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Prognostic factors for alveolar regeneration: bone formation at teeth and titanium implants

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Abstract

Objectives: There is a limited understanding of the effect of defect characteristics on alveolar bone healing. The objectives of this study were to assess the effect of alveolar bone width and space provision on bone regeneration at teeth and titanium implants, and to test the hypothesis that the regenerative potentials at teeth and implants are not significantly different.

Methods: Critical size, 5–6-mm, supra-alveolar, periodontal defects were surgically created in 10 young adult dogs. Similarly, critical size, 5-mm, supra-alveolar, peri-implant defects were created in four dogs. A space-providing expanded polytetrafluoroethylene device was implanted for guided tissue regeneration/guided bone regeneration. The animals were euthanized at 8 weeks postsurgery. Histometric analysis assessed alveolar bone regeneration (height) relative to space provision by the device and the width of the alveolar crest at the base of the defect. Statistical analysis used the linear mixed models.

Results: A significant correlation was found between bone width and wound area ($r = 0.55892$, $p < 0.0001$). Generally, bone width and wound area had statistically significant effects on the extent of bone regeneration ($p < 0.0005$ and $p < 0.0001$, respectively). Bone regeneration was linearly correlated with the bone width at periodontal ($p < 0.001$) and implant ($p = 0.04$) sites, and with the wound area at periodontal ($p < 0.0001$) and implant ($p = 0.03$) sites. The relationships of bone regeneration with these two variables were not significantly different between teeth and implants (bone width: $p = 0.83$; wound area: $p = 0.09$). When adjusted for wound area, bone regeneration was significantly greater at periodontal than at implant sites ($p = 0.047$).

Conclusions: The horizontal dimension of the alveolar bone influences space provision. Space provision and horizontal dimension of the alveolar bone appear to be important determinants of bone regeneration at teeth and implants. The extent of alveolar bone formation at implant sites is limited compared with that at periodontal sites.

Key words: alveolar bone; dogs; guided bone regeneration; guided tissue regeneration; tissue engineering; titanium implants

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Pathologic sequelae of periodontal disease are represented by various degrees of periodontal destruction. Therefore, the ultimate goal of periodontal therapy is not only to arrest the periodontal disease process but also to reconstruct, ''restitutio ad integrum'', the lost periodontium through regeneration of cementum, periodontal ligament, alveolar bone, and gingiva. Melcher (1976) formulated the hypothesis that eventually led to therapies conducive to periodontal regeneration suggesting that migration and proliferation of cells from the periodontal ligament onto the periodontally exposed root surface was fundamental for periodontal regeneration. Nyman et al. (1982) pioneered this concept in periodontal therapy. They showed that application of a device, a Millipore filter, during periodontal surgery to serve as a barrier to support migration and proliferation of cells and vascularity from the periodontal ligament and exclude gingival epithelial and connective tissue cells from contacting the root surface resulted in periodontal regeneration in a human intrabony defect. The same research group presented the first controlled preclinical and clinical studies evaluating this novel therapy, guided tissue regeneration (GTR) (Gottlow et al. 1984, 1986).

A meta-analysis of 18 clinical investigations on the outcomes of periodontal therapy in 342 deep intrabony defects showed that significant clinical attachment-level gains were observed following GTR (Tonetti & Cortellini 1997). However, a review of the literature also reveals that considerable variability in outcomes may be observed. Besides clinical variables already elucidated that may influence outcomes of GTR, other biological parameters may play a determinant role in the outcome of periodontal regenerative therapy. Preclinical evaluations in standardized model systems under optimal conditions for healing suggest that space provision may have a determinant influence on GTR outcomes. In a supra-alveolar periodontal defect model in dogs evaluating the effect of expanded polytetrafluoroethylene (ePTFE) devices with limited space provision, Haney et al. (1993) observed a mean vertical bone regeneration amounting to 1.4 mm for sites receiving the ePTFE device compared with 0.3 mm for the sham-surgery control. In the same animal model, utilizing a reinforced space-providing ePTFE device, Sigurdsson et al. (1994) showed a mean vertical bone regeneration of 2.9 mm for sites receiving the device compared with 0.6 mm for the surgical control. These biologic studies suggest that GTR has a significant influence on the regeneration of alveolar bone in periodontal defects.

The first attempt to translate the principles of GTR to regenerate alveolar bone in conjunction with titanium implants (guided bone regeneration (GBR)) was presented by Dahlin et al. (1989). In a rabbit model, 15 titanium implants with three to four threads exposed in a supracrestal fashion were covered with an ePTFE device; 15 implants received the same treatment without the device. New bone formation and osseointegration (bone–implant contact) were consistently observed at the GBR sites. Jovanovic et al. (1995) first reported vertical alveolar augmentation around implants in a dog model. Vertical augmentation of the alveolar bone ranged up to 1.9 mm in the GBR groups versus 0.5 mm in the surgical control. Caplanis et al. (1997) evaluated GBR in 5-mm supra-alveolar peri-implant defects in dogs. Contralateral defect sites were randomized to receive GBR alone or GBR combined with demineralized, freeze-dried, allogeneic bone (DFDBA). Vertical bone augmentation at 16 weeks postsurgery ranged from 1.1 (GBR) to 1.5 mm (GBR+ DFDBA) without significant differences between the protocols. It is apparent from these studies that GBR has a limited potential to enhance alveolar regeneration. Interestingly, DFDBA does not appear to have an osteoinductive or osteoconductive effect to further support alveolar augmentation.

Bone formation around titanium implants may differ from that in periodontal sites because of the absence of tissue resources sequestered in the periodontal ligament. If this were the case, it would be expected that under similar circumstances GTR will result in enhanced new bone formation when compared with GBR. The objectives of this study were to assess the effect of alveolar bone width and space provision on bone regeneration at teeth and titanium implants, and to test the hypothesis that the regenerative potentials at teeth and implants are not significantly different.

Material and Methods

Animals and experimental procedures

This study evaluated histologic specimens from three study groups including a total of 14 young adult dogs. Critical size, 6-mm, supra-alveolar, periodontal defects were surgically created in Groups 1 (six animals; Wikesjö et al. 2003a) and 2 (four animals; Wikesjö et al. 2003b), and critical size, 5-mm, supra-alveolar, peri-implant defects were created in Group 3 (four animals; Wikesjö et al. 2004) (Fig. 1). All surgical procedures were performed under general and local anesthesia.

For the supra-alveolar periodontal defects (Wikesjö & Selvig 1999), alveolar bone was removed around the circumference of the mandibular premolars and first molar using chisels and water-cooled rotating burs following sulcular incisions and reflection of buccal and lingual mucoperiosteal flaps. The first and second premolars were extracted and the first molar amputated at the level of the reduced alveolar crest. The root surfaces of the third and fourth premolars were instrumented with curettes, chisels, and water-cooled rotating diamonds to remove the cementum. The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento-enamel junction (CEJ) and the cut surfaces smoothed. Exposed pulpal tissues were sealed (Cavit®, ESPE, Seefeld/Oberbayern, Germany). Clinical defect height, from the CEJ to the reduced alveolar crest, was set to 6 mm as measured with a periodontal probe.

For the supra-alveolar peri-implant defects (Wikesjö et al. 2001), buccal and lingual mucoperiosteal flaps were reflected following buccal and lingual sulcular incisions from the canine to the second molar. Alveolar bone was removed around the circumference of the first, second, third, and fourth premolars using water-cooled rotating burs to a level of 6 mm from the CEJ and the teeth were then extracted. The first molar was amputated at the level of the surgically reduced alveolar crest. Custom titanium implants, two turned and one surface acid-etched (Implants Innovations, Inc., Palm Beach Gardens, FL, USA), were placed 5 mm within the surgically reduced alveolar ridge to the level of a reference thread, creating 5-mm, supra-alveolar, peri-implant defects. The turned implants were placed in the region of the fourth premolar and the surface acid-etched implant was placed in the region of the third premolar.

The defect sites in Group 1 received a space-providing porous ePTFE device (Reinforced GORE-TEX® ePTFE, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) (Fig. 1). The defect sites in Group 2 were treated in a similar fashion but, in addition, received a bioresorbable collagen sponge underneath the ePTFE device. The supra-alveolar peri-implant defects in Group 3 received the porous ePTFE device and the bioresorbable collagen sponge such as in Group 2. The porous ePTFE device, custom-made for the supra-alveolar, critical size, periodontal and peri-implant defect models exhibited laser-etched 300-μm pores at 0.8-mm (center to center) intervals and was reinforced with a laminated polypropylene mesh. These characteristics have been shown to support regeneration of alveolar bone and cementum regeneration in the supra-alveolar periodontal defect model (Wikesjö et al 2003a). The devices were fixed to the reduced alveolar bone with medical-grade stainless steel tacks (FRIOS® Augmentation system, Friadent, Mannheim, Germany). Following placement of the ePTFE device, the periostea were fenestrated at the base of the mucoperiosteal flaps to allow tension-free flap apposition. The flaps were advanced; the flap margins being adapted 3–4 mm coronal to the ePTFE device and sutured

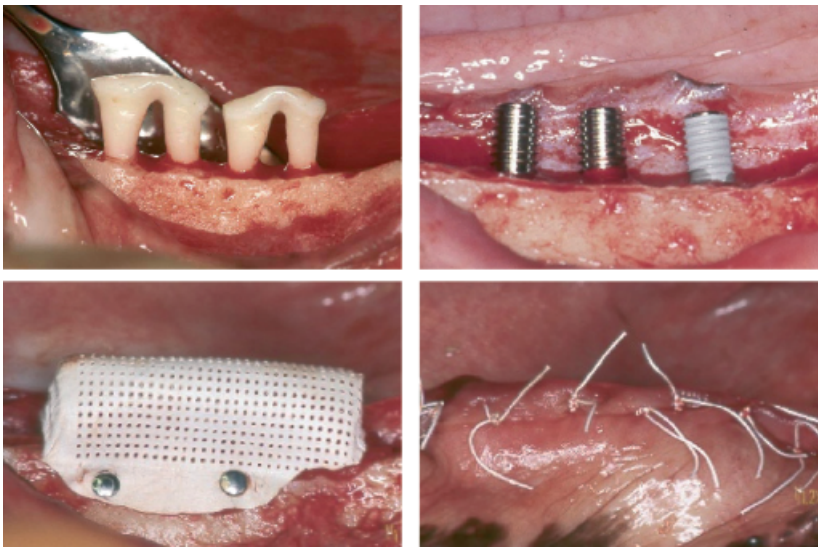

Fig. 1. Critical size, supraalveolar, periodontal or peri–implant defects were surgically created in 14 dogs. A porous ePTFE device, stabilized with stainless steel tacks, was used to cover the teeth or titanium implants. Mucoperiosteal flaps were released and advanced to cover the ePTFE device for tension-free wound closure.

(GORE-TEX™ Suture CV5, W.L. Gore & Associates Inc.). To alleviate potential trauma to the experimental mandibular periodontal or peri-implant sites postsurgery, the first, second and third maxillary premolars were surgically extracted and the maxillary fourth premolar reduced in height and exposed pulpal tissues sealed (Cavit®).

Postsurgery care

The animals were fed a canned soft dog food diet postsurgery. Buprenorphine HCl (0.015 mg/kg i.m. b.i.d. for 48 h) was administered for pain control. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg, i.m. b.i.d. for 14 days) was used for infection control. Plaque control was maintained by daily topical application of a 2% chlorhexidine dilution until gingival suture removal, thereafter once daily, Monday through Friday, until the completion of study. Sutures were removed under sedation at approximately 8 days postsurgery. The ePTFE device was not removed during the course of study. The healing interval was 8 weeks.

Histological processing and evaluation

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

Block specimens from the titanium implant sites were fixed in 10% buffered formalin for 3–5 days, dehydrated in alcohol, and embedded in methylmethacrylate resin (Technovit 7200 VLC, Heraeus Kulzer, Verheim, Germany). The implants were cut mid-axially in a buccal–lingual plane into sections of 200-μm thickness using the cutting–grinding technique (EXAKT Apparatebau, Norderstedt, Germany), and subsequently ground and polished to a final thickness of approximately 40 μm (Donath & Breuner 1982, Rohrer & Schubert 1992). The sections were stained with Stevenel's blue and van Gieson's picro fuchsin. The most central section from each implant was used for the histologic and histometric analysis.

One calibrated investigator (G.P.; intraclass correlation coefficient of repeated measurements = 0.984) performed the histometric analysis using incandescent and polarized light microscopy (BX 60, Olympus America, Inc. Melville, NY, USA), a microscope digital camera system (DP10, Olympus America, Inc.), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA) with custom programs for the supra-alveolar periodontal and peri-implant defect models. For the periodontal defects, the most central stained section of each root of the third and fourth premolar teeth was identified by the size of the root canal. This section was subjected to histometric analysis. For the peri-implant defects, the central section of each of the three implants in each jaw quadrant was selected and used for the histometric analysis. The following parameters were recorded for histologic sections of buccal and lingual surfaces of teeth and implants (Fig. 2):

- *Wound area*: area circumscribed by the planed root surface/supracrestal aspect of the titanium implant, the ePTFE device, and the base of the defect at the level of the apical extension of the root planing or the implant reference notch.
- *Bone width*: distance between the apical extension of root planing or the titanium implant reference notch and the lateral extension of the resident bone.
- *Bone regeneration (height)*: distance between the apical extension of root planing or the titanium implant reference notch and the coronal extension of regenerated alveolar bone along the planed root/supracrestal aspect of the implant.

Data analysis

Statistical analysis used the linear mixed models (Verbeke & Molenberghs 1997).

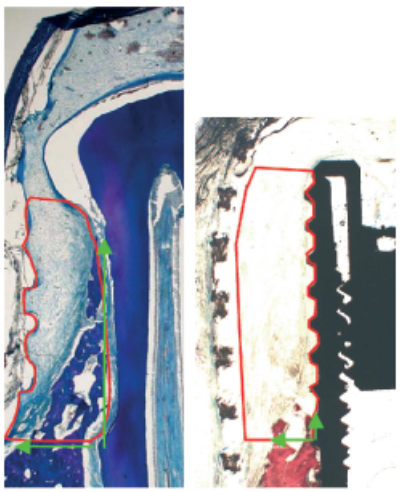

Fig. 2. Histometric parameters evaluated in the critical size, supraalveolar periodontal and peri-implant defect model in this study. The green arrows represent the measurements of bone width and bone regeneration (height), the red outline represents the wound area.

The individual sites were used as the unit of analysis, and the animals used as a random variable. The analytical model adjusted for the correlation between the sites of the same animal, and used bone regeneration (height) as the dependent variable (outcome) and bone width and wound area as independent variables. There were a total of 74 periodontal and 24 peri-implant sites in the model. There were no significant differences in the studied relationships of the dependent and independent variables between Groups 1 and 2. Therefore, data for these two groups were pooled, and this group will be referred to as ''periodontal sites''. Group 3 will be referred to as ''implant sites''. A high correlation was assessed between wound area and bone width (Pearson's $r = 0.56$, $p < 0.0001$), and the relationships of these two variables with the dependent variable were therefore modeled separately.

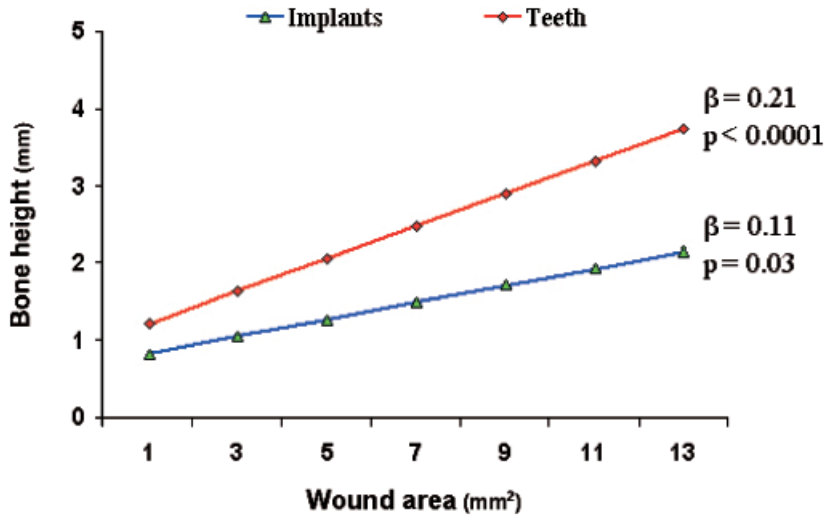

Fig. 3. The relationship between bone regeneration (height) and wound area at periodontal and implant sites.

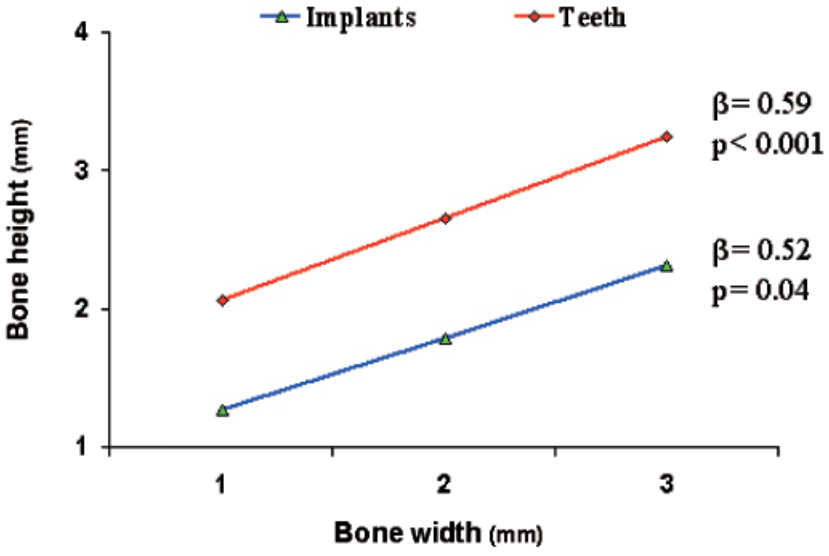

Fig. 4. The relationship between bone regeneration (height) and bone width at periodontal and implant sites.

Results

Main-effect analysis showed that both bone width and wound area had significant effects on the extent of bone regeneration ($p < 0.0005$ and $p < 0.0001$, respectively). The extent of bone regeneration had significant linear relationships with the bone width at periodontal ($\beta = 0.59$, $p < 0.001$) and implant sites ($\beta = 0.52$, $p = 0.04$) (Fig. 3), and also with the wound area at periodontal ($\beta = 0.21$, $p < 0.0001$) and implant sites ($\beta = 0.11$, $p = 0.03$) (Fig. 4). Furthermore, the relationships of bone regeneration with these two variables were not significantly different between teeth and implants (bone width: $p = 0.83$; wound area: $p = 0.09$).

The unadjusted mean bone regeneration (height) at periodontal and implant sites is shown in Table 1. Adjusting for bone width at the site showed that mean bone regeneration was somewhat higher at periodontal than implant sites, although the difference was not statistically significant ($p = 0.117$, Table 2). However, when adjusted for wound area at the site, bone regeneration was significantly greater at periodontal sites than at implants ($p = 0.047$, Table 3).

Table 1. Summary statistics for bone regeneration (mm), bone width (mm) and wound area (mm^2), by study group

Study group	Variable	*N*	Mean	SD	Min	Max
periodontal	bone height	74	2.16	0.96	0.0	4.28
	bone width	74	1.13	0.64	0.16	3.31
	wound area	74	5.31	2.73	1.0	12.78
implants	bone height	24	1.45	1.43	0.14	5.00
	bone width	24	1.34	0.69	0.13	2.48
	wound area	24	6.48	3.25	1.85	15.29

Discussion

The objectives of this study were to assess the effect of alveolar bone width and space provision on bone regeneration at teeth and titanium implants, and to test the hypothesis that the regenerative potentials at teeth and implants are not significantly different. Routine, critical size, 5–6-mm, supra-alveolar, periodontal defects were created in 10 dogs. Similarly, critical size, supra-alveolar, peri-implant defects were created in four dogs. A space-providing ePTFE device was implanted for GTR or GBR as appropriate. The animals

Table 2. Mean bone regeneration at periodontal and implant sites

Defect type	Mean	SE	*p*
implants	1.36	0.41	0.117
periodontal	2.17	0.26	

The means are adjusted for the effect of bone width.

Table 3. Mean bone regeneration at periodontal and implant sites

Defect type	Mean	SE	*p*
implants	1.28	0.35	0.047
periodontal	2.19	0.22	

The means are adjusted for the effect of wound area.

were euthanized following an 8-week healing interval for histometric analysis of alveolar bone regeneration. The results suggest that the horizontal dimension of the alveolar bone influences space provision. Space provision and horizontal dimension of the alveolar bone appear to be important factors for bone regeneration at teeth and implants. Alveolar bone formation at implant sites is limited compared with that at periodontal sites.

Space provision (evaluated as the wound area delineated by the ePTFE GTR/GBR device, the tooth/implant, and the alveolar crest at the base of the defect) and bone width were the dependent variables subject to statistical analysis in this study. It was shown that the width of the alveolar bone at the base of the defect might significantly influence space provision by the ePTFE device. In other words, a wide alveolar crest effectively supports the device resulting in a large wound area, while a narrow alveolar crest supports a smaller wound area. It was also shown that both wound area and bone width have significant effects on bone regeneration at teeth and implants. Thus, an increase in the magnitude of these two variables results in enhanced bone regeneration. Bone width is a variable that remains constant during the surgical procedure determined by the characteristics of the site. However, wound area can be subjected to various manipulations in order to support or expand space provision and thus enhance bone regeneration. Consistent with our biologic observations, Cortellini et al. (1995) have shown that structurally reinforced space-providing ePTFE devices may enhance periodontal regeneration. Others, applying GTR/GBR to treat periodontal defects or edentulous areas for implant placement, have utilized pins or tacks to support space provision (Buser et al. 1990, Becker et al. 1994, Simion et al. 1994, Tinti & Vincenzi 1994). It should be noted that placing slowly or non-resorbing biomaterials underneath a GTR or GBR device with the intent to support space provision might at the same time obstruct bone regeneration (Caplanis et al. 1997, Trombelli et al. 1999, Stavropoulos et al. 2001).

In the present study, 10 animals received ePTFE devices for GTR. Four of the animals additionally received a bioresorbable collagen sponge underneath the device following the specific protocol for Group 2. No significant differences in alveolar bone regeneration were observed between Group 1 (ePTFE alone) and Group 2 (ePTFE+ collagen sponge). The relationship between wound area/bone width and bone height did not result in significant differences between the protocols. Moreover, no other adjunctive or detrimental effects were observed relative to the collagen sponge, which appeared completely resorbed in all specimens evaluated, corroborating previous observations of this biomaterial in periodontal defects (Choi et al. 1993). Thus, it appears legitimate to group the observations from Group 1 with those from Group 2 for the present analysis.

The histometric analysis suggests similar patterns of bone regeneration following GTR and GBR. The width of the alveolar crest and the space provided by the ePTFE GTR or GBR device resulted in a significant relationship with the extent of alveolar bone regeneration for both protocols (Fig. 5). Thus, similarities in the behavior of factors influencing bone regeneration were observed for both teeth and implant sites. However, the histological evaluation pointed to dissimilarities in the morphology of bone regeneration. In the GTR group, bone regeneration exhibited a close proximity to root surface resembling the physiological appearance of the periodontal apparatus including alveolar bone, cementum, and a functionally oriented periodontal ligament. In contrast, the morphology of bone regeneration following GBR was somewhat different. Generally, the newly formed bone approximated the implant surface in the apical extent of the defect site to separate from the implant

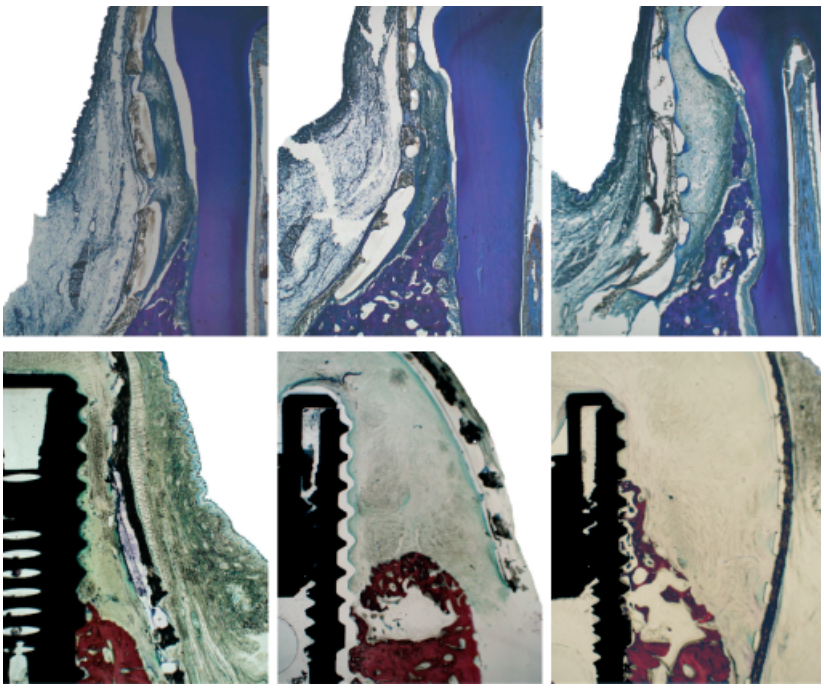

Fig. 5. Representative photomicrographs of supraalveolar periodontal and peri-implant defects with a space-providing porous ePTFE device. Synergistic effects between bone width and space provision on bone regeneration can be observed. A narrow alveolar crest (left) may not expand nor support the wound area resulting in limited bone formation. Wider ridges (center and right) effectively support/expand the wound area for enhanced bone formation.

surface more coronally, thus forming anatomical structures characteristic of the edentulous alveolar crest (Fig. 5, lower-center). These findings corroborate previous observations using the same experimental model to evaluate GTR or GBR ([3]Sigurdsson et al. 1994, Caplanis et al. 1997).

Adjusting for the effect of wound area, the magnitude of bone regeneration was significantly different between sites subject to GTR and GBR. The GTR sites exhibited significantly greater bone regeneration than that observed in the GBR sites. This observation suggests critical biologic differences between periodontal and peri-implant sites. While bone regeneration in periodontal sites may be induced by vascular and cellular elements sequestered in the periodontal ligament or by synergistic effects between the periodontal attachment and the resident alveolar bone, regeneration at peri-implant sites appears solely dependent on the evidently limited regenerative potential of the alveolar bone. In consequence, principles valid for GTR may not necessarily be immediately applied to GBR. Moreover, from a clinical perspective, these biologic observations suggest that bone regenerative procedures at implant sites may be considerably more challenging than in periodontal sites.

Conclusion

The results suggest that the horizontal dimension of the alveolar bone influences space provision. Space provision and horizontal dimension of the alveolar bone appear to be important determinants of bone regeneration at teeth and titanium implants. The extent of bone formation at implant sites is limited compared with that at periodontal sites.

References

Becker, W., Becker, B. E. & McGuire, M. K. (1994) Localized ridge augmentation using absorbable pins and e-PTFE barrier membranes: a new surgical technique. Case reports. *The International Journal of Periodontics & Restorative Dentistry* **14**, 48–61.

Buser, D., Brägger, U., Lang, N. P. & Nyman, S. (1990) Regeneration and enlargement of jaw bone using guided tissue regeneration. *Clinical Oral Implants Research* **1**, 22–32.

Caplanis, N., Sigurdsson, T. J., Rohrer, M. D. & Wikesjö, U. M. E. (1997) Effect of allogeneic, freeze-dried, demineralized bone matrix on guided bone regeneration in supra-alveolar peri-implant defects in dogs. *The International Journal of Oral & Maxillofacial Implants* **12**, 634–642.

Choi, S. Y., Nilvéus, R. E., Minutello, R. D., Zimmerman, G. J. & Wikesjö, U. M. E. (1993) Effect of a collagen matrix on healing in periodontal fenestration defects in dogs. *Journal of Periodontology* **64**, 878–882.

Cortellini, P., Pini Prato, G. & Tonetti, M. S. (1995) Periodontal regeneration of human intrabony defects with titanium reinforced membranes. A controlled clinical trial. *Journal of Periodontology* **66**, 797–803.

Dahlin, C., Sennerby, L., Lekholm, U., Linde, A. & Nyman, S. (1989) Generation of new bone around titanium implants using a membrane technique: an experimental study in rabbits. *The International Journal of Oral & Maxillofacial Implants* **4**, 19–25.

Donath, K. & Breuner, G. (1982) A method for the study of undecalcified bones and teeth with attached soft tissues. The Sage–Schliff (sawing and grinding) technique. *Journal of Oral Pathology* **11**, 318–326.

Gottlow, J., Nyman, S., Karring, T. & Lindhe, J. (1984) New attachment formation as the result of controlled tissue regeneration. *Journal of Clinical Periodontology* **11**, 494–503.

Gottlow, J., Nyman, S., Lindhe, J., Karring, T. & Wennström, J. (1986) New attachment formation in the human periodontium by guided tissue regeneration. Case reports. *Journal of Clinical Periodontology* **13**, 604–616.

Haney, J. M., Nilvéus, R. E., McMillan, P. J. & Wikesjö, U. M. E. (1993) Periodontal repair in dogs: expanded polytetrafluoroethylene barrier membranes support wound stabilization and enhance bone regeneration. *Journal of Periodontology* **64**, 883–890.

Jovanovic, S. A., Schenk, R. K., Orsini, M. & Kenney, E. B. (1995) Supracrestal bone formation around dental implants: an experimental dog study. *The International Journal of Oral & Maxillofacial Implants* **10**, 23–31.

Melcher, A. H. (1976) On the repair potential of periodontal tissues. *Journal of Periodontology* **47**, 256–260.

Nyman, S., Lindhe, J., Karring, T. & Rylander, H. (1982) New attachment following surgical treatment of human periodontal disease. *Journal of Clinical Periodontology* **9**, 290–296.

Rohrer, M. D. & Schubert, C. C. (1992) The cutting–grinding technique for histologic preparation of undecalcified bone and bone-anchored implants. Improvements in instrumentation and procedures. *Oral Surgery, Oral Medicine, and Oral Pathology* **74**, 73–78.

Sigurdsson, T. J., Hardwick, R., Bogle, G. C. & Wikesjö, U. M. E. (1994) Periodontal repair in dogs: space provision by reinforced ePTFE membranes enhances bone and cementum regeneration in large supraalveolar defects. *Journal of Periodontology* **65**, 350–356.

Simion, M., Trisi, P. & Piattelli, A. (1994) Vertical ridge augmentation using a membrane technique associated with osseointegrated implants. *The International Journal of Periodontics & Restorative Dentistry* **14**, 496–511.

Stavropoulos, A., Kostopoulos, L., Mardas, N., Nyengaard, J. R. & Karring, T. (2001) Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clinical Implant Dentistry & Related Research* **3**, 156–165.

Tinti, C. & Vincenzi, G. P. (1994) Expanded polytetrafluoroethylene titanium-reinforced membranes for regeneration of mucogingival recession defects. A 12-case report. *Journal of Periodontology* **65**, 1088–1094.

Tonetti, M. & Cortellini, P. (1997) Case selection and treatment considerations of guided tissue regeneration in deep intrabony defects. *Current Opinion in Periodontology* **4**, 82–88.

Trombelli, L., Lee, M. B., Promsudthi, A., Guglielmoni, P. G. & Wikesjö, U. M. E. (1999) Periodontal repair in dogs: histologic observations of guided tissue regeneration with a prostaglandin E_1 analog/methacrylate composite. *Journal of Clinical Periodontology* **26**, 381–387.

Verbeke, G. & Molenberghs, G. (1997) *Linear Mixed Models in Practice: A SAS-Oriented Approach. Lecture Notes in Statistics*. New York: Springer-Verlag.

Wikesjö, U. M. E., Lim, W. H., Thomson, R. C. & Hardwick, W. R. (2003a) Periodontal repair in dogs: gingival tissue exclusion, a critical requirement for guided tissue regeneration? *Journal of Clinical Periodontology* **30**, 655–664.

Wikesjö, U. M. E., Qahash, M., Thomson, R. C., Cook, A. D., Rohrer, M. D., Wozney, J. M. & Hardwick, W. R. (2004) rhBMP-2 significantly enhances guided bone regeneration. *Clinical Oral Implants Research* **15**, 194–204.

Wikesjö, U. M. E. & Selvig, K. A. (1999) Periodontal wound healing and regeneration. *Periodontology 2000* **19**, 21–39.

Wikesjö, U. M. E., Sorensen, R. G. & Wozney, J. M. (2001) Augmentation of alveolar bone and dental implant osseointegration: clinical implications of studies with rhBMP-2. *The Journal of Bone Joint Surgery American Volume* **2** (Suppl. 1, Part 2), S136–S145.

Wikesjö, U. M. E., Xiropaidis, A. V., Thomson, R. C., Cook, A. D., Selvig, K. A. & Hardwick, W. R. (2003b) Periodontal repair in dogs: rhBMP-2 significantly enhances bone formation under provisions for guided tissue regeneration. *Journal of Clinical Periodontology* **30**, 705–714.

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