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Prognostic factors for alveolar regeneration: osteogenic potential of resident bone

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Abstract

Objectives: There is a limited understanding of the role of resident bone in periodontal regeneration. The objective of this study was to evaluate the influence of the resident alveolar bone on bone regeneration in conjunction with guided tissue regeneration (GTR) in the presence or the absence of cell occlusivity.

Methods: Critical-size, 6-mm, supra-alveolar periodontal defects were created in six young adult Beagle dogs. Space-providing, occlusive or porous expanded polytetrafluaroethylene devices were implanted to provide for GTR. Treatments were alternated between left and right jaw quadrants in subsequent animals. The gingival flaps were advanced for primary intention healing. The animals were euthanized at week 8 postsurgery. The histometric analysis assessed regeneration of alveolar bone relative to space-provision by the GTR device and width of the alveolar crest at the base of the defect.

Results: There were no significant differences in mean alveolar regeneration between sites receiving the porous GTR device with a narrow versus a wide alveolar ridge after adjusting for wound area (2.22 versus 2.50 mm, respectively; $p = 0.36$). In contrast, analysis using sites receiving the occlusive GTR device revealed significantly greater bone regeneration at sites with a wide compared with a narrow alveolar ridge (3.34 versus 2.53 mm, respectively; $p = 0.02$). Regression analysis showed a significant relationship ($p \leqslant 0.05$) between space-provision and bone regeneration for all groups except for sites with a wide alveolar ridge receiving the occlusive GTR device ($p = 0.5$).

Conclusions: The resident alveolar bone may significantly influence the magnitude of alveolar bone regeneration. The relative presence of cells from the gingival connective tissue may attenuate this effect.

Key words: alveolar bone; dogs; expanded polytetrafluoroethylene devices; guided tissue regeneration; tissue engineering

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One important objective of periodontal therapy is the reconstruction of the periodontal apparatus including alveolar bone, cementum, periodontal ligament, and gingiva. Melcher (1976) presented a concept of selected cell repopulation and argued the periodontal ligament to be critical to periodontal regeneration; Nyman et al. (1982) first reported the clinical application of this concept. To date, a large body of clinical and histological evidence affirms the possibility for periodontal regeneration using this concept of guided tissue regeneration (GTR) (Karring et al. 1993, Cortellini & Tonetti 2000). A meta-analysis of 18 clinical investigations on the outcomes of periodontal therapy in 342 deep intrabony defects has shown that significant clinical attachment level gains can be observed following GTR (Tonetti & Cortellini 1997). Nevertheless, a review of the literature reveals that considerable variability in outcomes may be expected. A number of studies have concerned the role of various factors for the outcome of GTR (Tonetti et al. 1993, 1996, Kornman & Robertson 2000). Subsequent experimental studies have elucidated surgical and biological prognostic factors for regenerative outcomes (Polimeni et al. 2002, 2003a, b).

Space provision appears to be a critical factor for regeneration of alveolar bone. A statistically significant direct relationship has been shown between space provision by a GTR device and new bone formation (Haney et al. 1993, Polimeni et al. 2002, 2003a, b). Previous studies have pointed to the relative importance of the periodontal ligament

for regeneration of alveolar bone (Karring et al. 1980, Isidor et al. 1986, Polimeni et al. 2002). Moreover, recent observations suggest that the width of the alveolar bone may be an important determinant for space provision by a regenerative device (Polimeni et al. 2003a, b). Narrow alveolar ridges may limit the space-providing capacity of a GTR device, whereas wide ridges may effectively expand the space-providing capacity. In other words, an indirect effect of the morphology of the resident alveolar ridge may be observed relative to the magnitude of alveolar bone regeneration. However, it remains unclear whether the resident alveolar bone exerts a more direct role on bone formation in conjunction with GTR. The objective of this study was to evaluate the influence of the resident alveolar bone on bone regeneration in the presence or the absence of cell occlusivity.

Material and Methods

Animals

The experimental protocol of this study has been detailed elsewhere (Wikesjö et al. 2003). Briefly, six young adult male Beagle dogs obtained from an USDA-approved dealer were used; the experimental protocol was approved by the Animal Care and Use Committee, W.L. Gore & Associates Inc., Flagstaff, AZ, USA.

GTR devices

One space-providing occlusive or porous expanded polytetrafluoroethylene (ePTFE) GTR device (Reinforced GORE-TEX® ePTFE; W.L. Gore & Associates Inc.) was used to provide for GTR for each defect site. The occlusive device had a 15–25 μm nominal pore size and was reinforced with a laminated polypropylene mesh. The porous devices exhibited the same characteristics except for laser-etched 300-μm pores at 0.8 mm (center-to-center) intervals allowing for penetration of the gingival connective tissue.

Experimental surgery

Critical-size, 6-mm, supra-alveolar, periodontal defects were created around the third and fourth mandibular premolar teeth in the right and left jaw quadrants in each animal under general and local anesthesia (Wikesjö et al. 1994, 2003). The crowns of the teeth were reduced to approximately 2 mm coronal to the cemento–enamel junction and exposed pulpal tissues were sealed. The occlusive and porous GTR devices, one device/defect, were implanted into left and right jaw quadrants in a split-mouth design. The devices were placed to cover the defects without contacting the teeth (Fig. 1). Autologous blood drawn from an i.v. catheter was expelled underneath the GTR device to ensure an adequate blood clot. The GTR device was fixed to the reduced alveolar bone with medical-grade stainless steel tacks. The periostea were then fenestrated at the base of the gingival flaps to allow tension-free flap apposition. The flaps were advanced and the flap margins adapted 3–4 mm coronal to the GTR device and sutured.

Postsurgery protocol

The animals were fed a soft dog food diet postsurgery. Buprenorphine (0.04 mg/kg s.q. every 5 h) was used for postsurgery analgesia the first few days. A broad-spectrum antibiotic (enrofloxacin; 2.5 mg/kg, i.m., b.i.d.) was used for infection control for 14 days. Plaque control was maintained by twice daily topical application of a 2% chlorhexidine solution until gingival suture removal at approximately 10 days and once daily thereafter until the completion of study. The animals were euthanized at 8 weeks when the experimental teeth including surrounding soft and hard tissues were removed en bloc for histometric analysis. GTR devices were not removed during the healing interval.

Histological processing and analysis

The tissue blocks 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

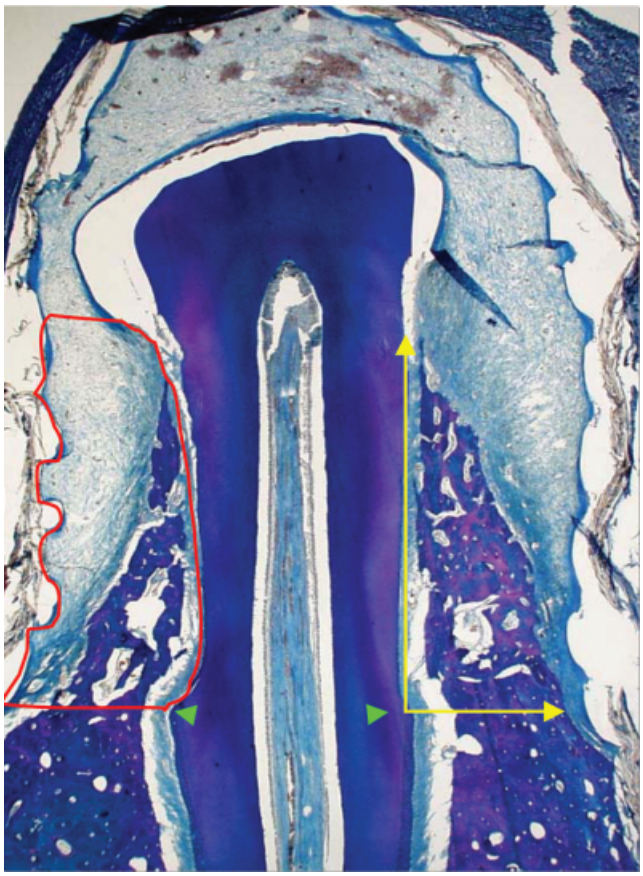

Fig. 1. Histologic section depicting the histometric parameters. The red outline shows the wound area. The yellow arrows show the vertical regeneration of the alveolar bone and the horizontal width of the alveolar bone at the base of the defect. Green arrowheads show the apical extent of the defect at the surgically reduced alveolar ridge. The self-supporting, space-providing guided tissue regeneration expanded polytetrafluoroethylene device does not contact the tooth.

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.

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). 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. The following measurements were recorded for the buccal and the lingual tooth surfaces for each section (Fig. 1):

- *Bone regeneration (height)*: This is the distance between the apical extension of the root planing and the coronal extension of alveolar bone regeneration along the planed root.
- *Wound area*: This is the area circumscribed by the planed root surface, the GTR device, and the base of the defect at the level of the apical extension of the root planning.
- *Bone width*: The width of the resident bone was defined as the distance from the tooth to the outer surface of alveolar bone at the apical extension of root planing. This variable was assessed in millimeters, and then categorized into ''narrow'' or ''wide''. Narrow bone width was defined as a measurement $\leqslant 1$ mm, and wide width was defined as > 1 mm.

Data analysis

The data were analyzed using bivariate and multivariate analyses (Verbeke & Molenberghs 1997). The multivariate analysis used the Mixed Models analysis of variance (Proc Mixed in SAS V8.1, SAS Institute Inc., Cary, NC, USA), which is designed for the analysis of correlated data and modeling of random effects. The analytical model used the site as the unit of analysis, and adjusted for the correlation among sites of the same animal. The analysis assessed the relationship of alveolar bone width and bone regeneration for defect sites receiving the occlusive or porous membranes standardizing for wound area.

Results

Defect sites in three animals implanted with the occlusive GTR device became exposed during the healing interval and were thus not included in the analysis. There was no significant difference in mean alveolar bone regeneration between sites receiving the porous GTR device with a narrow versus a wide alveolar ridge after adjusting for wound area (2.22 versus 2.50 mm, respectively; $p = 0.36$; Table 1). Analysis using sites receiving the occlusive GTR device revealed significantly greater bone regeneration in sites with a wide compared with sites with a narrow alveolar ridge (3.34 versus 2.53 mm, respectively; $p = 0.02$; Table 1). Regression analysis showed a significant relationship ($p \leqslant 0.05$) between space provision and bone regeneration for all groups except for sites with wide alveolar ridge receiving the occlusive GTR device ($p = 0.5$; Fig. 2).

Discussion

The objective of this study was to evaluate the influence of the resident alveolar bone on bone regeneration in the presence or the absence of cell occlusivity. Routine, 6-mm, critical-size, supra-alveolar, periodontal defects were created in six young adult Beagle dogs. Space-providing occlusive and porous ePTFE devices were implanted to provide for GTR. The animals were euthanized at week 8 postsurgery. The results of the histometric analysis suggest that the resident alveolar bone may significantly contribute to the magnitude of alveolar bone regeneration. The relative presence of cells from the gingival connective tissue may attenuate this effect.

Table 1. Mean alveolar bone regeneration (height) by treatment (porous/occlusive GTR device) and width of resident bone at the site

Treatment	Bone width	Estimate	SE	*p*
porous	narrow ($\leqslant 1$ mm)	2.22	0.28	0.36
	wide (> 1 mm)	2.50	0.29	
occlusive	narrow ($\leqslant 1$ mm)	2.53	0.33	0.02
	wide (> 1 mm)	3.34	0.34	

GTR, guided tissue regeneration.

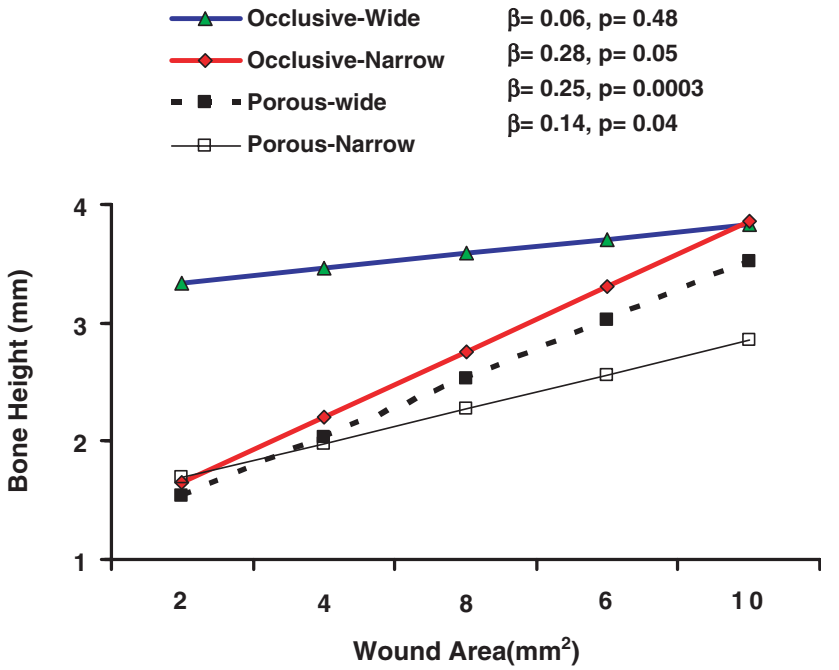

Fig. 2. The relationship of bone height and wound area by type of guided tissue regeneration device and width of resident bone.

This study used an experimental model including 6-mm, critical-size, supra-alveolar periodontal defects in dogs. The supraalveolar periodontal defect model has been shown to be a valuable tool to evaluate the regenerative potential of alveolar bone and the periodontal attachment in the assessment of candidate therapies prior to clinical application (Wikesjö & Selvig 1999). The defect dimensions provide for clinically relevant regeneration of alveolar bone and cementum. The defect morphology allows an unbiased, highly reproducible strategy of analysis (Koo et al. 2003a, b). Alveolar bone and cementum regeneration in sham-operated controls have been shown not to exceed 15% of the defect height over a 4- or 8-week healing interval (Wikesjö et al. 1994).

Space provision has been shown an important factor for the outcome of periodontal regenerative procedures (Haney et al. 1993, Sigurdsson et al 1994, Trombelli et al. 1999). Subsequent studies have shown a direct relationship between the space provided by GTR devices and the magnitude of newly formed bone under a variety of experimental conditions (Polimeni et al 2002, 2003a, b). These reports have also shown a highly significant correlation between the width of the resident bone at the base of the defect and space provision by the GTR device. In other words, sites with wide ridges are more likely to effectively support the GTR device resulting in enhanced space provision while sites with narrow ridges are more likely to experience a collapse of the device and limited space provision. In order to study the osteogenic potential of the resident alveolar bone as a prognostic factor for the outcome of GTR, one must consider the effect of the resident alveolar bone on space provision. Only comparisons of sites with different bone width but similar space provision may distinguish the osteogenic potential of the resident alveolar bone, from the effect of space provision.

In this study, in the presence of tissue occlusion and controlling for wound area, we established that sites exhibiting a wide alveolar ridge might have a greater osteogenic potential than sites with a narrow ridge. This observation suggests that the osteogenic potential of the resident bone plays a role in periodontal regeneration. This observation supplements those of previous studies evaluating regeneration of the periodontal attachment and alveolar bone in preclinical models (Karring et al. 1980, Isidor et al. 1986, Sigurdsson et al. 1994), which suggest that tissue elements originating from the periodontal ligament are critical to periodontal regeneration. However, these studies were not designed to investigate the contribution of the resident alveolar bone to the regenerative process. In the present study, assessment of the effect of the resident bone relative to other known factors influencing the outcome of periodontal regeneration was possible using the present experimental and statistical model.

In the absence of tissue occlusion (porous GTR devices) and controlling for wound area, sites exhibiting a wide alveolar ridge did not show an enhanced osteogenic potential compared with sites with a narrow ridge. This may be a consequence of the porous space-providing GTR device allowing the gingival connective tissue access to the wound area. It may be speculated that tissue resources including molecules, cells, and vascularity originating from the gingival connective tissue may have had an inhibitory effect on osteogenesis, and/or that migration and proliferation of tissue elements from the gingival connective tissue competitively occupied the space for bone to form into.

Observations from the present study show a positive correlation between space provision and bone regeneration at defect sites shielded by a porous GTR device, irrespective of the width of alveolar ridge and at defect sites with narrow alveolar ridge shielded by an occlusive GTR device. However, space provision did not significantly enhance bone regeneration at sites with a wide alveolar ridge treated with an occlusive GTR device. Provision of a wide wound area may contribute to an enhanced bone regeneration (Polimeni et al. 2002). Defect sites which possess a thick alveolar ridge usually feature a wide wound area, and this may be one of the rationales that such sites may experience enhanced bone regeneration (Polimeni et al. 2003b). However, in this study there was no significant correlation between wound area and the amount of bone regeneration at sites with a wide alveolar ridge that also received an occlusive GTR device. One may speculate that the regenerative potential at these sites may have been exhausted. In other words, once the healing potential of the site is exhausted, an increase in the magnitude of a prognostic factor, which under other circumstances would have resulted in increased bone regeneration, may not further influence the result.

Conclusion

The resident alveolar bone may significantly influence the magnitude of alveolar bone regeneration. The relative presence of cells from the gingival connective tissue may attenuate this effect.

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