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Prognostic factors for alveolar regeneration: effect of a space-providing biomaterial on guided tissue regeneration

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Abstract

Objectives: There is a limited understanding of the effect of bone biomaterials on the healing potential when used in conjunction with guided tissue regeneration (GTR). The objective of this study was to evaluate the effect of a space-providing coral-derived biomaterial on alveolar bone regeneration in conjunction with GTR.

Methods: Bilateral, critical-size, 6-mm, supra-alveolar, periodontal defects were created in four young adult Beagle dogs. In a split-mouth design, the animals received an ePTFE device to provide for GTR in contralateral defect sites with or without the coral biomaterial. The animals were euthanized at 4 weeks post surgery. A histometric analysis assessed vertical regeneration of alveolar bone relative to space-provision by the ePTFE device. Because of the correlation of within-dog measurements, a mixed model ANOVA was used to analyze the data.

Results: There was significantly greater mean bone regeneration in sites receiving calcium carbonate coral implant GTR (cGTR) compared to GTR ($p < 0.0001$). Sites providing larger wound areas exhibited greater bone regeneration compared to sites exhibiting smaller wound areas ($p < 0.0001$). However, grouping the sites by wound area thresholds showed that bone regeneration was not significantly different in sites receiving cGTR compared to sites receiving GTR alone, irrespective of the size of the wound area ($p > 0.5$).

Conclusions: Space-provision has a significant effect on bone regeneration following GTR. The coral biomaterial effectively enhances space-provision, and this appears to be the principal mechanism by which this biomaterial supports bone regeneration rather than postulated osteoconductive properties.

Key words: alveolar bone; bone biomaterials; experimental studies; guided tissue regeneration

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Studies in humans and experimental animals suggest that enhanced bone regeneration may be expected following reconstructive periodontal surgery including wound closure supported by guided tissue regeneration (GTR) devices (Nyman et al. 1982, Gottlow et al. 1984, 1986, 1990). There is also evidence to suggest that space-provision by the GTR device influences the total amount of new bone formation (Haney et al. 1993, Sigurdsson et al. 1994). Similar observations have been made in a clinical study investigating the effect of titanium-reinforced ePTFE devices positioned at the level of the CEJ compared to conventional ePTFE devices located at the alveolar crest in the treatment of intrabony defects (Cortellini et al. 1995). However, to date, no biological data are available to establish a direct relationship between space-provision and bone formation following GTR.

Various potentially osteogenic, osteoconductive, and osteoinductive therapies including the use of autogenous bone, bone derivatives, and bone substitutes have been suggested to support regeneration of alveolar bone, and for some therapies, the periodontal attachment (Mellonig 1996, Nasr et al. 1999). One of the rationales for using bone grafts or bone biomaterials has been to support space-provision in conjunction with the GTR device. In a recent study,

sites receiving a bioresorbable calcium carbonate coral implant (CI) in conjunction with GTR showed enhanced space-provision and bone formation (Wikesjö et al. 2003). However, it could not be discerned whether the observed effects may be attributed to GTR, space provision, or to osteoconductive properties of the CI biomaterial. Therefore, the objective of this study was to investigate the effect of the CI biomaterial on alveolar bone regeneration in conjunction with GTR.

Material and Methods

Animals

Four male Beagle dogs (age 18–24 months, weight 12–15 kg) exhibiting intact mandibular premolar dentition without crowding or evidence of periodontal disease were used. Animal selection and management, surgical protocol, and periodontal defect preparation followed a routine protocol approved for this study by the IACUC, Loma Linda University.

Biomaterials

ePTFE barrier devices (GORE-TEX® Regenerative Material Transgingival Configuration, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) were used. The tissue occlusive devices have a 15 to 25 μm nominal pore size. ePTFE sutures (GORE-TEX® Suture CV5, W.L. Gore & Associates Inc., Flagstaff, AZ, USA) were used for device fixation and wound closure.

A medical grade, resorbable, porous, particulate, calcium carbonate CI (Biocoral® 1000, Inoteb, Saint-Gonnery, France) was used. The coral implant was combined with a medical grade binding material that provided beneficial handling characteristics; hydroxyethyl starch was mixed with 0.5% gelatin and a 20 μM sodium acetate solution to form a visco-elastic gel to contain the calcium carbonate particles in a manageable mass.

Surgery procedure

Surgical procedures were performed using sodium pentobarbital anesthesia (20–30 mg/kg, i.v.) preceded by acepromazine sedation (1 mg/kg, i.m.). To maintain hydration, a sterile IV catheter was placed and animals received a constant rate infusion of LRS (10–20 mL/kg/h i.v.) when anesthetized. Routine dental infiltration anesthesia was used at the surgical sites. Thiopental sodium anesthesia (20–25 mg/kg, i.v.) was used for suture removal.

Routine, supra-alveolar, critical size, periodontal defects were created around the third and fourth mandibular premolar teeth in the right and left jaw quadrants in each animal (Wikesjö et al. 1994). Briefly, buccal and lingual mucoperiosteal flaps were reflected following buccal and lingual sulcular incisions from the canine tooth to the second molar. The first and second premolar teeth, and the first molar were extracted. Alveolar bone was removed around the circumference of the remaining premolar teeth using chisels and water-cooled rotating burs. The root surfaces were instrumented with curettes, chisels, and water-cooled rotating diamonds to remove the cementum. Clinical defect height from the cemento-enamel junction (CEJ) to the reduced alveolar crest was set to 6 mm as measured with a periodontal probe.

Experimental protocol

Experimental conditions included implantation of the resorbable, calcium carbonate CI in conjunction with GTR (cGTR) and GTR without the coral implant. A split-mouth design was used. Experimental conditions were alternated between left and right jaw quadrants in subsequent animals. A sham-surgery control was deemed unnecessary since previous studies using this model have demonstrated a limited potential for bone regeneration in surgical controls (Wikesjö et al. 1994).

Defects receiving the coral biomaterial had the implant molded around the premolar teeth to replace the surgically removed alveolar bone (actual implant volume/defect approximated 0.8 mL). The teeth were each fitted with an ePTFE device positioned and secured with an ePTFE suture immediately above the CEJ. Control defects received the ePTFE device without the coral implant. Periostea were fenestrated at the base of the flaps, the flaps advanced, and the flap margins adapted and sutured approximately 2 mm coronal to the CEJ.

Post-surgery care

Buprenorphine HCl (0.015 mg/kg, i.m., bid, 2 days) was administered for immediate post-surgery pain control. A broad-spectrum antibiotic (enrofloxacin, 2.5 mg/kg, i.m., bid, 14 days) was used for infection control. Plaque control was maintained by twice daily topical application of a chlorhexidine solution (chlorhexidine gluconate; 40 ml of a 2% solution). Sutures were removed at 10 days post-surgery. The animals were anesthetized and euthanized (concentrated thiopental sodium i.v.) at week 4 post-surgery and teeth with surrounding soft and hard tissues were removed *en bloc*. The ePTFE devices were not removed during the healing interval.

Histological processing and evaluation

The block sections were fixed in 10% buffered formalin for 3–5 days, decalcified in 5% formic acid for 8–10 weeks, trimmed, dehydrated, and embedded in butyl-methacrylate-paraffin. Serial sections (7 μm) were cut in a buccal-lingual plane throughout the mesial-distal extension of the teeth. Every 14th section was stained with Ladewig's connective tissue stain modified by Mallory allowing for observations at 100-μm intervals.

One calibrated investigator (G.P.; intraclass correlation coefficient = 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,), and a PC-based image analysis system (Image-Pro Plus™, Media Cybernetic, Silver Springs, MD, USA) customized for the supra-alveolar periodontal defect model. The most central-stained section for the mesial and distal root of the third and fourth premolar teeth was identified by the size of the root canal and subjected to histometric analysis. The following parameters were recorded for the buccal and the lingual tooth surfaces for the section:

- Bone regeneration (height): distance between the apical extension of the root planing and the coronal extension of alveolar bone formation along the planed root.
- Wound area: area circumscribed by the planed root surface, the ePTFE device, and the base of the defect at the level of the apical extension of the root planning.

Data analysis

The data were analyzed using univariate, bivariate, and multivariate analyses

using the Mixed Models ANOVA (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 univariate analysis assessed the effects of treatments and wound area, separately, on bone regeneration (height). The bivariate analysis assessed the effect of treatment methods on bone regeneration (height) within various thresholds of wound area. The multivariate analysis assessed the mean alveolar bone regeneration adjusted for wound area and type of treatment.

Results

Bone regeneration correlated with the size of the wound area, with sites providing larger wound areas exhibiting significantly greater bone regeneration compared to sites exhibiting smaller wound areas ($p<0.0001$; Table 1, Figs. 1 and 2). The unadjusted mean bone regeneration in sites receiving cGTR was significantly greater than that in sites receiving GTR alone ($p<0.0001$; Table 2). Standardizing the sites by wound area thresholds showed that bone regeneration was not significantly different in sites receiving cGTR compared to that in sites receiving GTR alone, irrespective of the size of the wound area ($p \cong 0.5$–0.99; Table 3). Similarly, there was no significant difference in the mean bone regeneration between sites receiving cGTR or GTR alone, after adjusting for wound area. However, a significant ($p<0.0001$) correlation between bone regeneration and the size of wound area still existed after adjusting for treatment protocol (Table 4), suggesting that the coral biomaterial did not provide additional effect on bone regeneration beyond what was provided by the size of wound area.

Discussion

The objective of this study was to evaluate the effect of a coral biomaterial on alveolar bone regeneration in conjunction with GTR. Critical size, supra-alveolar periodontal defects in contralateral jaw quadrants in 4 Beagle dogs received GTR and GTR combined with the space-providing coral biomaterial. The defect sites were subjected to histometric analysis following a 4-week healing interval. The results suggest that bone regeneration following GTR is dictated by space-provision. The coral biomaterial effectively enhances space-provision, and this appears to be the principal mechanism by which this biomaterial supports bone regeneration rather than postulated osteoconductive properties.

This study used a model system including 6-mm, critical size, supra-alveolar periodontal defects in dogs. The supra-alveolar periodontal defect model can be considered a ''litmus test'' for candidate protocols in the evaluation of their regenerative poten-

Table 1. Mean bone regeneration (height) by thresholds of wound area

Wound area (mm^2)	Mean	SE	*p*
<3	1.01	0.16	
3–7	2.03	0.16	0.0001
>7	2.34	0.17	0.0001

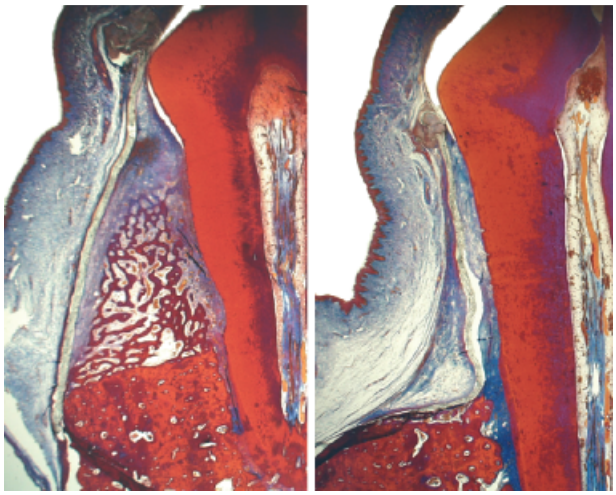

Fig. 1. Representative photomicrographs of supra-alveolar periodontal defects with space-providing ePTFE devices. Effect of space-provision can be observed. Sites providing a large wound area showed enhanced bone regeneration (left). Sites providing a small wound area showed limited bone regeneration (right).

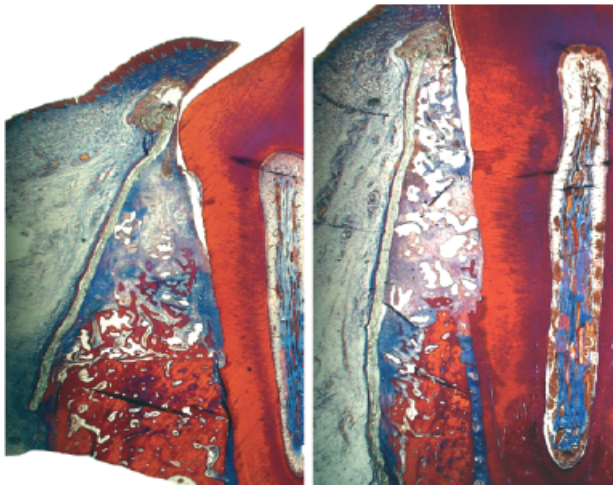

Fig. 2. Representative photomicrographs of supra-alveolar periodontal defects with space-providing ePTFE devices in conjunction with the coral biomaterial. Effect of space-provision can be observed. Sites providing a large wound area showed enhanced bone regeneration (left). Sites providing a small wound area showed limited bone regeneration (right).

Table 2. Mean bone regeneration (height) by treatment group

Treatment	Mean	S.E.	*p*
GTR	1.32	0.24	0.0001
cGTR	2.14	0.23	

GTR, guided tissue regeneration; cGTR, coral implant GTR.

Table 3. Mean bone regeneration (height) by treatment and wound area

Wound area (mm^2)	Treatment	Mean	SE	*p*
<3	GTR	1.02	0.18	0.998
	cGTR	1.02	0.45	
3–7	GTR	2.07	0.25	0.83
	cGTR	2.01	0.19	
>7	GTR	2.05	0.45	0.49
	cGTR	2.37	0.18	

GTR, guided tissue regeneration; cGTR, coral implant GTR.

Table 4. Mean bone regeneration (height) adjusted for treatment effect and wound area

Parameter	Group	Mean	SE	*p*
Treatment	GTR	1.78	0.18	0.89
	cGTR	1.81	0.16	
Wound area	<3 mm^2	1.03	0.19	
	3–7 mm^2	2.02	0.17	0.0001
	>7 mm^2	2.33	0.20	0.0001

GTR, guided tissue regeneration; cGTR, coral implant GTR.

tial of alveolar bone, cementum, and periodontal attachment (Wikesjö & Selvig 1999). The defect dimensions provide for clinically relevant regeneration of alveolar bone and cementum. The defect morphology allows for an unbiased and highly reproducible strategy of analysis (Koo et al. 2003a, b). Alveolar bone and cementum regeneration has been shown to not exceed 15% of the defect height in sham-operated controls over a 4- or 8-week healing interval, thus it appears that the regenerative potential of the site under such conditions are exhausted within 4 weeks (Wikesjö et al. 1994). This defect model appears adequate to evaluate the regenerative potential of alveolar bone within a 4-week healing interval such as in the present study.

This study suggests a critical role for space-provision on bone regeneration following GTR. Sites providing larger wound areas showed enhanced bone regeneration compared to sites with smaller wound areas irrespective of treatment protocol. This finding corroborates other studies elucidating the importance of space-provision for bone regeneration in periodontal defects (Sigurdsson et al. 1994, Wikesjö et al 2003). In contrast, small wound areas, including sites where the GTR devices were collapsed or compressed onto the root surface, appear to obstruct the regenerative process (Haney et al. 1993, Sigurdsson et al. 1994). One may only speculate on mechanisms controlling bone regeneration in conjunction with GTR. Previous studies indicate that tissue resources from within the periodontal ligament rather than the resident bone are critical to the regenerative potential of the periodontal attachment and alveolar bone (Isidor et al. 1986, Polimeni et al. 2002). However, this may not entirely explain space-related variations in bone regeneration under provisions for GTR. It appears that in the presence of space-provision exceeding the extent of alveolar bone regeneration, local factors affected by space-related variations may additionally influence bone regeneration. Hypothetically, a larger wound space may more effectively isolate the maturing granulation tissue in the wound space from micro-motion originated by physiologic and/or induced forces on the wound site, thus supporting bone regeneration. Further study is needed to address this hypothesis.

The data from the present study show that the coral biomaterial influences space-provision by enhancing the wound area. The physical structure of the biomaterial appeared to prevent the GTR device from collapsing onto the root surface. This effect overall supported enhanced bone formation in sites receiving cGTR compared to sites receiving GTR alone. However, when adjusted for the effect of wound area, a two-way ANOVA analysis did not show statistically significant differences between the protocols. Consistent with this observation, stratification of the wound area into subgroups did not reveal significant differences between protocols. This should be interpreted to indicate that the coral biomaterial did not exhibit adjunctive osteoconductive properties consistent with previous histopathological evaluations of the biomaterial (Koo et al. 2002). On the other hand, the coral biomaterial, as used in the present study, did not appear to obstruct bone formation in contrast to that observed for other particulate biomaterials used to support space-provision or serve as osteoconductive conduits for bone regeneration in conjunction with guided tissue/bone regeneration (Trombelli et al. 1999, Stavropoulos et al. 2001).

According to the observations provided in this study, future research evaluating osteoconductive properties of biomaterials used for bone augmentation should take into consideration the ''wound area effect'' of such biomaterials. A proper methodology and analysis should be applied in such studies to distinguish this effect from any osteoconductive effect of the biomaterial.

Conclusions

The results in this study suggest that space-provision has a significant effect on bone regeneration following GTR. The coral biomaterial effectively enhances space-provision, and this appears to be the principal mechanism by which this biomaterial supports bone regeneration rather than postulated osteoconductive properties.

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