CLINICAL ORAL IMPLANTS RESEARCH

Frank Schwarz
Ilja Mihatovic
Vladimir Golubovic
Andrea Hegewald
Jürgen Becker

Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs: part 1. Augmentation using bone graft substitutes and autogenous bone

Authors' affiliations:
Frank Schwarz, Ilja Mihatovic, Vladimir Golubovic, Andrea Hegewald, Jürgen Becker, Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany

Corresponding author:
Frank Schwarz
Department of Oral Surgery
Heinrich Heine University
Westdeutsche Kieferklinik
D-40225 Düsseldorf
Germany
Tel: +49 211 810 4471
Fax: +49 211 171 3542
e-mail: frank.schwarz@med.uni-dueseldorf.de

Date:
Accepted 15 February 2011

To cite this article:
Schwarz F, Mihatovic I, Golubovic V, Hegewald A, Becker J. Influence of two barrier membranes on staged guided bone regeneration and osseointegration of titanium implants in dogs: part 1. Augmentation using bone graft substitutes and autogenous bone.
Clin. Oral Impl. Res. **23**, 2012; 83–89.
doi: 10.1111/j.1600-0501.2011.02187.x

Key words: alveolar ridge defect, animal study, barrier membranes, bone substitutes, guided bone regeneration

Abstract

Objectives: To assess the influence of two barrier membranes and two bone graft substitutes mixed with autogenous bone (AB) on staged guided bone regeneration and osseointegration of titanium implants in dogs.

Materials and methods: Four saddle-type defects each were prepared in the upper jaw of six fox hounds and randomly filled with a natural bone mineral (NBM) + AB and a biphasic calcium phosphate (SBC) + AB and allocated to either an *in situ* gelling polyethylene glycol (PEG) or a collagen membrane (CM). At 8 weeks, modSLA titanium implants were inserted and left to heal in a submerged position. At 8 + 2 weeks, dissected blocks were processed for histomorphometrical analysis (e.g., treated area [TA], bone-to-implant contact [BIC]).

Results: The mean TA values (mm^2) and BIC values (%) tended to be higher in the PEG groups(TA: NBM + AB [10.4 ± 2.5]; SBC + AB [10.4 ± 5.8]/BIC: NBM + AB [86.4 ± 20.1]; SBC + AB [80.1 ± 21.5]) when compared with the corresponding CM groups (TA: NBM + AB [9.7 ± 4.8]; SBC + AB [7.8 ± 4.3]/ BIC: NBM + AB [71.3 ± 20.8]; SBC + AB [72.4 ± 20.3]). A significant difference was observed for the mean TA values in the SBC + AB groups.

Conclusion: It was concluded that all augmentation procedures investigated supported bone regeneration and staged osseointegration of modSLA titanium implants. However, the application of PEG may be associated with increased TA values.

The basic concept of guided bone regeneration (GBR) involves the placement of a barrier membrane to protect the blood clot and create a secluded space around the bone defect (BD), thus enabling access for bone-forming cells without competition from other tissues (Dahlin et al. 1988). Several design requirements such as biocompatibility, cell occlusivity, volume stability, tissue integration, nutrient transfer and also ease of use in the clinic have been proposed for a material that is intended for use as a barrier for GBR procedures (Hardwick et al. 1994). Nowadays, most investigations focus on the use of a porcine-derived native type I and type III collagen membrane (CM), featuring a pronounced tissue integration and semipermeability, thus facilitating nutrient transfer during the early stages of wound healing (Rothamel et al. 2005; Schwarz et al. 2006, 2008). However, a potential drawback particularly of native collagen is the fast biodegradation, resulting in a reduced ability to maintain space, thus compromising the secluded wound area (Sela et al. 2003; Rothamel et al. 2005). Recently, an *in situ* gelling hydrogel composed of two polyethylene glycol (PEG) components was introduced to serve as a new material for barrier membranes (Jung et al. 2006). Upon mixing, PEG is applied as a viscous liquid and gels on the application site within 20–50 s. During wound healing, the cross-linked PEG compounds are completely degraded by hydrolysis without acidic products or causing any foreign body reactions in the adjacent tissues (Wechsler et al. 2008; Herten et al. 2009). The hydrolytic disruption of PEG specimens was associated with an ingrowth of blood vessels at 4 weeks and a prolonged biodegradation at 16–24 weeks after subcutaneous implantation in rats (Herten et al. 2009). Experimental studies have indicated that this material is highly biocompatible and cell-occlusive, and demonstrated at least similar amounts of newly formed bone in the former

defect area when compared with other type of barriers (e.g. expanded polytetrafluorethylene, CM, polylactides, polyglykolides) (Jung et al. 2006, 2009b; Thoma et al. 2009; Schwarz et al. 2010). Because of its liquid consistency during application, it is, however, mandatory to combine the PEG membrane with grafting materials. In recent preclinical studies on localized ridge augmentation simultaneous with implant placement (i.e. one-stage GBR), the PEG membrane was applied over two types of bone fillers including particulated autogenous bone (AB) or a biphasic calcium phosphate (SBC) (Jung et al. 2009b; Schwarz et al. 2010). After 6 months of submerged healing, both groups revealed similar amounts of new bone formation and bone-to-implant contact (BIC), which appeared to be on a level equivalent to that noted when both types of bone fillers were combined with CM (Jung et al. 2009b). In a randomized, controlled clinical trial, a comparable vertical bone fill around dental implants was also noted when both PEG and CM were combined with a bovine-derived natural bone mineral (NBM) (Jung et al. 2009a). Based on these findings, it might be suggested that one-stage GBR using PEG in combination with either AB, NBM or SBC may result in similar histological and/or clinical outcomes as the application of CM. However, when considering the improved volume stability and prolonged barrier function noted for PEG in comparison with CM (Herten et al. 2009), one may speculate that these specific physicochemical properties improve bone regeneration in more advanced defect sites, requiring a staged GBR and implant placement. Even though the survival rates of implants placed in augmented bone have been reported to be comparable to the rates of implants placed in pristine bone, it still remains unknown to what extent a staged GBR procedure may influence the initial process of osseointegration (Jensen & Terheyden 2009). So far, these issues have not been addressed either for PEG or for CM.

Therefore, the aim of the present study was to assess the histological outcome (i.e. new bone formation, BIC) of a staged GBR procedure using a combination of PEG and CM either with NBM + AB or SBC + AB for localized ridge augmentation and subsequent implant placement at saddle-type defects in a dog model.

Material and methods

Animals

In the present study, a total of six fox hounds (age 18–22 months, weight 34–42 kg) were included. All animals exhibited a fully erupted permanent dentition. During the experiment, the dogs were fed once per day with soft-food diet and water *ad libitum*. Animal selection, management and surgery protocol were approved by the Animal Care and Use Committee of the Heinrich Heine University and the local government of Düsseldorf. The experimental segment of the study started after an adaption period of 4 weeks.

Study design and randomization

The study was performed in three surgical phases.

In the first phase, the mandibular and maxillary first, second, third, fourth premolar as well as the first and second molar (P1–M2) were extracted. After a healing period of 10 weeks, a total of four standardized saddle-type defects (mesio-distal width: 10 mm; height: 8 mm) were bilaterally prepared in the upper jaw of each dog (i.e. $n = 4$ defects per animal in the upper jaw).

The defects were filled using NBM + AB and SBC + AB with a random assignment of both treatment procedures to anterior and posterior sites. Subsequently, treated defects were randomly allocated in a split-mouth design to the application of either PEG or CM. Accordingly, all dogs received the following treatment procedures:

NBM + AB + PEG and SBC + AB + PEG vs.
NBM + AB + CM and SBC + AB + CM

At 8 weeks, modSLA titanium implants ($n = 4$ per animal in the upper jaw) were inserted at the respective treated defect sites and left to heal in a submerged position for 2 weeks.

Randomization was performed according to a computer-generated list (RandList®, DatInf GmbH, Tübingen, Germany). The animals were killed after a healing period of 8 + 2 weeks.

Surgical procedure

Before each surgical intervention, intramuscular sedation was accomplished with 0.17 mg/kg acepromazine (Vetranquil 1%, Ceva Tiergesundheit, Düsseldorf, Germany). Subsequently, anesthesia was initiated using 21.5 mg/kg thiopental-sodium (Trapanal 2.5%, Altana GmbH, Konstanz, Germany). During all the surgical procedures, inhalation anesthesia was performed using oxygen and nitrous oxide and isoflurane. To maintain hydration, all animals received a constant-rate infusion of lactated Ringer's solution while anesthetized. Intraoperative analgesia was performed by an intravenous injection of 0.4 mg/kg piritramid (Dipidolor®, Janssen-Cilag GmbH, Neuss, Germany) and 4.5 mg/kg carprofene (Rimadyl®, Pfitzer Pharma GmbH, Karlsruhe, Germany). For postoperative treatment, piritramid and carprofene were applied subcutaneously for 3 days at the same dose as described before.

Surgical phase 1 (tooth extraction)

In the first surgery, mucoperiosteal flaps were reflected bilaterally in both jaws and P1–M2 were carefully removed after tooth separation. Wound closure was accomplished by means of mattress sutures and the sites were allowed to heal for 10 weeks. Prophylactic administration of clindamycine (11 mg/kg body weight, Cleorobe®, Pharmacia Tiergesundheit, Erlangen, Germany) was performed intra- and postoperatively for 10 days.

Surgical phase 2 (defect creation and GBR)

After 3 months of healing, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the alveolar bone in the upper jaws. Vertical releasing incisions were placed about 4–5 mm distant to the designated experimental sites. Four standardized saddle-type defects including the vestibular and oral aspect of the alveolar ridge were subsequently prepared bilaterally at a distance of at least 5 mm using a straight fissure carbide bur. After bone block removal, the final dimension of all defects revealed a mesio-distal width of 10 mm and an apico-coronal height, as measured from the crestal bone, of 8 mm (Fig. 1a). The defect sizes were standardized using a periodontal probe (PCP12, Hu-Friedy Co., Chicago, IL, USA). All osteotomy procedures were performed under copious irrigation with a sterile 0.9% physiological saline. Finally, each defect site was rinsed thoroughly with sterile saline to completely remove any residual debris. Subsequently, the respective defects were homogeneously filled with a particulate NBM (Geistlich BioOss® spongiosa granules, particle size 0.25–1 mm, Geistlich Biomaterials, Wolhusen, Switzerland) or SBC (60% HA + 40% β-TCP, Straumann Bone Ceramic®, pore diameters: 100–500 μm, Institut Straumann AG, Basel, Switzerland). Particular care was taken that the graft particles did not exceed the contour of the bordering bone walls in both the vestibular/oral and the cranial directions. Before treatment, both NBM and SBC were homogeneously mixed (ratio 1 : 1) with particulated AB (particle size 0.5–1 mm) harvested from the cancellous aspect of the bone blocks. Following treatment, CM (Geistlich BioGide®, Geistlich Biomaterials) was adapted over the respective defect areas so as to cover 1–2 mm of the surrounding alveolar bone. Neither sutures nor pins were used for membrane fixation or stabilization (Fig. 1b). At the contralateral sites, excess of blood was removed from the surrounding bone. Afterwards, the PEG hydrogel (MembraGel®, Institut Straumann AG) was applied in a viscous form, also extending 1–2 mm beyond the margins of the defect walls. After approximately 60 s, the PEG membrane had set to its gelated status (Jung et al. 2009b) (Fig. 1c). Following periosteal-releasing incisions, the mucoperiosteal flaps were advanced, repositioned tension-free in a

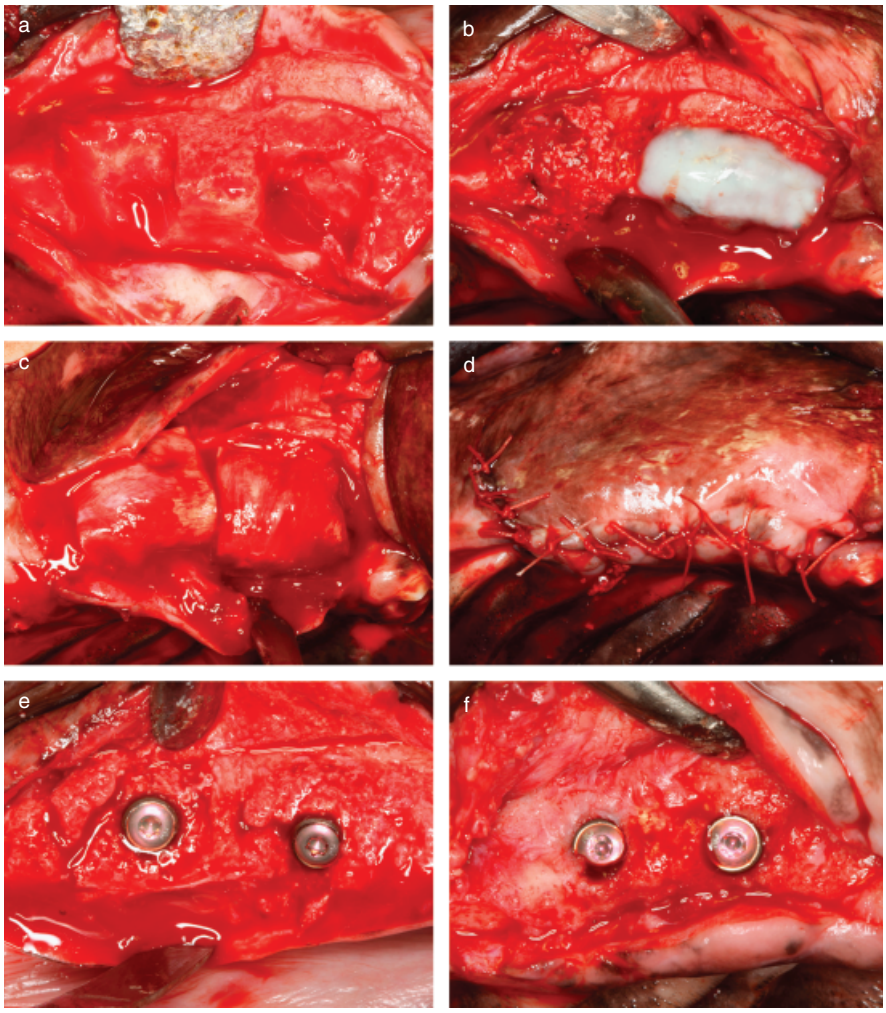

Fig. 1. (a) At 10 weeks after tooth extraction, a total of $n = 4$ standardized saddle-type defects (width: 10 mm; height: 8 mm) were bilaterally created at a distance of at least 5 mm in each upper jaw of 6 dogs. Occlusal view indicating that both vestibular and lingual bone plates were removed. (b) In each hemimandible, the defects were homogeneously filled with NBM + AB and SBC + AB (ratio 1 : 1). Experimental sites receiving the PEG hydrogel after the removal of excess blood from the surrounding bone. Situation at about 60 s after application indicating a gelated status of the material. PEG application at the anterior defect is not shown in this figure. (c) Contralateral sites receiving CM that were adapted in such a way as to cover 1–2 mm of the surrounding alveolar bone. (d) Experimental sites were left to heal in a submerged position for 8 weeks. (e) At reentry, the defect borders were clearly demarcated by residual particles of the respective bone substitutes. Implants were placed at the central aspect of each defect site in such a way that IS at best coincided with the regenerated bone crest at both vestibular and oral aspects (left site: NBM + AB + CM; right site: SBC + AB + CM). (f) A corticalization of the newly formed bone was more frequently observed at PEG-treated sites (left site: SBC + AB + PEG; right site: NBM + AB + PEG). NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol; CM, collagen membrane; IS, implant shoulder.

coronal position and fixed with vertical or horizontal mattress sutures (Resorba®, Resorba Wundversorgung GmbH & Co. KG, Nürnberg, Germany) in such a way as to ensure a submerged healing condition (Fig. 1d).

Surgical phase 3 (implant placement)

In the third surgery, midcrestal incisions were made and mucoperiosteal flaps were reflected to expose the experimental sites for implant placement. All granulation tissue was carefully removed from the residual defect areas. A total of $n = 4$ implant sites were prepared bilaterally, at the central aspect of each experimental site, using a low-trauma surgical technique under copious irrigation with sterile 0.9% physiological saline (surgery protocol by Institut Straumann AG). Thereafter, screw-type modSLA (Bone Level® SLActive®, ∅4.1 mm, length 10 mm, Institut Straumann AG) titanium implants were inserted with good primary stability (i.e. lack of clinical implant mobility) in such a way that the implant shoulder (IS) at best coincided with the regenerated bone crest at both vestibular and oral aspects (Fig. 1e and f). Following the application of closure screws, the mucoperiosteal flaps were repositioned and fixed with vertical or horizontal mattress sutures (Resorba®) in such a way as to ensure a submerged healing condition.

All surgical procedures were performed by two experienced surgeons (F. S. and I. M.).

Animal sacrifice and retrieval of specimens

After a healing period of 8 + 2 weeks, the animals were killed by an overdose of sodium pentobarbital 3%, respectively. The oral tissues were fixed by perfusion with 10% buffered formalin administered through the carotid arteries. The jaws were dissected and blocks containing the experimental specimens were obtained. All specimens were fixed in a 10% neutral-buffered formalin solution for 4–7 days.

Histological preparation

The specimens were dehydrated using ascending grades of alcohol and xylene, infiltrated and embedded in methylmethacrylate (MMA, Technovit 9100 NEU, Heraeus Kulzer, Wehrheim, Germany) for nondecalcified sectioning. During this procedure, any negative influence of polymerisation heat was avoided due to a controlled polymerization in a cold atmosphere (−4°C). After 20 h, the specimens were completely polymerized. Each implant site was cut in the buccooral direction along with the long axis of the implant using a diamond band saw (Exakt®, Apparatebau, Norderstedt, Germany). Serial sections were prepared from the central defect area, resulting in four sections approximately 300 µm in thickness each (Donath 1985). Only implant sections showing an inner thread were chosen for the histological evaluation. Subsequently, all specimens were glued with acrylic cement (Technovit 7210 VLC, Heraeus Kulzer, Wehrheim, Germany) to silanized glass slides (Super Frost, Menzel GmbH, Braunschweig, Germany) and ground to a final thickness of approximately 40 µm. All sections were stained with toluidine blue to evaluate new bone formation. With this technique, old bone stains light blue, whereas newly formed bone stains dark blue because of its higher protein content (Schenk et al. 1984).

Histomorphometrical analysis

Histomorphometrical analyses as well as microscopic observations were performed by one experienced investigator masked to the specific experimental conditions (A. H.). For image acquisition, a color CCD camera (Color View III, Olympus, Hamburg, Germany) was mounted on a binocular light microscope (Olympus BX50, Olympus). Digital images (original magnification × 200) were evaluated using a software program (Cell D®, Soft Imaging System, Münster, Germany).

The following landmarks were identified in the stained sections at both vestibular and oral aspects: the IS and the bottom of the BD. Defect length (DL) was measured from IS to BD (mm); the amount of new BIC in the defect was measured as percentage of the distance from BD to IS, serving as 100% (Fig. 2). Additionally, the treated

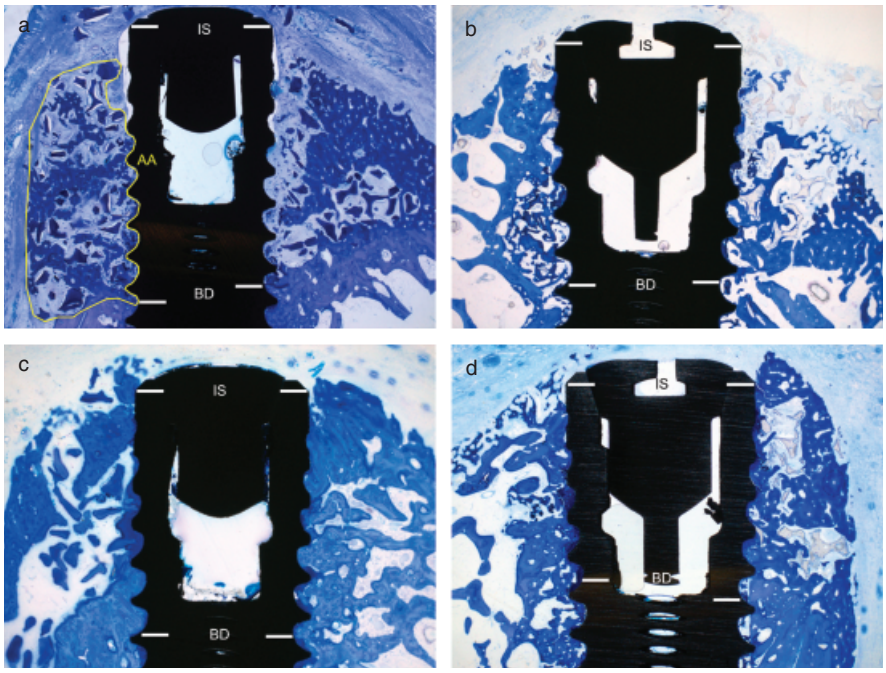

Fig. 2. Representative histological views (vestibulo-oral sections, toluidine blue stain) of wound healing at 8 + 2 weeks (original magnification × 25). Histological observation commonly revealed a homogeneous bone formation and osseointegration within the secluded volume created by both CM and PEG barrier membranes. A corticalization of the cancellous bone was noted in the periphery of the treated area. (a) NBM + AB + CM, (b) SBC + AB + CM, (c) NBM + AB + PEG, (d) SBC + AB + PEG. BD, bottom of the bone defect; TA, treated area; IS, implant shoulder; NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol; CM, collagen membrane.

area (TA) (mm²) was measured from BD to IS. Within TA, the surface area of mineralized- (MT) and nonmineralized tissue (NMT) as well as residual NBM/SBC particles (BS) were automatically assessed (mm²) by the image analysis software. Before the start of the morphometrical analysis, a calibration procedure was initiated for the image analysis software and revealed that repeated measurements of $n = 12$ different sections were similar at >95% level.

Statistical analysis

The statistical analysis was performed using a commercially available software program (PASW Statistics 19.0, SPSS Inc., Chicago, IL, USA). The mean values and standard deviations among animals were calculated for each variable and group. The data rows were examined using the Kolmogorow–Smirnow test for a normal distribution. Within-group comparisons were performed using the paired *t*-test (i.e. vestibular and oral aspects). For the comparisons between groups at 8 + 2 weeks, the unpaired *t*- test was used. The α error was set at 0.05.

Results

Clinical healing

The postoperative healing was considered as generally uneventful in all dogs. No complications such as allergic reactions, swellings, abscesses or infections were observed throughout the entire study period. None of the defect sites revealed a premature exposure of the wound area (i.e. exposure of barrier membranes or titanium implants). Surgical reentry at 8 weeks indicated that the volume of the treated sites had been fully maintained in all groups investigated, thus resulting in a homogeneous bony contour at either vestibular or oral aspects. Both NBM and SBC particles appeared to be well integrated into a newly formed hard tissue. A corticalization of the newly formed bone was more frequently observed at PEG-treated sites (Fig. 1e and f).

Descriptive histology

Histological observation at 8 + 2 weeks revealed a bony filling of the secluded wound area confined by both CM and PEG membranes. In particular, a scaffold of cancellous bone with numerous blood vessels and bone-forming cells homogeneously invaded the former defect area and appeared to be in close contact with both NBM and SBC as well as residual AB particles. A transformation of the cancellous bone into cortical bone was commonly observed in the periphery of the former defect area (Fig. 2a–d). Moreover, it was observed that SBC particles revealed an increased contact with NMT, while NBM particles were more frequently surrounded by MT (Fig. 3a–c). In general, histological observation failed to demonstrate any osteoclastic activity at the surface of both bone graft particles. However, a dissolution of SBC resulting in a superficial disintegration of particles into individual grains was commonly observed in areas where the bone graft particles were surrounded by NMT (Fig. 3d). In all groups investigated, modSLA implants seemed to be surrounded by a firmly attached mature, parallel-fibered woven bone. Occasionally, the implant surfaces were in direct contact with the NBM and SBC particles. These areas did not reveal any interposition of NMT between the implant surface and the respective bone graft particles (Fig. 3a and c). The maturity of the woven bone was identifiable by the development of primary osteons and appeared to be comparable in both regenerated and pristine areas (Fig. 3a–c).

Histomorphometric analysis

The mean values of DL, TA, MT, NMT, BS and BIC in both groups after 8 + 2 weeks of healing are presented in Tables 1–3. Basically, within-group comparisons in the CM group revealed comparable ($P > 0.05$; paired *t*-test, respectively) mean DL, TA, MT, NMT and BIC values at both NBM + AB- and SBC + AB-treated sites. A significant difference between vestibular and oral aspects was only observed for the mean BS values in the SBC + AB + CM group ($P < 0.05$; paired *t*-test) (Table 1). Similarly, the PEG groups also revealed comparable mean TA, MT, BS and BIC values ($P > 0.05$; paired *t*-test, respectively) at both vestibular and oral aspects. Within-group comparisons only revealed a significant difference for the mean DL and NMT values ($P < 0.01$, $P < 0.05$; paired *t*-test, respectively) in the NBM + AB + PEG group (Table 2). Both NBM + AB + PEG and SBC + AB + PEG groups tended to reveal increased TA, MT and BIC values when compared with the respective CM groups (Fig. 3a–c). However, statistical analysis only revealed a significant difference for the mean TA values between SBC + AB + CM- and SBC + AB + PEG-treated sites (Table 3).

Discussion

The present experimental study was designed to histologically evaluate the outcome of a staged GBR procedure using a combination of PEG and CM either with NBM + AB or SBC + AB for localized ridge augmentation and subsequent implant placement at saddle-type defects in a dog model. In this context, it must be emphasized that this type of defect model is commonly used and well accepted to evaluate GBR procedures in canines (Schenk et al. 1994; Simion et al. 1999; Bornstein et al. 2007). Within its limitations, the present data have indicated that all augmentation procedures investigated resulted in a homoge-

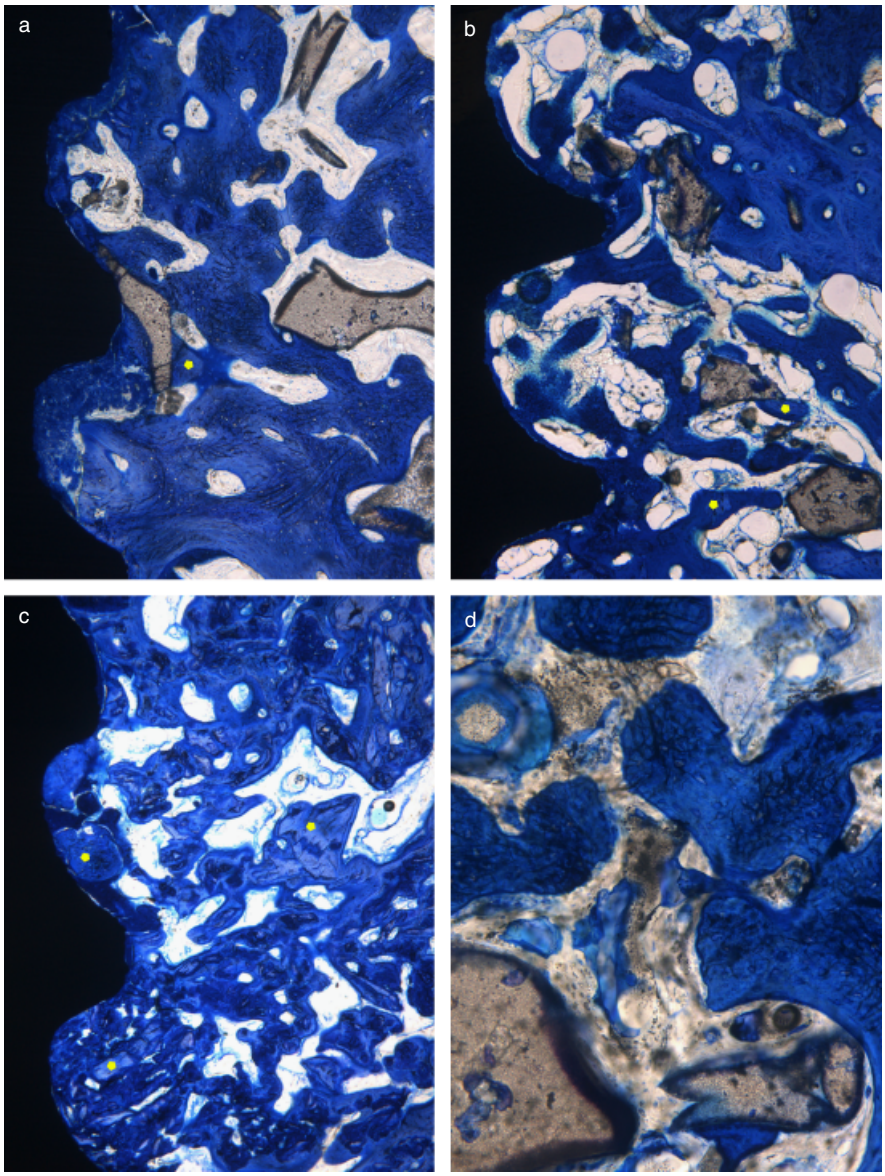

Fig. 3 Both NBM and SBC particles as well as residual AB were homogeneously integrated into a dense network of cancellous bone. The application of PEG appeared to be associated with an increased density of MT within TA (a–c). (a) SBC + AB + PEG (original magnification × 100), (b) SBC + AB + CM (original magnification × 100), (c) NBM + AB + PEG (original magnification × 100). SBC particles were more frequently surrounded by NMT than either NBM or AB particles. In these areas, however, a dissolution of SBC was commonly observed. (d) SBC + AB + PEG (original magnification × 200). Yellow polygons indicate residual autogenous bone particles. NBM, natural bone mineral; AB, autogenous bone; MT, mineralized tissue; PEG, polyethylene glycol; TA, treated area; CM, collagen membrane; NMT, nonmineralized tissue.

Table 1. **Mean values (± SD) of DL (in mm), TA, MT, NMT, BS (in mm^2) and BIC (in %) in the CM group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing ($n = 6$ dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM + AB						
Vestibular	5 ± 1.8	10.2 ± 4.8	5.3 ± 3.9	4.1 ± 1.5	0.9 ± 0.4	61.8 ± 26.5
Oral	5.2 ± 2.5	9.1 ± 5.3	4.1 ± 2.2	4.2 ± 3	0.9 ± 0.6	80.8 ± 6.8
P value*	NS	NS	NS	NS	NS	NS
SBC + AB						
Vestibular	3.7 ± 0.6	6.8 ± 2.9	3.9 ± 2.4	2.4 ± 0.9	0.5 ± 0.4	72.4 ± 10.2
Oral	5.5 ± 3	8.7 ± 3.7	3.7 ± 1.3	3.4 ± 2.8	1.5 ± 0.7	72.4 ± 27.2
P value*	NS	NS	NS	NS	<0.05	NS

*Comparisons within groups (paired *t*-test).
DL, defect length; TA, treated area; MT, mineralized tissue; NMT, nonmineralized tissue; BS, bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; NBM, natural bone mineral; AB, autogenous bone.

neous bone formation and subsequently osseointegration of modSLA implants within the confined wound area at 8 + 2 weeks. Within-group comparisons revealed comparable TA values at both vestibular and oral aspects, thus indicating that both types of barrier membranes provided a sufficient stabilization of the wound area over the entire observation period. However, histological analysis has pointed to increased TA values at PEG-treated sites when compared with the corresponding CM sites, even reaching statistical significance in the SBC + AB groups. Basically, the observation that NBM + AB + CM may be associated with a predictable bone formation in saddle-type defects is in agreement with previous experimental data (Bornstein et al. 2007). In particular, after 8 and 16 weeks of submerged healing, a dome-shaped bone regeneration was observed above the bottom of the defects. The mean TA values at 8 weeks ranged from about 28.8 mm^2 in the control group (i.e. NBM + AB without membrane application) to about 30.2 mm^2 at NBM + AB + CM-treated sites (values calculated from the data provided in the publication) and remained almost stable in both groups at 16 weeks (Bornstein et al. 2007). The lower overall mean TA values, as noted in the present study, can mainly be attributed to the surface area occupied by modSLA titanium implants, which was not considered for the histomorphometrical analysis. The observation that the application of CM was not associated with an improved outcome of healing (Bornstein et al. 2007) coupled with the finding of the present study that PEG treated sites revealed increased TA scores in comparison with the corresponding CM groups may point to the beneficial effect of a prolonged barrier function, as noted for the *in situ* gelling hydrogel (Herten et al. 2009; Thoma et al. 2009). In this context, it must be emphasized that chemical cross-linking of CM was also proven to be associated with improved membrane stability and bone regeneration in both animal and human studies (Bornstein et al. 2007; Schwarz et al. 2008; Becker et al. 2009). However, in the case of a premature membrane exposure, cross-linking obviously impaired soft-tissue healing or caused wound infections (Bornstein et al. 2007; Becker et al. 2009). In contrast, the *in situ* formed PEG membrane was safely used in a variety of indications, revealing no biologically significant abnormal soft tissue reactions compared with different GBR membranes (e.g. CM) (Jung et al. 2006, 2009b, 2009a; Thoma et al. 2009). The observation that the application of both CM and PEG may be associated with minimal or even no membrane exposures is also in agreement with previous experimental animal studies (Jung et al. 2006; Bornstein et al. 2007; Schwarz et al. 2010).

Table 2. **Mean values (± SD) of DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in%) in the PEG group at both vestibular and oral aspects after 8 + 2 weeks of submerged healing (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
NBM + AB						
Vestibular	5.5 ± 0.8	12.8 ± 4.2	6.1 ± 2.6	5.8 ± 2.6	0.9 ± 0.4	85.7 ± 23.9
Oral	3.9 ± 0.7	7.9 ± 6.5	4.7 ± 3.9	2.3 ± 1.9	0.9 ± 0.8	87 ± 17.7
P value*	<0.01	NS	NS	<0.05	NS	NS
SBC + AB						
Vestibular	6.8 ± 1.9	10.5 ± 2.6	4.9 ± 2.2	4.5 ± 2.2	1.1 ± 0.5	83.9 ± 16.4
Oral	5.2 ± 1.1	10.2 ± 2.5	5.4 ± 1.7	4 ± 1.7	0.8 ± 0.7	76.3 ± 26.7
P value*	NS	NS	NS	NS	NS	NS

*Comparisons within groups (paired *t*-test): $P > 0.05$.

DL, defect length; TA, treated area; MT, mineralizedtissue; NMT, nonmineralized tissue; BS, bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; NBM, natural bone mineral; AB, autogenous bone.

Table 3. **Between-group comparison of mean (± SD) DL (in mm), TA, MT, NMT, BS (in mm²) and BIC (in %) values at 8 + 2 weeks (*n* = 6 dogs)**

Group	DL	TA	MT	NMT	BS	BIC
CM						
NBM + AB	5.1 ± 2.1	9.7 ± 4.8	4.7 ± 3	4.1 ± 2.2	0.9 ± 0.5	71.3 ± 20.8
SBC + AB	4.7 ± 2.4	7.8 ± 3.4*	3.9 ± 1.7	2.9 ± 2.2	1 ± 0.8	72.4 ± 20.3
PEG						
NBM + AB	4.7 ± 1.1	10.4 ± 5.8	5.4 ± 3.3	4.1 ± 2.8	0.9 ± 0.6	86.4 ± 20.1
SBC + AB	5 ± 1.7	10.4 ± 2.5*	5.2 ± 1.9	4.3 ± 1.9	0.9 ± 0.6	80.1 ± 21.5
P value*	NS	<0.05	NS	NS	NS	NS

*Comparisons between groups (unpaired t-test).

DL, defect length; TA, treated area; MT, mineralized tissue; NMT, nonmineralized tissue, BS, Bone substitute (i.e residual NBM/SBC particles); BIC, bone to implant contact; CM, collagen membrane; NBM, natural bone mineral; AB, autogenous bone; PEG, polyethylene glycol.

Histological observation has indicated that both NBM + AB and SBC + AB scaffolds were equally and homogeneously integrated into a newly formed scaffold of cancellous bone at 8 + 2 weeks. Currently, the osteoconductive properties of both bone graft substitutes have only been compared for human maxillary sinus augmentation (Cordaro et al. 2008; Froum et al. 2008; Lindgren et al. 2010). In particular, it was observed that NBM and SBC produced comparable amounts of newly formed bone, exhibiting a similar histologic appearance. However, it was also noted that SBC-treated sites revealed an increased amount of NMT when compared with the NBM sites (Cordaro et al. 2008; Lindgren et al. 2009). This observation was not supported by the present histomorphometrical analysis, as all the groups investigated revealed comparable mean NMT values. One might speculate that the osteoinductive and osteogenetic properties noted for AB (Chiriac et al. 2005) may have increased the mean MT and subsequently reduced the mean NMT values in the SBC group. Nevertheless, the present data clearly support previous findings that both types of bone graft particles reveal different resorption characteristics (Cordaro et al. 2008). An initial dissolution of SBC was also observed after 8 weeks of healing in the mandible of minipigs (Jensen et al. 2007). In particular, histological observation revealed the presence of multinucleated giant cells on the surface of particles that were not covered by MT. Even though there were no signs of any cell-mediated resorption lacunae, these surfaces revealed a higher penetration of the staining agent, thus pointing to an initial dissolution of the graft material (Jensen et al. 2007). In this context, however, it is important to emphasize that the mean BIC values, as noted in the present study, were comparable in both NBM + AB and SBC + AB groups, thus pointing to an undisturbed osseointegration, even in the presence of the almost nonresorbing NBM (Mordenfeld et al. 2010). Even though the application of PEG tended to be associated with an increase in the mean BIC values when compared with the corresponding CM groups, these differences did not reach statistical significance. Basically, the BIC values assessed in all groups investigated are within the range of that noted for modSLA titanium implants after 2 weeks of healing in the canine upper jaw (Schwarz et al. 2007a, 2007b), thus pointing to an equal potential of both regenerated and pristine bone to support osseointegration. This assumption is also supported by the results of a recent experimental study, indicating that BIC at dual-acid etched titanium implants was comparable at both NBM + CM-treated defects and pristine sites (Artzi et al. 2010). At present, the quantitative and qualitative capacities of NBM + PEG-, SBC + CM- and SBC + PEG-treated sites to support the initial process of osseointegration are yet to be determined.

Within the limits of the present study, it was concluded that all augmentation procedures investigated supported bone regeneration and staged osseointegration of modSLA titanium implants. However, the application of PEG may be associated with increased TA values.

Acknowledgements: We appreciate the skills and commitment of Mr Fienitz, Mr Kaiser and Mr Lommen (Department of Oral Surgery, Heinrich Heine University, Düsseldorf, Germany) in the preparation of the histological specimens. *Source of funding:* The study was funded in part by a grant from Institut Straumann AG. The study materials were kindly provided by Institut Straumann AG.

Conflict of interests: The authors report no conflicts of interest related to this study.

References

Artzi, Z., Nemcowsky, C.E., Tal, H., Weinberg, E., Weinreb, M., Prasad, H., Rohrer, M.D. & Kozlovsky, A. (2010) Simultaneous versus two-stage implant placement and guided bone regeneration in the canine: histomorphometry at 8 and 16 months. *Journal of Clinical Periodontology* **37**: 1029–1038.

Becker, J., Al-Nawas, B., Klein, M.O., Schliephake, H., Terheyden, H. & Schwarz, F. (2009) Use of a new cross-linked collagen membrane for the treatment of dehiscence-type defects at titanium implants: a prospective, randomized-controlled double-blinded clinical multicenter study. *Clinical Oral Implants Research* **20**: 742–749.

Bornstein, M.M., Bosshardt, D. & Buser, D. (2007) Effect of two different bioabsorbable collagen membranes on guided bone regeneration: a comparative histomorphometric study in the dog mandible. *Journal of Periodontology* **78**: 1943–1953.

Chiriac, G., Herten, M., Schwarz, F., Rothamel, D. & Becker, J. (2005) Autogenous bone chips: influence of a new piezoelectric device (piezosurgery) on chip morphology, cell viability and differentiation. *Journal of Clinical Periodontology* **32**: 994–999.

Cordaro, L., Bosshardt, D.D., Palattella, P., Rao, W., Serino, G. & Chiapasco, M. (2008) Maxillary sinus grafting with Bio-Oss or Straumann Bone Ceramic: histomorphometric results from a randomized controlled multicenter clinical trial. *Clinical Oral Implants Research* **19**: 796–803.

Dahlin, C., Linde, A., Gottlow, J. & Nyman, S. (1988) Healing of bone defects by guided tissue regeneration. *Plastic and Reconstructive Surgery* **81**: 672–676.

Donath, K. (1985) The diagnostic value of the new method for the study of undecalcified bones and teeth with attached soft tissue (Säge-Schliff (sawing and grinding) technique). *Pathology Research Practice* **179**: 631–633.

Froum, S.J., Wallace, S.S., Cho, S.C., Elian, N. & Tarnow, D.P. (2008) Histomorphometric comparison of a biphasic bone ceramic to anorganic bovine bone for sinus augmentation: 6- to 8-month postsurgical assessment of vital bone formation. A pilot study. *International Journal of Periodontics and Restorative Dentistry* **28**: 273–281.

Hardwick, R., Scantlebury, T.V., Sanchez, R., Whitley, N. & Ambruster, J. (1994) Membrane design criteria for guided bone regeneration of the alveolar ridge In: Buser, D., Dahlin, C., Schenk, R.K., eds. *Guided bone regeneration in implant dentistry* 1st edition, 101–137. Chicago: Quintessence Publishing Co.

Herten, M., Jung, R.E., Ferrari, D., Rothamel, D., Golubovic, V., Molenberg, A., Hammerle, C.H., Becker, J. & Schwarz, F. (2009) Biodegradation of different synthetic hydrogels made of polyethylene glycol hydrogel/RGD-peptide modifications: an immunohistochemical study in rats. *Clinical Oral Implants Research* **20**: 116–125.

Jensen, S.S. & Terheyden, H. (2009) Bone augmentation procedures in localized defects in the alveolar ridge: clinical results with different bone grafts and bone-substitute materials. *International Journal of Oral and Maxillofacial Implants* **24** (Suppl.): 218–236.

Jensen, S.S., Yeo, A., Dard, M., Hunziker, E., Schenk, R. & Buser, D. (2007) Evaluation of a novel biphasic calcium phosphate in standardized bone defects: a histologic and histomorphometric study in the mandibles of minipigs. *Clinical Oral Implants Research* **18**: 752–760.

Jung, R.E., Halg, G.A., Thoma, D.S. & Hämmerle, C.H. (2009a) A randomized, controlled clinical trial to evaluate a new membrane for guided bone regeneration around dental implants. *Clinical Oral Implants Research* **20**: 162–168.

Jung, R.E., Lecloux, G., Rompen, E., Ramel, C.F., Buser, D. & Hämmerle, C.H. (2009b) A feasibility study evaluating an in situ formed synthetic biodegradable membrane for guided bone regeneration in dogs. *Clinical Oral Implants Research* **20**: 151–161.

Jung, R.E., Zwahlen, R., Weber, F.E., Molenberg, A., van Lenthe, G.H. & Hämmerle, C.H. (2006) Evaluation of an in situ formed synthetic hydrogel as a biodegradable membrane for guided bone regeneration. *Clinical Oral Implants Research* **17**: 426–433.

Lindgren, C., Hallman, M., Sennerby, L. & Sammons, R. (2010) Back-scattered electron imaging and elemental analysis of retrieved bone tissue following sinus augmentation with deproteinized bovine bone or biphasic calcium phosphate. *Clinical Oral Implants Research* **21**: 924–930.

Lindgren, C., Sennerby, L., Mordenfeld, A. & Hallman, M. (2009) Clinical histology of microimplants placed in two different biomaterials. *International Journal of Oral and Maxillofacial Implants* **24**: 1093–1100.

Mordenfeld, A., Hallman, M., Johansson, C.B. & Albrektsson, T. (2010) Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clinical Oral Implants Research* **21**: 961–970.

Rothamel, D., Schwarz, F., Sager, M., Herten, M., Sculean, A. & Becker, J. (2005) Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clinical Oral Implants Research* **16**: 369–378.

Schenk, R.K., Buser, D., Hardwick, W.R. & Dahlin, C. (1994) Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. *International Journal of Oral and Maxillofacial Implants* **9**: 13–29.

Schenk, R.K., Olah, A.J. & Herrmann, W. (1984) Preparation of calcified tissues for light microscopy. In: Dickson, G.R., (ed.) *Methods of Calcified Tissue Preparation*, pp 1–56. Amsterdam: Elsevier.

Schwarz, F., Ferrari, D., Herten, M., Mihatovic, I., Wieland, M., Sager, M. & Becker, J. (2007a) Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. *Journal of Periodontology* **78**: 2171–2184.

Schwarz, F., Herten, M., Sager, M., Wieland, M., Dard, M. & Becker, J. (2007b) Bone regeneration in dehiscence-type defects at chemically modified (SLActive) and conventional SLA titanium implants: a pilot study in dogs. *Journal of Clinical Periodontology* **34**: 78–86.

Schwarz, F., Jung, R.E., Fienitz, T., Wieland, M., Becker, J. & Sager, M. (2010) Impact of guided bone regeneration and defect dimension on wound healing at chemically modified hydrophilic titanium implant surfaces: an experimental study in dogs. *Journal of Clinical Periodontology* **37**: 474–485.

Schwarz, F., Rothamel, D., Herten, M., Sager, M. & Becker, J. (2006) Angiogenesis pattern of native and cross-linked collagen membranes: an immunohistochemical study in the rat. *Clinical Oral Implants Research* **17**: 403–409.

Schwarz, F., Rothamel, D., Herten, M., Wustefeld, M., Sager, M., Ferrari, D. & Becker, J. (2008) Immunohistochemical characterization of guided bone regeneration at a dehiscence-type defect using different barrier membranes: an experimental study in dogs. *Clinical Oral Implants Research* **19**: 402–415.

Sela, M.N., Kohavi, D., Krausz, E., Steinberg, D. & Rosen, G. (2003) Enzymatic degradation of collagen-guided tissue regeneration membranes by periodontal bacteria. *Clinical Oral Implants Research* **14**: 263–268.

Simion, M., Dahlin, C., Blair, K. & Schenk, R.K. (1999) Effect of different microstructures of e-PTFE membranes on bone regeneration and soft tissue response: a histologic study in canine mandible. *Clinical Oral Implants Research* **10**: 73–84.

Thoma, D.S., Halg, G.A., Dard, M.M., Seibl, R., Hämmerle, C.H. & Jung, R.E. (2009) Evaluation of a new biodegradable membrane to prevent gingival ingrowth into mandibular bone defects in minipigs. *Clinical Oral Implants Research* **20**: 7–16.

Wechsler, S., Fehr, D., Molenberg, A., Raeber, G., Schense, J.C. & Weber, F.E. (2008) A novel, tissue occlusive poly(ethylene glycol) hydrogel material. *Journal of Biomedical Materials Research A* **85**: 285–292.