animals were observed daily and a detailed clinical follow-up was performed. The clinical aspect of soft tissue in the operated areas was recorded and photographs were taken once a week during the first month.

Histological preparation

One month before sacrifice, a single tetracycline labeling (Terramycin 100, Pfizer, France) injection was administered in all dogs to allow an evaluation of the bone mineralization on histological sections. After 2 and 6 months following implantation and regenerative surgery, six and five dogs were sacrificed, respectively. The animals were anesthetized by intramuscular injection in order to perform a fixation of the tissues by perfusion using approximately 300 ml of a 10% buffered formalin solution for each dog. The animals were then sacrified by a lethal dose of pento-

Fig. 1. Defects created at the time of teeth extraction – buccal view.

Fig. 2. Occlusal view of the defects.

Fig. 3. Implants placed into the defects after 3 months of defect healing – buccal view.

Fig. 4. Occlusal view of the implants after installation.

Fig. 5. Application of the polyethylene glycol (PEG) gel on autogenous bone chips.

Fig. 6. Sites after augmentation procedures: autogenous bone chips alone (left) (group 4) and HA/TCP granules, covered with the polyethylene glycol (PEG) membrane (group 2).

Fig. 7. Sites after augmentation procedure: autogenous bone chips, covered with bioresorbable collagen membrane, fixed with polylactide pins (left) (group 3) and polyethylene glycol (PEG) membrane, applied onto autogenous bone chips (group 1).

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barbitual. For histological evaluation, all sites were harvested with the intact surrounding soft tissues. A total of 44 sites were immersed in a 10% buffered formalin solution. Samples were dehydrated in alcohol solutions of increased concentrations and embedded in polymethylmethacrylate. From each specimen, two central orofacial sections through the implant were prepared for histological assessment. The final thickness (20–30 μm) of longitudinal sections through the defect was obtained by a micro-cutting and grinding technique [Donath & Breuner 1982]. Four of the sections from each dog were stained with modified Paragon for qualitative, semi-quantitative and quantitative light microscopic analysis. The remaining four sections were kept unstained for UV light observation of the bone labeling.

### Semi-quantitative histological evaluation
A semi-quantitative evaluation of the local soft-tissue tolerance according to the ISO 10993-6 standards was performed for each site (evaluation of fibrin, necrosis, osteolysis, tissue degeneration, inflammatory reaction, neutrophils, cosinophilic, lymphocytes, plasma cells, macrophages, giant cells – fibrous encapsulation, cartilaginous tissue formation, neovascularization and any other relevant parameters). The grading scale used was: 0 = absent, 1 = slight, 2 = moderate, 3 = marked, 4 = severe.

### Quantitative histomorphometric analysis
From each specimen, the central orofacial section through the implant was selected for quantitative assessment by applying standard morphometrical techniques [Wei-bel 1980; Gundersen et al. 1988]. Measurements were performed with a light microscope equipped with an analyzing system for color images (SAMBA®, SAMBA Technologies, Lyon, France).

The amount of newly formed bone was evaluated by the percentage of vertical bone gain at the buccal aspect of the implant. This was based on measuring the vertical distance (VD) between the original buccal bone crest and the crest of the newly formed bone as well as the vertical defect measurements of the former dehiscence:

\[
\text{Vertical bone gain (%) =} \frac{\text{VD of newly formed bone}}{\text{VD of the former defect}} \times 100
\]

The degree of BIC was evaluated in the former defect area. The assessment was made from the most coronal level of BIC to the bottom of the former defect according to previously published protocols [Bot-ticelli et al. 2003, Jung et al. 2003].

### Statistical analysis
Mean values and standard deviations were calculated for vertical bone gain, as well as for BIC. For statistical analysis, repeated measures analysis of variance (ANOVA) and subsequent pairwise Student's t-test with corrected P-values according to Bonferroni were used to detect the differences between the four treatment modalities. The level of significance chosen in all statistical tests was set at \( P < 0.05 \).

### Results

#### Two months results

**Clinical observations**
During the experiment no reductions in body weights were noted, and no adverse effects were reported regarding all six animals. After 30 days, all sites were healed normally except one site treated with the PEG membrane and autogenous bone revealing a very small opening \( \leq 1 \text{ mm} \) of the soft tissue. The observation at sacrifice revealed no macroscopic signs of local intolerance at any of the treated sites.

**Descriptive histology and semi-quantitative histological evaluation**
Because of angulations and malpositioning of three implants, a histological analysis of the sections could not be performed because of missing tissue parts. These three implant sites, making up one site of groups 1, 2 and 4, were therefore excluded from further analysis. The remaining 21 implants were well osseointegrated and showed in general a marked apposition of cortical bone or newly formed trabecular bone to the lingual implant surface with an endosteal bone ingrowth. The semi-quantitative histological observation revealed no biologically significant abnormal inflammatory response, infection or cellular change in the soft tissues in association with any of the treatment groups (Table 1). However, the inflammatory reaction observed around the PEG membrane was slightly greater to the one observed with the collagen membrane.

**Group 1** showed a few signs of autogenous bone chips that were embedded within the newly formed bone. Residual PEG hydrogel material was observed in three out of five samples. This group showed inconsistent results with slight to marked bone growth (Fig. 8).

In group 2, HA/TCP granules were visible in varying amounts. They showed poorly osseointegrated and were encapsulated into a fibroconnective tissue. This treatment group showed slight to marked bone regeneration and was the most consistent compared with other groups. Two sites clearly showed presence of fragments of the PEG material. The number of inflammatory cells observed around the fragments was slightly greater than the one observed around the collagen membrane (Fig. 9).

In group 3, the autogenous bone chips were not evident. The collagen membrane was visible in all sites and was infiltrated by a limited number of macrophages. This treatment group showed inconsistent results with slight to marked bone regeneration (Fig. 10).

**Group 4** revealed no observable autogenous bone chips after 2 months of healing. This group demonstrated inconsistent bone growth with slight to marked bone regeneration (Fig. 11).

**Quantitative histomorphometric analysis**
Groups 2 and 3 showed the highest vertical bone gain (45% and 44%, respectively), although no statistically significant difference was observed between the four treatment groups due to inconsistent results obtained in each group (Table 2).

The BIC within the former defect area was similarly high in groups 1, 2 and 4 and ranged between 81% and 83%. Group 3 revealed a BIC of 71% with no statistically significant differences across the four treatment groups (Table 2).

#### Six months results

**Clinical observations**
Three days after implantation and regenerative surgery signs of inflammation of the wound and desquamation of the surrounding epithelium were observed in almost all treated sites. The inflammation
Table 1. Semi-quantitative histological observation after 1 month of healing

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>PEG</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 8. Representative histological slide after 2 months of healing of group 1 treated with autogenous bone chips + polyethylene glycol (PEG) membrane (arrow indicating the most coronal portion of newly formed bone) (Paragon stain, original magnification × 1).

Fig. 9. Representative histological slide after 2 months of healing of group 2 treated with HA/TCP-granules + polyethylene glycol (PEG) membrane (arrow indicating the most coronal portion of newly formed bone) (Paragon stain, original magnification × 1).
disappeared completely between 10 and maximum 20 days post-surgery. The inflammation was found to be attributable to the use of 3% CHX instead of 0.3%. The daily application of the too highly concentrated CHX was stopped approximately 7 days after surgery. After this event, 3 out of 10 sites treated with the PEG membrane (1 × group 1 and 2 × group 2) showed an exposure of the membrane. These sites with membrane exposure healed uneventfully by secondary wound healing leaving part of the implant healing cap slightly visible at 30 days post-surgery. No other clinical abnormality was observed in the five animals in the course of the study following the initial inflammation.

Table 2. Quantitative histomorphometric analysis after 1 month of healing

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SD</th>
<th>PEG Membrane</th>
<th>HA, TCP Membrane</th>
<th>Collagen Membrane</th>
<th>Autologous Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical</td>
<td>30 ± 5</td>
<td>45 ± 20</td>
<td>50 ± 15</td>
<td>25 ± 20</td>
<td>30 ± 15</td>
</tr>
<tr>
<td>Bone</td>
<td>60 ± 10</td>
<td>49 ± 12</td>
<td>51 ± 10</td>
<td>38 ± 12</td>
<td>49 ± 12</td>
</tr>
</tbody>
</table>

Quantitative histomorphometric analysis
Measurement of the vertical bone gain from the previous baseline showed no statistically significant differences across the four groups with a range between 31% and 38% bone gain (Table 3).

The BIC measured within the former defect showed the highest BIC values for groups 2 and 3 [90 ± 9% and 91 ± 11%, respectively] followed by group 1 [76 ± 21%] and group 4 [49 ± 42%]. No statistically significant differences were detected across the groups (Table 4).

Discussion
With regard to the first aim of the present study, evaluating multiple parameters of the soft-tissue reaction, no local adverse effects in association with the newly developed PEG membrane compared with a standard collagen membrane were observed after 2 and 6 months. Concerning the second aim, the sites treated with the PEG membrane documented a similar amount of newly formed bone compared with defects regenerated with a collagen membrane. However, both membranes did not show a statistically significant difference compared with sites treated with autogenous bone without a membrane.
Fig. 12. Representative histological slide after 6 months of healing of group 1 treated with autogenous bone chips + polyethylene glycol (PEG) membrane [arrow indicating the most coronal portion of newly formed bone] (Paragon stain, original magnification × 1).

Fig. 13. Representative histological slide after 6 months of healing of group 2 treated with HA/TCP-granules + polyethylene glycol (PEG) membrane [arrow indicating the most coronal portion of newly formed bone] (Paragon stain, original magnification × 1).
The PEG material has been successfully investigated in several preclinical studies for the use as a matrix system to release bioactive molecules (Lutolf et al. 2003; Jung et al. 2007a, 2007b). The difference between the PEG material utilized as a matrix system and the presently used membrane lies in the chemical network structure. For membrane purposes, the PEG hydrogel consists of multi-arm PEG molecules with more and shorter arms than those used for the matrix system. It was demonstrated that the PEG gels consisting of multi-arm PEG molecules are cell-occlusive, due to the fact that the distances between the cross-linking points are several orders of magnitude smaller than the dimensions of a cell (Wechsler et al. 2008). The barrier function was examined after subcutaneous placement of the PEG gels in rats. Histological analysis revealed prevention of cellular penetration in the membrane group up to 4 months (Wechsler et al. 2008). In a recent study, the presently used PEG material has been successfully used to treat non-critical size bone defects in the rabbit skull (Jung et al. 2006). After 4 weeks of healing, histomorphometrical analysis and micro-computed tomography demonstrated similar amounts of newly formed bone for defects treated with the PEG membranes compared with defects treated with a standard ePTFE membrane. The ePTFE and the PEG membranes revealed statistically significantly more bone formation compared with defects treated without a membrane.

In addition to PEG as a matrix or a membrane for bone regeneration, it is presently used in other medical disciplines. The safety and effectiveness of a sprayable PEG material used as a barrier system in laparoscopic surgery has been assessed in randomized, prospective, controlled, clinical trials (Mettler et al. 2005). It was demonstrated that the PEG material was safe, well tolerated and there were no adverse effects attributed to the material and no patients in whom it could not be applied. These findings are in agreement with the present study evaluating multiple parameters of the soft-tissue reaction to the PEG material. The clinical and semi-quantitative histological evaluation did not reveal any biologically significant abnormal reaction in association with the tested membrane materials. The presently used collagen membrane was chosen as control treatment because it is the standard of care in terms of resorbable membranes in GBR (for review see Hämmerle & Jung 2005), and it showed good soft-tissue integration as well as cellular proliferation (Rothamel et al. 2004, 2005). In the present study, 6 months after regenerative surgery the collagen membranes were infiltrated with fibrocytes and macrophages whereas the PEG membrane revealed only macrophages. This points out that the PEG membrane is cell-occlusive for a prolonged time, also efficient during the course of degradation, but it might also indicate a less favorable soft-tissue integration.

The clinical evaluation of the soft-tissue coverage revealed a total of 9 % dehiscences within all 44 sites after 28 days. However, all soft-tissue dehiscences were found in the 22 sites treated with the PEG membrane revealing a membrane exposure rate of 18 %. This rate lies within the range of similar dog studies, showing an incidence of non-resorbable and resorbable membrane exposures from 20 % to 60 % [Buser et al. 1995; Von Arx et al. 2001; Oh et al. 2003]. Two reasons may explain the findings in the present study. First, three out of four soft-tissue dehiscences occurred in the group of animals treated with a very high

**Fig. 14.** Representative histological slide after 6 months of healing of group 3 treated with autogenous bone + collagen membrane (arrow indicating the most coronal portion of newly formed bone) (Paragon stain, original magnification × 1).

**Fig. 15.** Representative histological slide after 6 months of healing of group 4 treated with autogenous bone chips alone (arrow indicating the most coronal portion of newly formed bone) (Paragon stain, original magnification × 1).

**Table 4.** Quantitative histomorphometric analysis after 6 months of healing

<table>
<thead>
<tr>
<th></th>
<th>PEG</th>
<th>ePTFE</th>
<th>Collagen Membrane</th>
<th>Autogenous Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical bone</td>
<td>32 ± 17</td>
<td>22 ± 14</td>
<td>37 ± 15</td>
<td>20 ± 18</td>
</tr>
<tr>
<td>Horizontal bone</td>
<td>38 ± 18</td>
<td>36 ± 15</td>
<td>39 ± 17</td>
<td>29 ± 19</td>
</tr>
<tr>
<td>Bone to membrane ratio</td>
<td>0.68 ± 0.02</td>
<td>0.62 ± 0.03</td>
<td>0.70 ± 0.05</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Bone to implant ratio</td>
<td>1.04 ± 0.03</td>
<td>1.01 ± 0.02</td>
<td>1.10 ± 0.04</td>
<td>1.12 ± 0.03</td>
</tr>
</tbody>
</table>
concentration of CHX. After changing the CHX concentration to 0.2%, the soft tissue recovered and only parts of the implant cover screw revealed visible. Hence, the sites with exposed PEG membranes healed subsequently without additional complications, demonstrating that no membrane removal was necessary. Second, at both time-points (2 and 6 months), a slightly greater inflammatory reaction was observed for the PEG membrane compared with the collagen membrane. This delay in the soft-tissue integration together with a high dosage of CHX might explain premature exposures of the synthetic PEG membrane.

The dog model is well established to evaluate the outcome of bone regeneration with and without implant placement [Buser et al. 1995; Kohal et al. 1999; Von Arx et al. 2001; Lima et al. 2003; Oh et al. 2003]. However, a large variety of defect shapes and sizes have been tested. The majority of these defects are acute defects prepared at the time of regeneration surgery. In contrast, the present study utilized defect situations mimicking healed dehiscence-type osseous defects. Consequently, the inserted implants revealed their buccal surface exposed outside the bony envelope and no adjacent bone walls were present to support the membranes or protect the graft material. This defect can be considered to be challenging.

The use of a PEG membrane or a collagen membrane in the present study revealed a vertical bone gain on the buccal side of the exposed implants of 32–45% after 2 and 6 months. These values are similar or smaller compared with dog studies using smaller and acute dehiscence-type defects [Lima et al. 2003; Oh et al. 2003]. One study using a non-resorbable ePTFE membrane revealed a vertical bone gain of 45–86% after 4 and 6 months [Lima et al. 2003], and the other study showed a vertical bone gain of 57–67% for two resorbable collagen membranes after 4 months [Oh et al. 2003]. In a further dog study using a similar acute dehiscence-type defect, a synthetic resorbable and a non-resorbable ePTFE membrane were investigated without the use of grafting materials [Kohal et al. 1999]. After 6 months, a vertical bone gain of 51% could be observed in the non-resorbable membrane group whereas the resorbable membrane did not show any new bone formation. Bone regeneration was only detected at the base of the defect but minimal or no new bone formation was observed in the coronal, shallower defect part. These findings were attributed to a membrane collapse and the fact that the bony borders of the defects were too small to allow osteo-progenitor cells to repopulate the implant surface [Kohal et al. 1999]. In contrast, a recent dog study using saddle-type osseous defects demonstrated that a similar synthetic resorbable membrane was able to protect the defect and allowed a greater amount of bone regeneration compared with a standard collagen membrane [Stavropoulos et al. 2004]. All these different and sometimes contradictory results within similar dog studies indicate that the outcome of bone regeneration using a variety of GBR materials depends very much on the size and shape of the bone defects. In the present study, the membrane groups were not able to show a statistically significant increase of the amount of vertical bone gain compared with autogenous bone without a membrane. Possible reasons are: [a] the challenging defect design offering no adjacent bone walls to reduce the pressure of the alveolar mucosa on the augmentate, [b] very little bone activity due to the healed dehiscence type of bone defect, [c] the tested resorbable membranes in combination with a grafting material showed insufficient mechanical properties to maintain the entire space for regeneration and [d] because of gravity the graft material has been sagged apically.

Although the vertical bone gain was moderate in the present study, the BIC in the area of newly formed bone was very high in all treatment groups ranging from 49% to 91% after 2 and 6 months [Fig. 16]. Other dog studies reported BIC values of 11–45% [Oh et al. 2003], 29–47% [Kohal et al. 1999] and 68–77% [Botticelli et al. 2004]. The BIC values of the present study indicate that a high-quality bone and good implant integration was reached in the area of newly formed bone. This was true not only in the groups with autogenous bone but also in the group using a grafting material consisting of HA/TCP. However, the graft material was found to be mainly surrounded by fibroconnective tissue and showed a relatively low bone-to-substitute contact. This is in contrast to other clinical and animal studies reporting good osteo-conductive properties of the same or similar graft material in different indications [Piattelli et al. 1996; Schwartz et al. 1999; Boix et al. 2004; Jung et al. 2007b]. The reason for this observation is unclear. One possible explanation could be that some parts of the surface of this graft material undergo significant remodeling and hence mineralized bone is not directly deposited on the surface in those areas. This finding is supported by a recent
clinical and histomorphometric study, showing relatively high amount of newly formed bone with a low osteoconductivity when using HA/TCP (Cordaro et al. 2008). In addition, it is also known that the stability of the grafted area is a prerequisite for bone regeneration (Witkiewicz & Nilveus 1990). Hence, it might be speculated that the insufficient mechanical protection of the graft material by the membranes and the gravity led to micro movements of the granules and, therefore, to a fibrous encapsulation.

One major advantage of the presently used PEG membrane is the clinical handling and the possibility to apply the membrane directly intra-operatively. Hence, no time is needed to cut and shape a standard preformed GBR membrane. In recent human and animal studies, a membrane composed of polyactic acid dissolved in N-methyl-pyrrolidone was investigated as a barrier in procedures aimed at regeneration of lost periodontal tissues (Bogle et al. 1997; Garrett et al. 1997).

Although this material can be precipitated from solution in situ by adding water [Rosen & Reynolds 1999], in the majority of the studies a membrane made of the polymer was formed extraorally, before it was applied to cover the periodontal defect [Garrett et al. 1997; Jepsen et al. 2000]. The favorable viscosity of the presently used PEG material makes this gel suitable for its clinical application without leakage of material into the adjacent areas. Hence, the PEG hydrogel used as a barrier membrane would represent an improvement for future GBR procedures.

In conclusion, with regard to the first aim the in situ forming synthetic membrane made of PEG was documented as safe in the present study, revealing no biologically significant abnormal soft-tissue reaction compared with a standard collagen membrane. Regarding the second aim, histomorphometrical analysis demonstrated similar amounts of newly formed bone for defects treated with the PEG membrane compared with defects treated with a standard collagen membrane. However, both membranes did not show a statistically significant difference compared with sites treated with autogenous bone without a membrane. All inserted implants obtained after 2 and 6 months of healing, a high percentage of BIC, irrespective of which of the four tested grafting techniques was used.

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References


Chapter 4


