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Influence of defect dimensions on periodontal wound healing/regeneration in intrabony defects following implantation of a bovine bone biomaterial and provisions for guided tissue regeneration: an experimental study in the dog

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Abstract

Objective: To evaluate the influence of defect dimensions on periodontal wound healing/regeneration in intrabony defects following implantation of a deproteinized bovine bone/collagen matrix under provisions for guided tissue regeneration.

Material and Methods: Contra-lateral one-wall intrabony [6 × 6 mm (wide/deep) *versus* 4 × 4 mm (narrow/shallow)] periodontal defects were surgically created at the edentulated mesial aspect of the mandibular first molars in three Labradors, i.e., three defects in each category. The defects were implanted with the bovine bone/collagen matrix and covered with a collagen membrane. Histologic/histometric analysis followed an 18-month healing interval.

Results: New cementum encompassed the entire intrabony component in both wide/deep (5.6 ± 0.5 mm) and narrow/shallow (4.2 ± 0.1 mm) defects; bone formation amounted to 5.6 ± 0.6 and 4.0 ± 0.8 mm, respectively. Mineralized bone encompassed 57.5% *versus* 65% and the bone biomaterial 11.6% *versus* 13.1% of the defect space. A periodontal ligament with a width and composition similar to that of the resident periodontal ligament encompassing the entire aspect of the defects was observed. Root resorption/ankylosis was rare.

Conclusions: Both wide/deep and narrow/shallow intrabony defects showed a substantial potential for periodontal regeneration in this pre-clinical model. The contribution of the bovine bone/collagen matrix and guided tissue regeneration to this regenerative potential is not clear.

Key words: animal; bovine bone biomaterial; guided tissue regeneration; membrane; periodontal regeneration; pre-clinical

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Conflict of interest and source of funding statement

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A number of clinical protocols, considerably different in approach and rationale including surgical implantation of autogenous bone grafts, bone biomaterials either synthetic or sourced from

cadaver bone, matrix/growth/differentiation factors, root conditioning, coronal flap advancement, etc., have been associated – occasionally incorrectly – with a potential to support periodontal regeneration (Wang & Cooke 2005, Lee et al. 2010). One such clinical protocol comprises the application of a barrier device, an occlusive membrane, to separate the wound from the gingival epithelium and connective tissue while creating a secluded space for the migration and proliferation of cells sequestered in the periodontal ligament and alveolar bone. This surgical approach known as guided tissue regeneration (GTR) has been evaluated in a plethora of pre-clinical and human histologic studies supporting the concept that clinical improvements observed following GTR therapy in patients largely reflect regeneration of the periodontal attachment (Nyman et al. 1982, Gottlow et al. 1984, Haney et al. 1993, Sigurdsson et al. 1994).

The current clinical protocol for GTR often includes the use of bioresorbable membranes combined with bone biomaterials implanted underneath the membrane with the intent to prevent collapse or compression of the membrane onto the root surface or into the defect, thus assuring space provision and possibly contributing osteoconductive qualities. One such device/biomaterial combination that has received wide recognition includes a porcine Type I collagen membrane and a particulate deproteinized bovine bone biomaterial. Histologic observations from pre-clinical models (Yamada et al. 2002, Sakata et al. 2006) and clinical cases (Camelo et al. 1998, Mellonig 2000, Paolantonio et al. 2001, Nevins et al. 2003, Sculean et al. 2004) suggest that healing following this clinical protocol, at least in part, may be characterized as periodontal regeneration. The pattern of new bone formation has been described as predominantly slender trabeculae in continuity with resident bone often contacting, occasionally immersing, the particulate bovine bone biomaterial. The bone biomaterial occupies a major portion of the defect sites, in particular away from resident bone. In the more coronal aspects of the sites, the bone biomaterial largely appears embedded in fibrovascular connective tissue. New bone formation does not always juxtapose new cementum and a regenerated periodontal ligament often appears irregular in shape and width without distinct periodontal fibre bundles connecting the newly formed cementum and bone (Camelo et al. 1998, Mellonig 2000, Paolantonio et al. 2001, Yamada et al. 2002, Nevins et al. 2003, Sculean et al. 2004, Sakata et al. 2006).

An analogous pattern of bone formation following the use of the bovine bone biomaterial has been observed in bone defects in a variety of pre-clinical models, as well as in human biopsies, also following extended healing intervals (Hämmerle et al. 1997, Schmid et al. 1997, Slotte & Lundgren 1999, Araujo et al. 2002, Carmagnola et al. 2002, 2003, Artzi et al. 2003, Stavropoulos et al. 2003, 2004, Simion et al. 2006). Notably, defect sites receiving the membrane without the biomaterial show increased bone formation compared with sites receiving the bovine bone/membrane combination. It appears that osteogenic bone formation in defect sites implanted with the bovine bone biomaterial might proceed to a certain, limited distance from the resident bone, and that the outcome of healing might depend on the volume and geometry of the defect site. Possibly, implantation of the bovine bone biomaterial in large defects and/or defects with limited number of bone walls would not enhance but rather obstruct or delay bone formation and maturation. The aim of the present study was to evaluate the influence of defect dimensions on periodontal wound healing/regeneration in intrabony defects following implantation of the deproteinized bovine bone biomaterial dispersed in a collagen matrix under provisions for GTR.

Material and Methods

Animals

Three female Labrador dogs (age 22–24 months, weight 25–30 kg) obtained from a licensed vendor were used according to a protocol approved by the Danish Inspectorate for Animal Experiments. The animals were acclimated for 1 week and accustomed to a standard dog-pellet diet slightly softened in water twice daily. They had ad libitum access to water throughout the study.

Biomaterials

The bone biomaterial used in the present study consisted of deproteinized bovine bone particles incorporated in a highly purified porcine Type I collagen matrix block (Bio-Oss Collagen®, Geistlich Pharma, Wohlhusen, Switzerland). The GTR membrane consisted of a double-layered porcine Type I collagen structure (Biogide®, Geistlich Pharma).

Surgical protocol

Surgical interventions, i.e., tooth extractions and experimental surgeries, were performed as follows: food was withheld the night preceding surgery. The animals were pre-anaesthetized with atropine (Atropin, DAK, Nycomed, Copenhagen, Denmark; 0.02–0.04 mg/kg i.m.), buprenorphine HCl (Buprenorphine, Nycomed; 0.01–0.03 mg/kg i.m.), and acepromazine (Plegicil, Pharmacia Animal Health, Copenhagen, Denmark; 0.2–0.3 mg/kg i.m.). After sedation, the animals were moved to the operating room and anaesthesia was induced with propofol (Propofol, Braun AG, Melsungen, Germany; 5–7 mg/kg i.v.) and maintained on gas inhalation anaesthesia (Isoflurane, Baxter, Allerød, Denmark; 1.5–2% isoflurane/O_2 to effect). The animals received a slow constant rate infusion of lactated Ringer's solution (10–20 ml/kg/h i.v.) to maintain hydration during surgery. The depth of anaesthesia was monitored by a lack of corneal reflex, and by monitoring the depth of respiration and heart rate.

Surgical procedures

Left and right second, third, and fourth mandibular premolars were extracted and the extraction sites were allowed to heal for 4 months. Next, mucoperiosteal flaps were elevated at the mandibular first molar following buccal/lingual intra-sulcular incisions and a connecting mesial crestal incision. Box-type intrabony defects were then created at the mesial aspect of the left and right mandibular first molars using low-speed round burs and sterile saline irrigation, and using bone chisels. Bone removal extended to the mid-buccal and mid-lingual portion of the mesial root (Figs 1 and 2). The surgically exposed root was instrumented with curettes to remove the periodontal ligament and the cementum. Defect dimensions (width × depth) 6 × 6 mm (wide/deep defects) and 4 × 4 mm (narrow/shallow defects), measured using a periodontal probe, were alternated between right and left jaw quadrants in subsequent animals. The width of the defect was measured from the most prominent

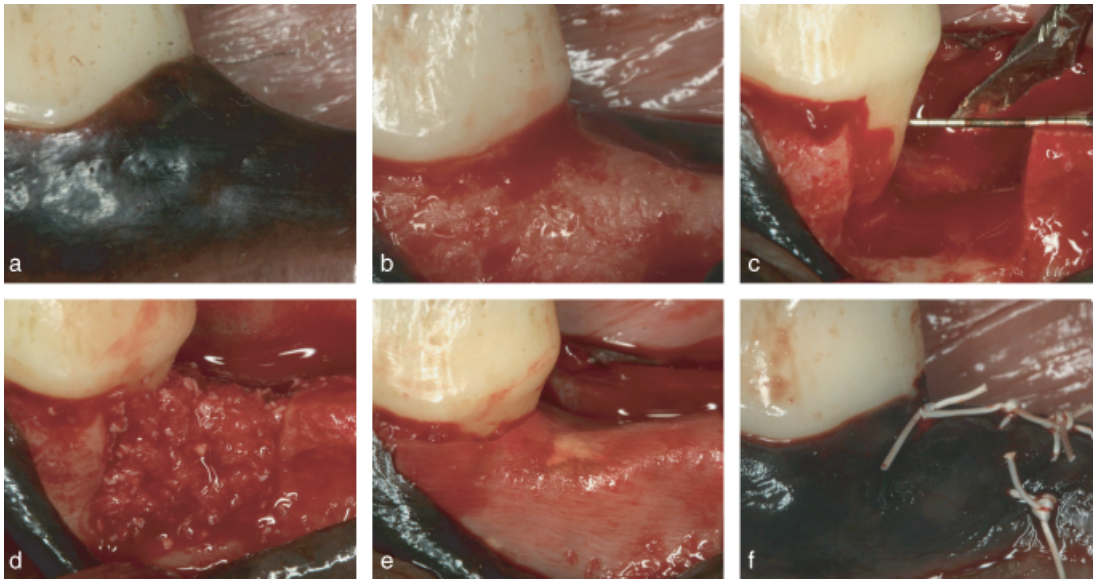

Fig. 1. Pre-surgery view of the experimental area (a), after flap elevation (b), and creation of a box-type, wide/deep (6 × 6 mm) intrabony periodontal defect at the mesial aspect of the mandibular first molar (c). The defect was filled with a piece of a bovine bone/collagen matrix (d), covered with a porcine Type I collagen membrane (e), and the flaps were repositioned and sutured for primary intention healing (f).

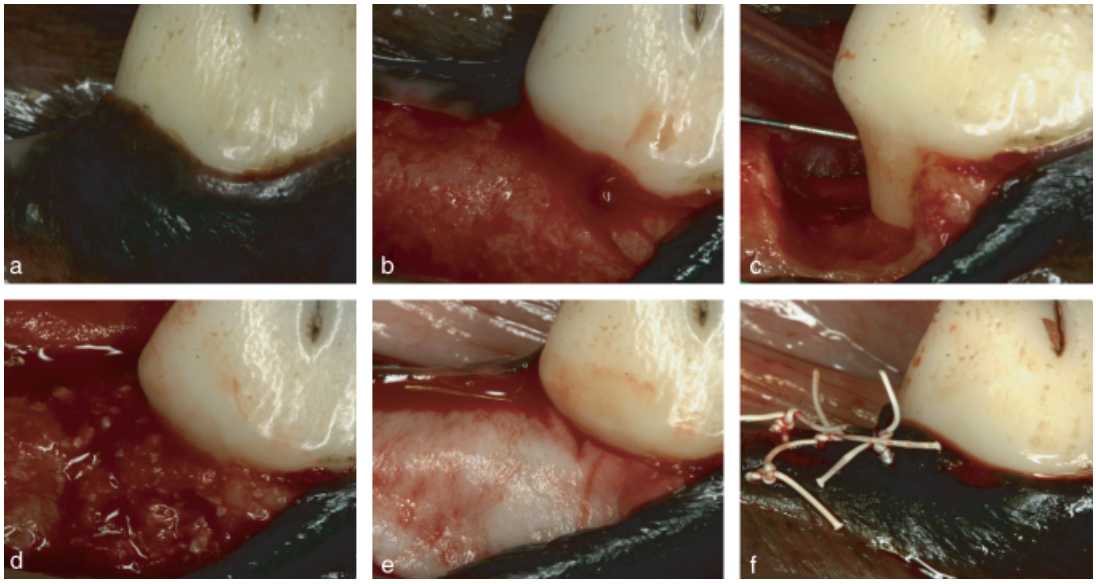

Fig. 2. Pre-surgery view of the experimental area (a), after flap elevation (b), and creation of a box-type, narrow/shallow (4 × 4 mm) intrabony periodontal defect at the mesial aspect of the mandibular first molar (c). The defect was filled with a piece of a bovine bone/collagen matrix (d), covered with a porcine Type I collagen membrane (e), and the flaps were repositioned and sutured for primary intention healing (f).

mesial aspect of the root to the mesial vertical bone wall. The depth of the defect was measured along the mesial vertical bone wall at the middle aspect of the residual alveolar crest. A notch was made in the root surface at the most apical aspect of the defect using a small diameter round bur. The defects were then filled each with one piece of the bovine bone/collagen matrix block adapted to roughly reconstitute the geometry of the alveolar ridge without overfilling the defect, and were then covered using the collagen membrane that extended approximately 3 mm beyond the defect margins. The coronal level of the membrane was positioned 1–2 mm below the cemento-enamel junction at the mesial aspect of the molar teeth. No sutures were used to stabilize the membranes. The mucoperiosteal flaps were then adapted to cover the membrane and sutured using expanded polytetrafluoroethylene sutures (CV4, W.L. Gore & Associates Inc., Flagstaff, AZ, USA), the gingival margins being positioned slightly coronal to the cemento-enamel junction.

Post-surgery protocol

Pain control was managed using a fentanyl patch (50 μg; Durogesic,

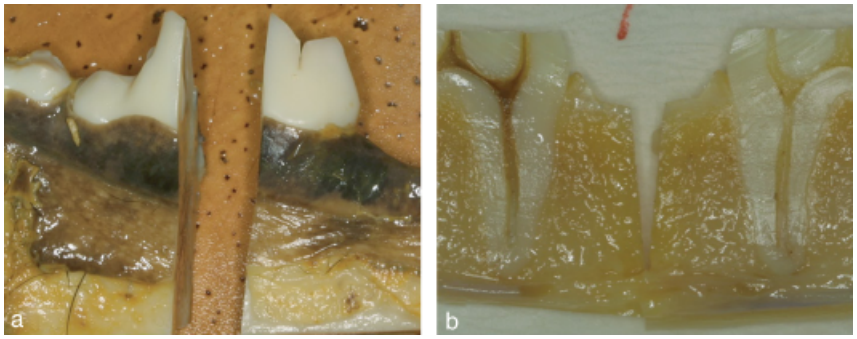

Fig. 3. Before embedding, the mesial root of the mandibular first molar including the defect site was separated from the block (a) and split into two halves along a mesio-distal plane at the centre of the defect using a surgical blade (b).

Janssen-Cilag, Birkerød, Denmark). Infection control included rinsing the teeth daily with a 0.2% chlorhexidine solution (Corsodyl, SmithKline Beecham, Copenhagen, Denmark; 10 days) and systemic amoxicillin/clavoulanic acid (Synulox Orion Pharma, Nivå, Denmark; 20 mg/kg i.m., 1 h pre-surgery, b.i.d. post-surgery 7 days). Sutures were removed after 10–13 days. Thereafter, chlorhexidine rinses were performed two to three times weekly. The animals were euthanized after an 18-month healing interval using an over-dose of sodium pentobarbital (Mebumal, Nycomed DAK) under anaesthesia induced by propofol. Biopsies including the defects and teeth with surrounding tissues were harvested and fixed in 70% alcohol.

Histotechnical procedures

The specimens were decalcified in EDTA, dehydrated and prepared for embedding in paraffin. Before embedding, the blocks were split at the centre of the defect along a mesio-distal plane using a surgical blade (Fig. 3). Thus, sections representing the immediate centre of the defect could be produced without the need for exhaustive sectioning. Twenty-eight-micrometre-thick sections were collected from each buccal and lingual block, consecutively stained with haematoxylin and eosin, van Gieson's picrofuchsin, or Ladewig's connective tissue stain.

Histologic analysis

At least four stained central sections per specimen were evaluated by one experienced investigator (A. S.) using incandescent light microscopy (BH-50, Olympus Denmark AS, Ballerup, Denmark). The analysis included a gross evaluation of the extent and qualitative characteristics of cementum and bone regeneration within the defect space; the presence of functionally oriented periodontal ligament fibres within the regenerated tissues; the presence of root resorption/ankylosis; spatial distribution of the bone biomaterial and its association with the various regenerated tissues; evidence for bone biomaterial resorption; and presence of inflammatory reactions.

Histometric analysis

The histometric analysis was performed by the same experienced investigator (A. S.) using a computer-assisted image-analysis system (VIS®, Visiopharm, Hørsholm, Denmark) connected to a light microscope (BH-50, Olympus Denmark AS) fitted to a video camera (Olympus DP70, Olympus Denmark AS). Four central sections per specimen were evaluated as follows:

(1) Defect height: distance between the apical notch and the cemento-enamel junction.
(2) Connective tissue repair: distance between the apical notch and the apical extension of the junctional epithelium.
(3) Cementum regeneration (height): distance between the apical notch and the coronal extension of a continuous layer of cementum or cementum-like deposit on the planed root.
(4) Bone regeneration (height): distance between the apical notch and the coronal extension of the newly formed alveolar bone juxtaposed the planed root.
(5) Root resorption: combined linear heights of distinct resorption lacunae on the planed root.
(6) Ankylosis: combined linear heights of ankylotic union between the regenerated alveolar bone and the planed root.

Area fractions of mineralized bone, marrow and connective tissue, and bovine bone particles were estimated using a point-counting protocol (Gundersen et al. 1981). Briefly, a square digital area of interest containing a set of digital points was projected over the section image and juxtaposed to the root, with one side level with the apical extension of the root planing and one side parallel to the root surface. The dimensions of the area of interest were 6×6 mm for wide/deep or 4×4 mm for narrow/shallow defects. Positive scores were expressed as a percentage of the total number of points within the area of interest.

The composition of the newly formed periodontal ligament including width, vascular elements, and collagen fibre bundles was evaluated and compared with that of the native periodontal ligament. A digital box 0.2 mm in height and width circumscribing the periodontal ligament space was projected (1) immediately apically to the notch (resident periodontal ligament), (2) immediately coronally to the notch (apical extent of defect), (3) 0.1 mm apical to the bone crest (coronal extent of defect), and (4) at the mid-distance between the alveolar crest and the notch (centre of defect). Using a point-counting technique, viewing the specimens at a $\times$ 400 magnification, the relative proportions of vascular elements and collagen fibre bundles were estimated and expressed as a percentage of the total number of superimposed points. The width of the periodontal ligament was measured at three equidistant levels of each digital box (coronal, centre, and apical).

Statistical analysis

Because of the small number of animals, only descriptive statistics (means $\pm$ SD) were used to present the results.

Results

Clinical observations

Clinical healing was uneventful. Gingival margins remained slightly above the cemento-enamel junction without overt signs of inflammation throughout the 18-month healing interval.

Histologic observations

The histologic analysis revealed extensive periodontal regeneration for both wide/deep and narrow/shallow defects; cementum and bone formation approximated the level membrane placement, slightly apical to the cemento-enamel junction. The newly formed periodontal ligament generally exhibited similar dimensions (width) and composition (fraction vascular elements and collagen fibre bundles) compared with the native periodontium. Cementum formation reaching the apical extension of the junctional epithelium appeared thin and cellular (Figs 4, 6, 7).

A major portion of the original bone defect was filled with lamellar trabecular bone and fatty marrow. Only a fraction of the defect, at the level of the alveolar crest, was filled with fibrous connective tissue. The bovine bone biomaterial was proportionally represented in wide/deep and narrow/shallow defects, and in most occasions the bone particles appeared in contact with or immersed in bone. Not uncommonly, the bovine bone particles were observed immediately coronal to the alveolar crest or in large numbers segregated inside bone cavities, usually covered with/entrapped in dense connective tissue capsules resembling a foreign body reaction (Figs 4–6 and 9). There was no obvious evidence of bioresorption/degradation of the bovine bone biomaterial or of any associated inflammatory reaction. The bovine bone particles were never found in immediate contact with the tooth, although in a couple of occasions bone particles were found lateral to the bone within a locally slightly widened periodontal ligament. A mostly amorphous, acellular organic material was often observed in the centre of the ghost images of the bovine bone particles (Figs 5 and 6). Evidence of root resorption/ankylosis was observed in one specimen (Fig. 8), but it did not appear to be associated with the bovine bone biomaterial, the bovine bone particles found at a distance from the root being completely incorporated in the lamellar bone.

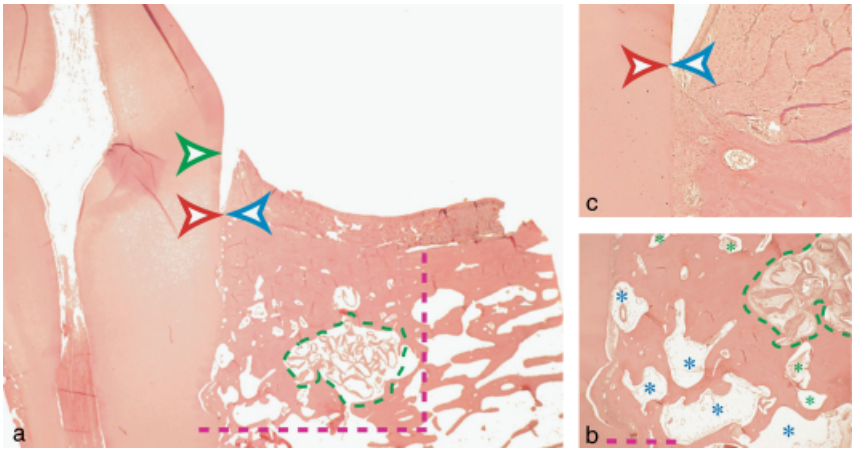

Fig. 4. Representative photomicrographs of a wide/deep defect site; overview (a; × 8) and apical (b; × 20) and coronal (c; × 20) magnifications. The red arrowhead indicates the coronal level of continuous new cementum formation with inserting collagen fibres approximating the level of membrane placement, approximately 2 mm apical to the cemento-enamel junction (green arrowhead). The blue arrowhead indicates the apical termination of the junctional epithelium. The major portion of the intrabony defect (violet dashed line) is filled with lamellar trabecular bone and fatty marrow (blue asterisks). The bovine bone biomaterial (green asterisks) contacting or appearing immersed in bone or in large numbers segregated within bone cavities (green dashed line) occupies a fraction of the defect. The violet dashed line in (b) indicates the apical level of the defect. Haematoxylin and eosin.

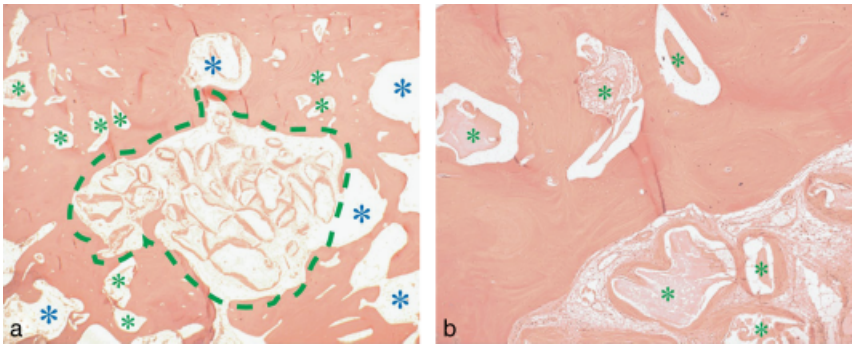

Fig. 5. Photomicrographs showing additional aspects of the wide/deep defect site in Fig. 4 (a; × 35 and b; × 125) The particulate bovine bone biomaterial (green asterisks) contacts or appears immersed in bone; and not uncommonly, in large numbers, segregated within bone cavities (green dashed line) entrapped in dense connective tissue capsules resembling a foreign body reaction. A mostly amorphous, acellular organic material was often observed in the centre of the ghost images of the particulate bovine bone biomaterial. Blue asterisks indicate bone marrow cavities. Haematoxylin and eosin.

Histometric observations

The results from the histometric evaluation are presented in Tables 1 and 2. Cementum and bone formation reached the same level, approximately 2 mm below the cemento-enamel junction, in both wide/deep and narrow/shallow defects. Mineralized bone averaged 58% and 65% of the defect area in wide/deep and narrow/shallow, respectively. Residual bone biomaterial averaged 12% and 13% of the defect area in wide/deep and narrow/shallow defects, respectively. The corresponding values for connective tissue (excluding bone marrow) approximated 4% and 1%. Ankylosis, observed in one single specimen, averaged 1.5 mm.

Discussion

Only few reports have considered the long-term (5–6 years) clinical outcome of the deproteinized bovine bone

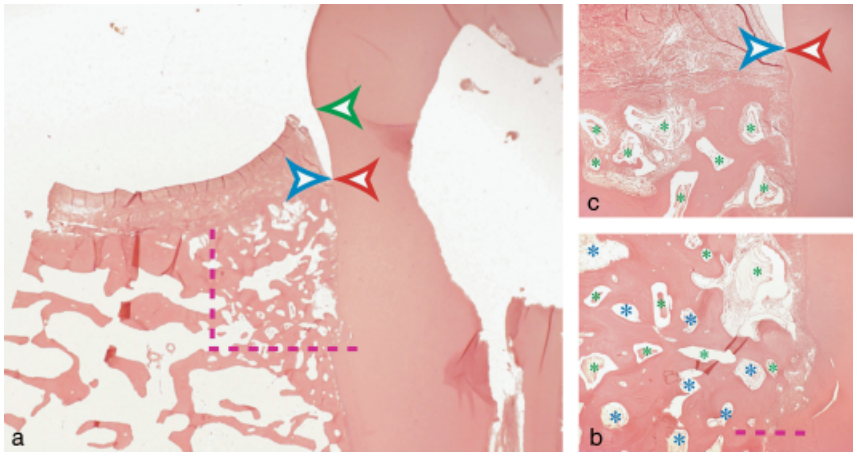

Fig. 6. Representative photomicrographs of a narrow/shallow defect site; overview (a; × 8) and apical (b; × 30) and coronal (c; × 30) magnifications. The red arrowhead indicates the coronal level of continuous new cementum formation with inserting collagen fibres approximating the level of membrane placement, approximately 2 mm apical to the cemento-enamel junction (green arrowhead). The blue arrowhead indicates the apical termination of the junctional epithelium. The major portion of the original bone defect (violet dashed line) is filled with lamellar trabecular bone and fatty marrow (blue asterisks). The bovine bone biomaterial (green asterisks) contacting or appearing immersed in bone or in large numbers segregated within bone cavities occupies a fraction of the defect. The violet dashed line in (b) indicates the apical level of the defect. Haematoxylin and eosin.

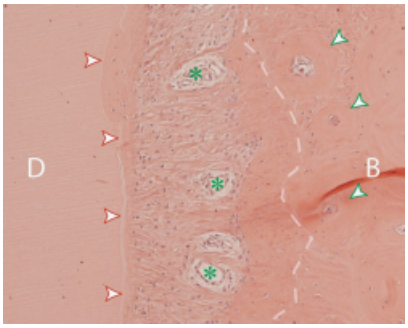

Fig. 7. High-magnification (× 220) photomicrograph of an aspect of the regenerated periodontium. A thin regenerated cementum layer (red arrowheads) consisting of acellular extrinsic fibre cementum and cellular mixed stratified cementum can be observed on the instrumented dentin surface (D). The newly formed periodontal ligament exhibits dimensions (width) and composition, i.e., a fraction of vascular elements (green stars) and collagen fibre bundles, comparable to native periodontium. The dashed line indicates the border between the alveolar bone proper and the alveolar bone (B). Green arrowheads indicate osteons in the regenerated alveolar bone. Haematoxylin & eosin.

biomaterial combined with GTR in the treatment of periodontal intrabony defects (Sculean et al. 2007, Slotte et al. 2007, Stavropoulos & Karring 2010). The present study is the first to report the histological long-term outcome following the use of the bovine bone/collagen matrix combined with GTR. The observations herein indicate that defect dimensions may not critically direct the potential for periodontal regeneration following implantation of the deproteinized bovine bone/collagen matrix under provisions for GTR. Both wide/deep and narrow/shallow one-wall intrabony defects – both of a clinically significant magnitude – showed comparable almost complete regeneration of the periodontal attachment. The new periodontal ligament exhibited characteristics of the native periodontium. These observations should come as no surprise considering the substantial innate potential for regeneration of the periodontium provided that the important biological directives of wound stability, unobstructed space provision, and primary intention healing are met (Polimeni et al. 2006). Indeed, extensive, gradually maturing, periodontal regeneration assuming features of the resident periodontal attachment has been observed in a variety of settings, including non-human primate buccal dehiscence and intrabony defects (Graziani et al. 2005, Laurell et al. 2006), as well as conceptually more challenging 5–6 mm canine supra-alveolar periodontal defects, following GTR as a stand-alone protocol (Haney et al. 1993, Sigurdsson et al. 1994, Wikesjö et al. 2003a, b, Polimeni et al. 2009).

The double-layered porcine Type I collagen membrane used in the present study may not posses adequate structural qualities to be used as a successful stand-alone device for GTR. This collagen membrane requires the support of a secondary structure when applied to large defects and/or defects without a space-providing morphology. A major difference thus between the above-mentioned pre-clinical studies evaluating GTR and the present study is the implantation of the deproteinized bovine bone/collagen matrix underneath the membrane. Because no GTR, bovine bone/collagen matrix, and sham-surgery controls were included in the present study, the contribution, if any, of the bovine bone/collagen matrix to the observed periodontal wound healing/regeneration remains unknown. A previous pre-clinical study evaluated the bovine bone biomaterial (without the collagen matrix) combined with the collagen membrane also using large one-wall, box-type (4 × 7 mm; width × depth) intrabony defects at the mesial aspect of the mandibular first molars in dogs (Sakata et al. 2006). Histological evaluation following a 6-month healing interval showed enhanced cementum and bone formation for the bovine bone/GTR combination compared with the sham-surgery control; however, GTR and bovine bone controls were again not included in this study. In another study using surgically induced two-wall intrabony defects in dogs (5 × 5 mm; width × height), significantly larger amounts of bone regeneration were reported following the bovine bone/collagen membrane application compared with GTR alone following an 8-week healing interval, while no differences in cementum regeneration were observed (Yamada et al. 2002). Collectively, these studies point the regenerative potential of a periodontal site released under conditions for GTR while remaining indecisive relative to the contribution, if any, from implanted bovine bone biomaterials. In context, a recent systematic review of pre-clinical studies involving combinations of barrier membranes and grafting materials concluded that additional benefits of combination treatments over the use of membranes alone were detected only in non-contained two-wall intrabony or supra-alveolar defects (Sculean et al. 2008).

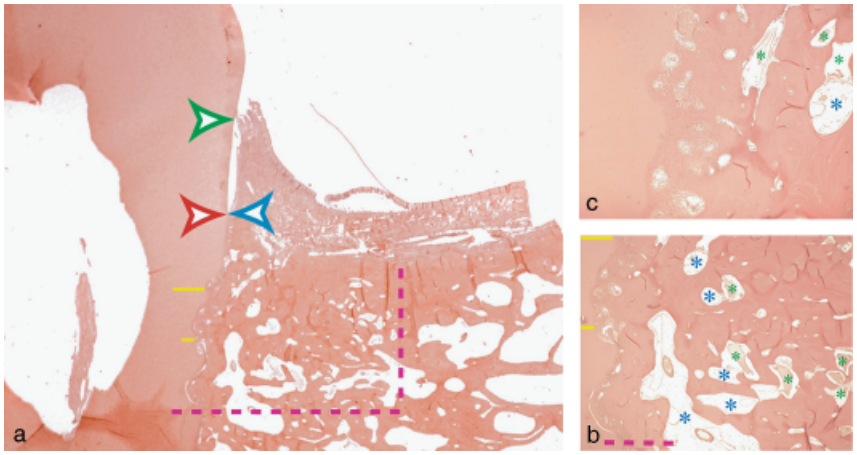

Fig. 8. Photomicrograph of one defect site (wide/deep) showing root resorption/ankylosis (within the yellow lines); overview (a; × 8) and apical (b; × 20) and coronal (c; × 50) magnifications. The red arrowhead indicates the coronal level of continuous new cementum formation with inserting collagen fibres; the green arrowhead the cemento-enamel junction, and the blue arrowhead the apical termination of the junctional epithelium. The major portion of the original bone defect (violet dashed line) is filled with lamellar trabecular bone and fatty marrow (blue asterisks). The bovine bone biomaterial (green asterisks) contacting or appearing immersed in bone occupies a fraction of the defect. The violet dashed line in (b) indicates the apical level of the defect. Haematoxylin and eosin.

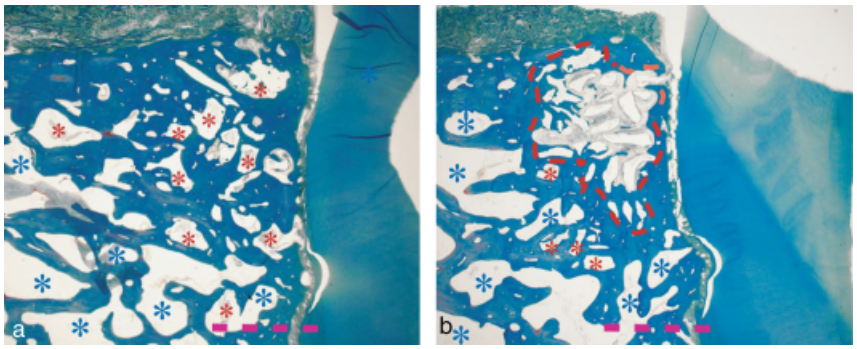

Fig. 9. Photomicrographs of a narrow/shallow site (× 10). In section (a), the particulate bone biomaterial (red asterisks) appears in contact with/immersed in bone. In an adjacent section (b), approximately 0.1 mm more laterally a large number of bovine bone particles appear segregated within a bone cavity (red dashed line) covered with/entrapped in dense connective tissue capsules. The violet dashed line indicates the apical level of the defect. Blue asterisks indicate bone marrow cavities. Ladewig's connective tissue stain.

Previous studies indicate that implantation of slowly or non-resorbing bone biomaterials in conjunction with periodontal regenerative procedures, including GTR, may delay wound maturation and/or obstruct periodontal wound healing/regeneration, the extent of bone formation being inversely correlated to the particle density of an implanted bone biomaterial (Sigurdsson et al. 1996, Trombelli et al. 1999). The use of a single long-term healing period and lack of controls in the current study do not allow assumptions whether the bovine bone biomaterial ameliorates or deters periodontal regeneration. It has been documented that this particular bone biomaterial is biocompatible but it is unclear whether it is osteoconductive, i.e., it enhances bone formation. In several animal and human studies, bone formation in defects implanted with the bovine bone biomaterial and covered with a membrane was confined to the vicinity of the native bone, while defect sites receiving only a membrane showed larger amounts of new bone (Hämmerle et al. 1997, Schmid et al. 1997, Slotte & Lundgren 1999, Araujo et al. 2002, Carmagnola et al. 2002, 2003, Artzi et al. 2003, Simion et al. 2006). It appears that the outcome of healing in bone defects implanted with the particulate deproteinized bovine bone biomaterial might largely depend on the volume and geometry of the defect site. Presence of the bone biomaterial was permissive to substantial periodontal regeneration in the current study, stressing out the fact that significant biological differences exist between alveolar bone regeneration in the presence and absence of teeth (Polimeni et al. 2004). The observation that both shallow/narrow and deep/wide defects showed a complete fill should not be interpreted as lack of significant difference in size between the two defect groups; the 2 × 2 mm (depth × width) difference in defect dimensions between the two groups corresponded to a 2.25 times larger volume for deep/wide defects compared with narrow/shallow defects. In addition, both narrow/shallow and wide/deep defects might by inference qualify as critical-size defects, i.e., defects that do not heal to completion unless subjected to a regenerative treatment (Schmitz & Hollinger 1986), even if using an 18-month healing interval and molar location. It has been shown that surgically created one-wall, intrabony 4 × 4 mm (width × depth) premolar defects in dogs, i.e., a defect with configuration and dimensions corresponding to the narrow/shallow one-wall molar defects in the present study, will support cementum and bone formation approximating 30–35% of the defect height following a 2-month healing interval (Kim et al. 2005) and sham surgery. Extending the healing interval to 6 months does not appear to have a significant impact on cementum and bone formation (Choi et al. 2002). In context, the observation of complete regeneration in both shallow/narrow and deep/wide defects should rather be interpreted as an illustration of the strong innate regenerative potential of the periodontium (Polimeni et al. 2004).

The use of a single long-term healing period in the current experiment does not allow for any assumptions relative to resorption of the bovine bone biomaterial. Both narrow/shallow and wide/deep defects in the present study displayed a small fraction (12–13%) of the bovine bone biomaterial compared with what

Table 1. Mean (± SD) histometric results: defect height, cementum and bone formation, and root resorption/ankylosis (mm); and fraction bone, connective tissue (CT), and bovine bone biomaterial (%) in wide/deep and narrow/shallow periodontal defects ($N = 3$)

	Defect height	Cementum height	Bone height	Root resorption/ankylosis	Fraction bone	Fraction CT	Fraction bovine bone
Wide/deep	7.9 ± 0.7	5.6 ± 0.5	5.6 ± 0.6	1.5 ± 0.8	57.5 ± 20.9	4.2 ± 4.9	11.6 ± 3.4
Narrow/shallow	6.3 ± 0.3	4.2 ± 0.1	4.0 ± 0.8	–	65.0 ± 12.9	1.4 ± 1.3	13.1 ± 8.9

Table 2. Mean (± SD) histometric results: periodontal ligament width (mm) and fraction fiber bundles and vascular elements (%) at the crestal, center, and apical aspect of the wide/deep and narrow/shallow periodontal defects, and within the native periodontal ligament ($N = 3$)

	Wide/deep				Narrow/shallow			
	crestal	center	apical	native	crestal	center	apical	native
Width	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Fibers	79.2 ± 7.9	88.8 ± 4.2	87.6 ± 6.7	82.8 ± 3.9	79.9 ± 14.5	80.8 ± 13.6	71.8 ± 4.5	91.1 ± 3.2
Vascular elements	20.8 ± 7.9	11.2 ± 4.2	12.4 ± 6.7	17.3 ± 3.9	20.1 ± 14.5	19.2 ± 13.6	28.2 ± 4.5	8.8 ± 3.2

was estimated from photomicrographs of previous animal (Yamada et al. 2002, Sakata et al. 2006) and human studies (Camelo et al. 1998, Mellonig 2000, Paolantonio et al. 2001, Nevins et al. 2003, Sculean et al. 2004); specific data regarding residual biomaterial in these studies were not provided. Other reports with short/shorter healing intervals suggest that the bovine bone biomaterial undergoes osteoclastic resorption (Berglundh & Lindhe 1997, Hämmerle et al. 1998, Araujo et al. 2001, Cardaropoli et al. 2005) implying that the material would eventually be cleared from the defect site. The small fraction of bovine bone biomaterial in the present study may not be due to biodegradation of the biomaterial but may merely represent a reflection of the bovine bone/collagen matrix containing less particulate bovine bone biomaterial per volume than what is typically observed when the particulate biomaterial is used as is (i.e., without the collagen matrix) to loosely fill a defect. In context, the relative proportion and/or spatial distribution of the biomaterial inside the bovine bone/collagen matrix have not been reported. In any case, no obvious evidence of active or past osteoclastic resorption including cells and scalloped borders or other evidence of biodegradation of the bovine bone particles was observed in the present study. This finding corroborates a multiple of both short and long-term animal and human reports suggesting that this bovine bone biomaterial is inert and remains sequestered in bone, marrow, and fibrovascular tissue (up to 10 years) (Schlegel & Donath 1998, Stavropoulos et al. 2001, 2003, Sartori et al. 2003, Araujo et al. 2008). The observations in the present study and in recent reports about the long-term preservation of clinical improvements obtained after treatment with the bovine bone biomaterial/GTR combination (Sculean et al. 2007, Slotte et al. 2007, Stavropoulos & Karring 2010) may suggest that the mere presence of the bovine bone particles inside the regenerated periodontal tissues have no influence on the stability of clinical conditions. In context, the long-term effect of the residual biomaterial both with regard to the biomechanical properties of bone and the possibility of an accelerated inflammatory process following re-infection of the regenerated site remain unknown but should also be considered.

In conclusion, both wide/deep and narrow/shallow intrabony defects have a substantial potential for periodontal regeneration in this pre-clinical model. The contribution of the bovine bone biomaterial and guided tissue regeneration to this regenerative potential is not clear.

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Clinical Relevance

Scientific rationale for study: Previous evidence suggests that the outcome of healing in defects implanted with a deproteinized bovine bone biomaterial in combination with GTR might depend on the volume and geometry of the defect site. Implantation of this bone biomaterial in combination with GTR in large periodontal defects with limited number of bone walls might not enhance but rather obstruct/delay periodontal wound healing/regeneration even on the long term.

Principal findings: Substantial periodontal regeneration encompassing almost the entire intrabony defect was observed in both wide/deep and narrow/shallow defects implanted with a bovine bone/collagen matrix under provisions for GTR following an 18-month healing interval.

Practical implications: Implantation of intrabony periodontal defects with the deproteinized bovine bone/collagen matrix in combination with GTR is permissive to substantial periodontal regeneration.