

# Periodontal Repair in Dogs: A Bioabsorbable Calcium Carbonate Coral Implant Enhances Space Provision for Alveolar Bone Regeneration in Conjunction with Guided Tissue Regeneration

Ulf M.E. Wikesjö,\* Won Hee Lim,\* Saghi S. Razi,† Thorarinn J. Sigurdsson,‡ Michael B. Lee,§  
Dimitris N. Tatakis,|| and W. Ross Hardwick¶

**Background:** Collapse or compression of a barrier device into a periodontal defect or onto the root surface compromises outcomes following guided tissue regeneration (GTR). Bone biomaterials have been suggested to support regeneration of alveolar bone and to improve space provision with GTR devices. The objective of this study was to evaluate space provision, alveolar bone, and cementum regeneration following use of a bioabsorbable, calcium carbonate biomaterial in conjunction with GTR.

**Methods:** Routine, critical size, 5 to 6 mm, supraalveolar, periodontal defects were created in 5 young adult beagle dogs. Alternate jaw quadrants in consecutive animals received GTR and the coral biomaterial (cGTR) or GTR alone. The animals were euthanized 4 weeks postsurgery and tissue blocks processed for histometric analysis.

**Results:** The coral implant particles were surrounded by newly formed bone or immersed in connective tissue and appeared to resorb and be replaced by bone. There was limited, if any, appreciable cementum regeneration. Space provision was enhanced in cGTR compared to GTR sites ( $6.1 \pm 1.6$  versus  $2.4 \pm 0.8$  mm<sup>2</sup>;  $P < 0.05$ ). Bone regeneration (height) was significantly increased in cGTR compared to GTR sites averaging  $1.9 \pm 0.6$  and  $1.2 \pm 0.6$  mm, respectively ( $P < 0.05$ ). Bone regeneration (area) was 2-fold greater in cGTR sites compared to the GTR control ( $3.3 \pm 1.8$  versus  $1.4 \pm 0.5$  mm<sup>2</sup>), however the difference was not statistically significant ( $P > 0.05$ ).

**Conclusions:** The coral implant significantly enhanced space provision for GTR while alveolar bone formation appeared to be enhanced by its use. Increased healing intervals are needed to fully understand the biologic value of the coral implant as an adjunct to GTR. *J Periodontol* 2003;74:957-964.

## KEY WORDS

Alveolar bone; bone regeneration; calcium carbonate; dental cementum; guided bone regeneration; membranes, bioabsorbable.

Guided tissue regeneration (GTR) is based on the concept that tissue resources located in the periodontal ligament are critical for regeneration of the periodontal attachment including alveolar bone, cementum, and a functionally oriented periodontal ligament. It has further been postulated that regeneration of the periodontal attachment may only occur if the gingival connective tissue and epithelium are prevented access to the tooth-gingival flap interface.<sup>1</sup> Accordingly, GTR devices that act as passive barriers have been developed, manufactured, and marketed to support proliferation and migration of periodontal ligament cells onto the periodontally compromised tooth surface and to prevent influx from the gingival connective tissue and epithelium.<sup>2,3</sup> Studies have also shown that alveolar bone and cementum regeneration is critically dependent on space provision by the GTR device.<sup>4-9</sup> With limited space provision by the barrier membrane, alveolar bone will fill the space provided by the device.<sup>4,9</sup> In presence of a larger space, the alveolar bone adopts a physiologic form along the entire root surface, the remainder of the space being filled by fibrous connective tissue.<sup>5,6,8,9</sup>

Collapse or compression of the barrier device into a periodontal defect or onto the root surface will necessarily

\* Laboratory for Applied Periodontal and Craniofacial Regeneration, Department of Periodontology, Temple University, Philadelphia, PA.

† Private practice, Beverly Hills, CA.

‡ University of Akureyri, Akureyri, Iceland.

§ Department of Periodontics, Loma Linda University, Loma Linda, CA.

|| Department of Periodontology, Ohio State University, Columbus, OH.

¶ W.L. Gore & Associates, Inc., Flagstaff, AZ.

compromise regeneration of alveolar bone and cementum following GTR procedures.<sup>4-9</sup> The space provided by the device at the time of surgery is lost and the device becomes a physical obstacle to bone and cementum regeneration. Thus, structurally reinforced GTR devices have been developed to safeguard wound stability and maintain necessary space provision.<sup>5,6,8-11</sup> Autogenous bone grafts, bone derivatives, and bone substitutes have been suggested to support regeneration of alveolar bone and, for some biomaterials, periodontal attachment.<sup>12,13</sup> Bone grafts, bone derivatives, and bone substitutes may also potentially offer advantages in supporting stability and space provision of GTR devices. The objective of this study was to evaluate space provision and alveolar bone and cementum regeneration following use of a bioabsorbable, porous, particulate, calcium carbonate coral implant in conjunction with GTR.

## MATERIALS AND METHODS

### Animals

Animal selection and management, surgical protocol, and periodontal defect preparation followed a routine protocol approved for this study by the Institutional Animal Care and Use Committee, Loma Linda University.<sup>14</sup> Five male beagle dogs (age 18 to 24 months; weight 12 to 15 kg) exhibiting intact mandibular premolar dentition without crowding or evidence of periodontal disease were used. The animals were fed a soft-consistency dog food supplemented with vitamins. A soft diet was chosen to reduce potential mechanical trauma to the experimental sites postsurgery.

### Biomaterials

Expanded polytetrafluoroethylene (ePTFE) barrier devices<sup>#</sup> were used. The tissue occlusive devices have a 15 to 25  $\mu\text{m}$  nominal pore size. These characteristics have been shown to support alveolar bone and cementum regeneration in the supraalveolar periodontal defect model.<sup>4-8</sup> ePTFE sutures<sup>\*\*</sup> were used for membrane fixation and wound closure.

A medical grade, resorbable, porous, particulate, calcium carbonate coral implant<sup>††</sup> 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  $\mu\text{M}$  sodium acetate solution to form a viscoelastic gel to contain the calcium carbonate particles in a manageable mass.

### Surgical Protocol

Food was withheld the night before surgery. Surgical procedures were performed using sodium pentobarbital anesthesia (20 to 30 mg/kg, IV) preceded by acepromazine sedation (1 mg/kg, IM). Routine dental infiltration anesthesia was used at the surgical sites. To

maintain hydration, a sterile IV catheter was placed and animals received a constant rate infusion of lactated Ringer's solution (10 to 20 ml/kg/hr IV) while anesthetized. Thiopental sodium anesthesia (20 to 25 mg/kg, IV) was used for suture removal and radiographic registrations.

Routine, supraalveolar, critical size, periodontal defects were created around the third and fourth mandibular premolar teeth in the right and left jaw quadrants in each animal.<sup>14</sup> 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 premolars 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 curets, 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 (Fig. 1). The maxillary first, second, and third premolar teeth were surgically extracted bilaterally, and the maxillary fourth premolars were reduced in height and exposed pulpal tissues sealed<sup>‡‡</sup> to alleviate potential mechanical trauma from the maxillary teeth to the experimental sites.

### Experimental Protocol

Experimental conditions included implantation of the bioabsorbable, calcium carbonate coral implant in conjunction with GTR (cGTR) and GTR without the coral implant (control). Defects receiving the coral biomaterial had the implant molded around the premolar teeth to replace removed alveolar bone (actual implant volume/defect approximated 0.8 ml). The teeth were each fitted with an ePTFE barrier device positioned and secured with an ePTFE suture immediately above the CEJ. Control defects received the ePTFE device without the coral implant. Periosteum were fenestrated at the base of the flaps, the flaps were advanced and the flap margins adapted and sutured approximately 2 mm coronal to the CEJ (Fig. 1).

A split-mouth design was used. Experimental conditions were alternated between left and right jaw quadrants in subsequent animals. The critical size, supraalveolar, periodontal defect model<sup>14</sup> has been extensively evaluated, thus, a surgical control (gingival flap surgery alone) was not considered necessary.

### Postsurgery Protocol

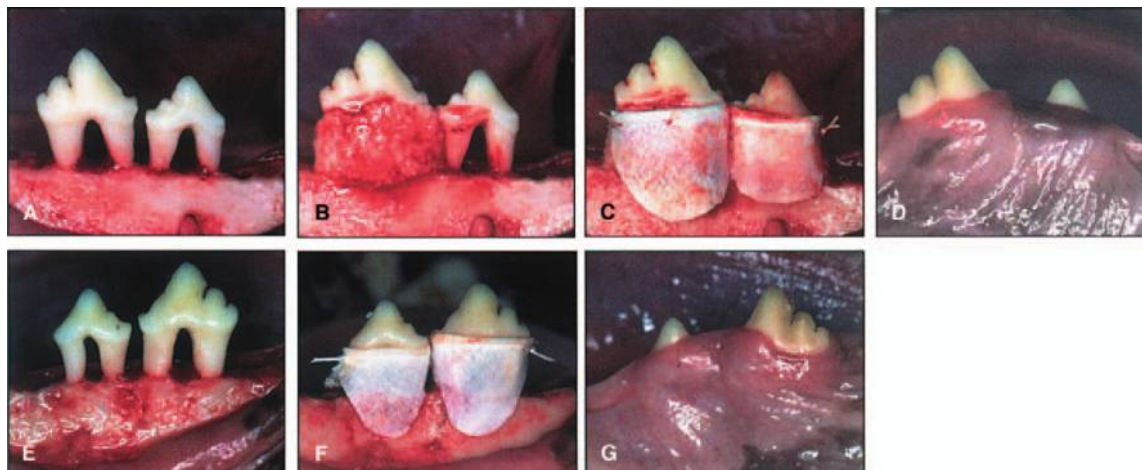
Buprenorphine HCl (0.015 mg/kg, IM, bid, 2 days) was administered for immediate postsurgery pain control. A broad-spectrum antibiotic (enrofloxacin,

# Gore-Tex Regenerative Material, W.L. Gore & Associates, Inc., Flagstaff, AZ.

\*\* Gore-Tex Suture CV5, W.L. Gore & Associates, Inc.

†† Biocoral 1000, Inotek, Saint-Gonny, France.

‡‡ Cavit, ESPE, Seefeld/Oberbayern, Germany.



**Figure 1.**

Critical size, supraalveolar periodontal defects following surgical reduction and root preparation of the mandibular premolar teeth (**A** and **E**); following placement of the porous, particulate coral biomaterial (**B**); following placement of the ePTFE devices (**C** and **F**); and at euthanasia 4 weeks postsurgery (**D** and **G**). **A** through **D** represent the cGTR protocol; and **E** through **G** the GTR protocol.

2.5 mg/kg, IM, b.i.d., 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 postsurgery. The animals were anesthetized and euthanized (concentrated thiopental sodium IV) at week 4 postsurgery and teeth with surrounding soft and hard tissues were removed *en bloc*. Barrier devices were not removed during the healing interval.

Photographs and radiographs were obtained at defect induction, suture removal (photographs only), and at 2 and 4 weeks postsurgery. Observations of experimental sites with regards to gingival health, flap adaptation, edema, and purulence were made daily.

#### **Histological Processing and Evaluation**

Block sections including teeth, bone, and soft tissues were fixed in 10% buffered formalin for 3 to 5 days, decalcified in 5% formic acid for 8 to 10 weeks, trimmed, dehydrated, and embedded in butyl-methacrylate-paraffin. Serial sections (7  $\mu$ m) were cut in a buccal-lingual plane throughout the mesial-distal extension of the teeth. Every fourteenth section was stained with Ladewig's connective tissue stain modified by Mallory allowing for observations at 100  $\mu$ m intervals.

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. This section and the immediate stained step serial section on either side were subject to histometric analysis. Thus, 3 subsequent step serial sections, representing 0.2 mm of the mid-portion of the mesial and distal root for each

premolar tooth, were used for analysis. One experienced investigator, masked to the specific experimental conditions, performed the histometric analysis using incandescent and polarized light microscopy, a microscope digital camera system, and a PC-based image analysis system<sup>§§</sup> customized for the supraalveolar periodontal defect model. The following parameters were recorded for the buccal and the lingual tooth surfaces for each section:

**Defect height.** Distance between the apical extension of root planing and the CEJ.

**Barrier device height.** Distance between the apical extension of the root planing and the most coronal aspect of the ePTFE device.

**Defect area.** Area under the ePTFE device circumscribed by the planed root, the width of the alveolar bone at the apical extension of the root planing, and the device.

**Connective tissue repair.** Distance between the apical extension of the root planing and the apical extension of a junctional epithelium along the planed root.

**Cementum regeneration.** Distance between the apical extension of the root planing and the coronal extension of a continuous layer of new cementum or cementum-like deposit on the planed root.

**Bone regeneration (height).** Distance between the apical extension of the root planing and the coronal extension of alveolar bone formation along the planed root.

**Bone regeneration (area).** Area represented by new alveolar bone along the planed root.

§§ Image-Pro Plus, Media Cybernetic, Silver Spring, MD.

**Bone regeneration (density).** Ratio of mineralized bone matrix/total bone area.

**Biomaterial density.** Ratio of residual biomaterial/total bone area.

**Root resorption.** Combined linear heights of distinct resorption lacunae on the planed root.

**Ankylosis.** Combined linear heights of ankylotic unions between new alveolar bone and the planed root.

### Data Analysis

Summary statistics (means  $\pm$  SD) based on animal means were calculated using selected step serial sections. Differences between experimental conditions were analyzed using paired *t* test. Correlation coefficients were estimated for defect area versus bone regeneration area.

## RESULTS

### Clinical and Radiographic Observations

Healing was generally uneventful. Exposures of the ePTFE devices were not observed. The height of the gingival contour was similar for cGTR and GTR defect sites, whereas alveolar width appeared increased for the cGTR compared to the GTR control sites. Two animals exhibited gingival inflammation at defect sites receiving the cGTR protocol.

cGTR sites exhibited granular radiopacity consistent with the coral biomaterial. Bone formation within the coral biomaterial appeared continuous with the contiguous resident alveolar bone. GTR sites exhibited evidence of bone deposition within the apical third of the defect. The radiopacity appeared integrated with

the contiguous resident alveolar bone and more distinct along the root surfaces.

### Histological Observations

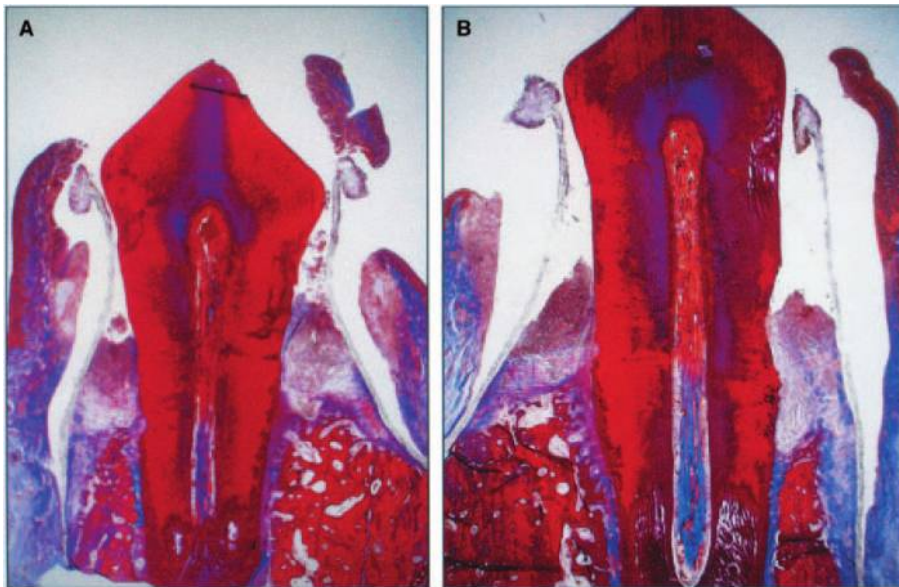
One animal exhibited an extensive inflammatory infiltrate occupying the buccal and lingual wound space above and below the ePTFE device for both premolar teeth in the defect site receiving the cGTR protocol (Fig. 2). The aspect of the ePTFE device facing the gingival flap exhibited epithelization. cGTR defect sites in the remaining animals did not display evidence of an acute inflammatory reaction, and the epithelia appeared arrested at the CEJ. The resorbable, porous, particulate coral implant appeared to support ample space between the tooth surface and the ePTFE device (Fig. 3).

Bone regeneration of trabecular nature, variable from defect to defect and from animal to animal, ranged from negative (bone resorption in defect sites exhibiting the inflammatory infiltrate) to encompass approximately two-thirds of the defect height. Defects with apparent uneventful healing exhibited evidence of residual coral biomaterial. The coral particles were surrounded by newly formed bone or immersed in connective tissue. Particles embedded in newly formed bone appeared to be resorbing and replaced by bone (Fig. 4).

Cementum regeneration was limited. When present, cementum regeneration was observed in the very apical extension of the planed root. Limited root resorption was observed. The animal exhibiting an inflammatory reaction presented extensive root resorption concomitant with the inflammatory infiltrate. One defect site exhibited limited ankylosis.

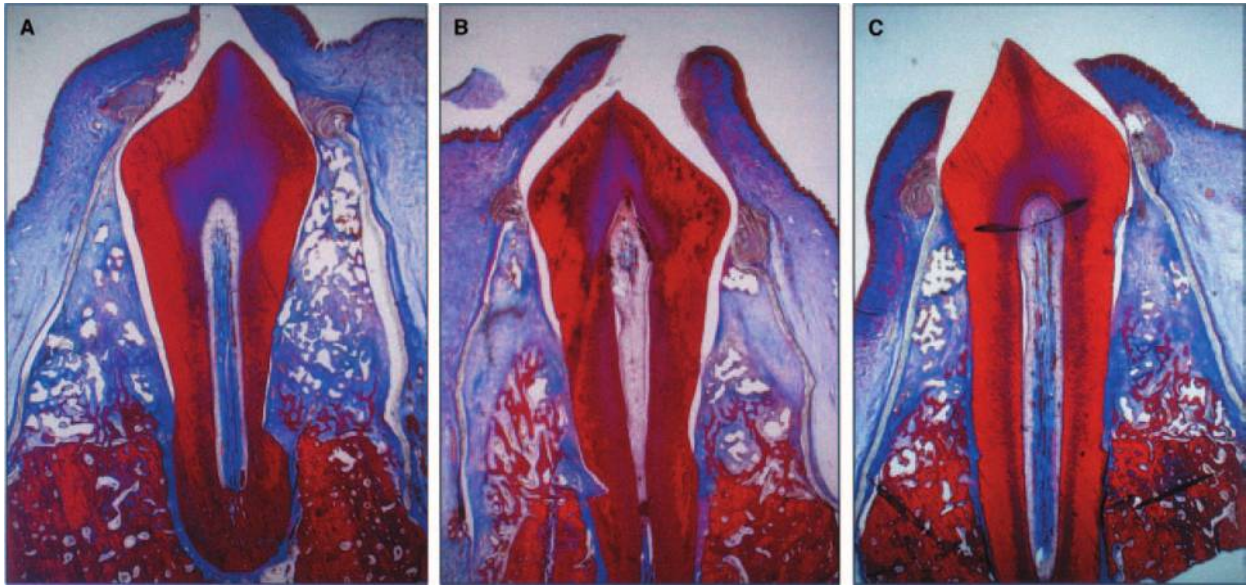
Space provision by the GTR control was limited (Fig. 5). The lingual aspect of the sites exhibited greater space provision compared to the buccal aspect, due to a generally more pronounced lingual alveolar base. One defect site exhibited a limited inflammatory infiltrate. All other sites appeared to have healed without complication. Epithelia appeared arrested at the CEJ for all defect sites.

Generally, the GTR control sites exhibited limited amounts of newly formed trabecular bone ranging up to one-third of the defect height; bone regeneration commonly appearing more pronounced at the lingual aspect of the teeth. The trabecular bone displayed relatively wide marrow spaces.



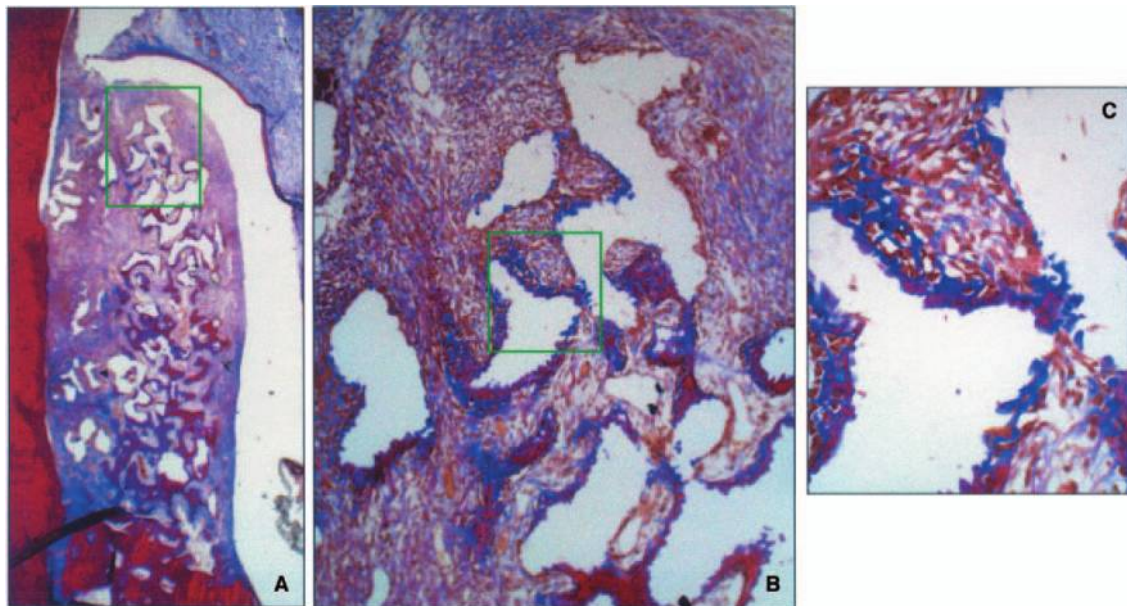
**Figure 2.**

Photomicrographs of the premolar teeth at the defect site experiencing subclinical exposure of the ePTFE device and an associated inflammatory reaction (original magnification 2.5 $\times$ ; Ladewig's connective tissue stain modified by Mallory).



**Figure 3.**

Representative photomicrographs of defect sites receiving the coral biomaterial in conjunction with GTR (original magnification 2.5 $\times$ ; Ladewig's connective tissue stain modified by Mallory).



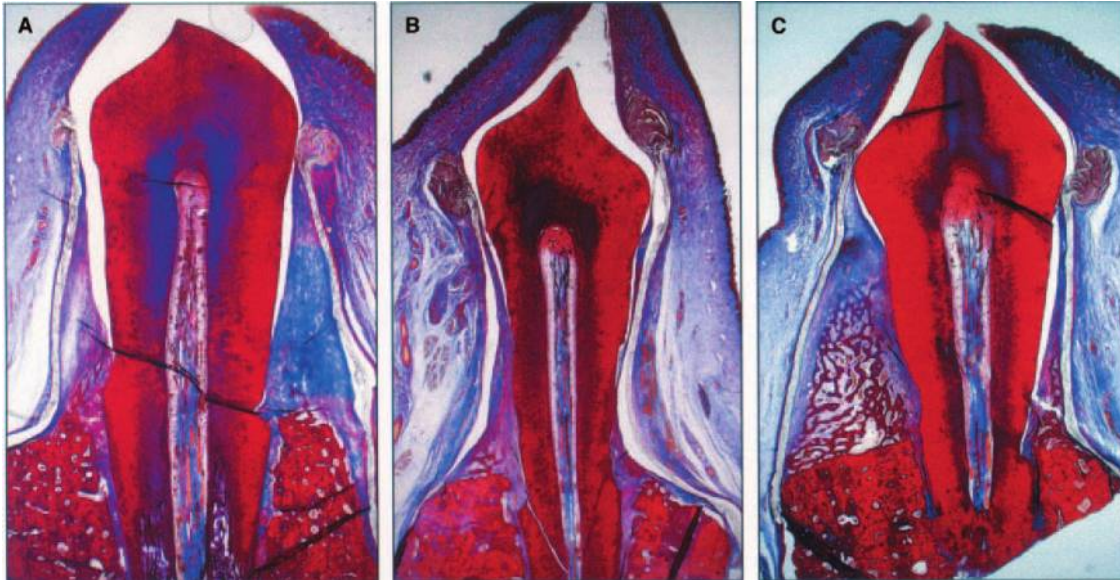
**Figure 4.**

Photomicrograph of defect site receiving the coral biomaterial in conjunction with GTR (A, original magnification 2.5 $\times$ ) and detail in higher magnification (B, 20 $\times$ ; C, 80 $\times$ ) showing bone formation associated with the biomaterial (Ladewig's connective tissue stain modified by Mallory).

The GTR control sites exhibited limited cementum regeneration. When present, newly formed cementum appeared at the very apical extension of the root planing. Limited root resorption was observed. Ankylosis was not observed.

#### **Histometric Analysis**

Table 1 shows group means  $\pm$  SD and *P* values from the histometric analysis of the cGTR and GTR defect sites. To enable analysis of the biologic potential of the experimental protocols under optimal conditions for



**Figure 5.**

Representative photomicrographs of defect sites receiving the GTR protocol (original magnification 2.5 $\times$ ; Ladewig's connective tissue stain modified by Mallory).

healing, the animal exhibiting wound failure resulting in an acute inflammatory infiltrate under the membrane was excluded from the analysis. Space provision was enhanced in cGTR compared to GTR sites ( $6.1 \pm 1.6$  versus  $2.4 \pm 0.8$  mm<sup>2</sup>;  $P = 0.0491$ ). Bone regeneration (height) was increased in cGTR compared to GTR sites averaging  $1.9 \pm 0.6$  and  $1.2 \pm 0.6$  mm, respectively ( $P = 0.0429$ ). Bone regeneration (area) was 2-fold greater in sites receiving the cGTR protocol compared to the GTR control ( $3.3 \pm 1.8$  versus  $1.4 \pm 0.5$  mm<sup>2</sup>), however, the difference between the protocols was not statistically significant ( $P > 0.05$ ). Bone regeneration (density) was greater in the GTR control compared to that in the cGTR sites ( $23.0\% \pm 5.4\%$  versus  $12.7\% \pm 4.9\%$ ;  $P = 0.0436$ ). There was a statistically significant correlation between defect area and bone regeneration area for the GTR control defects ( $r^2 = 0.9843$ ,  $P < 0.001$ ).

## DISCUSSION

The objective of this study was to evaluate space provision and alveolar bone and cementum regeneration following surgical implantation of a medical grade, bioabsorbable, porous, particulate, calcium carbon-

**Table 1.**

### Summary Statistics (group means $\pm$ SD) for the Histometric Parameters; N = 4 Excluding the Animal Exhibiting Wound Failure

Parameter	cGTR	GTR	<i>P</i> cGTR vs. GTR
Defect height (mm)	4.5 $\pm$ 0.5	4.6 $\pm$ 0.5	0.8662
Membrane height	5.0 $\pm$ 0.7	4.9 $\pm$ 0.7	0.8198
Defect area (mm <sup>2</sup> )	6.1 $\pm$ 1.6	2.4 $\pm$ 0.8	<b>0.0491</b>
Connective tissue repair (mm)	4.5 $\pm$ 0.5	4.6 $\pm$ 0.5	0.7789
Cementum regeneration (mm)	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	—
Bone regeneration (height) (mm)	1.9 $\pm$ 0.6	1.2 $\pm$ 0.6	<b>0.0429</b>
Bone regeneration (area) (mm <sup>2</sup> )	3.3 $\pm$ 1.8	1.4 $\pm$ 0.5	0.1806
Bone regeneration (density) (%)	12.7 $\pm$ 4.9	23.0 $\pm$ 5.4	<b>0.0436</b>
Biomaterial density (%)	10.1 $\pm$ 4.4	—	—
Root resorption (mm)	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	0.6574
Ankylosis	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	—

Boldface type indicates statistical significance.

ate, coral biomaterial in conjunction with GTR. Routine, critical size, supraalveolar, periodontal defects were created in 5 beagle dogs. Alternate jaw quadrants in consecutive animals received GTR and the coral biomaterial or GTR alone. The animals were

ethanized at 4 weeks postsurgery and tissue blocks processed for histometric analysis. The overall results suggest that the coral implant enhanced space provision and alveolar bone regeneration over the 4-week healing interval.

The bioabsorbable calcium carbonate implant is derived from the coral genus *Porites*. The implant consists of resorbable aragonite crystals with an average pore size of 250  $\mu\text{m}$  (range 150 to 400  $\mu\text{m}$ ) resembling spongy bone. The architecture of the aragonite crystals allows ingrowth of the coralline structure by granulation tissue. When implanted into bone tissue, the open pore structure allows formation of a fibrovascular tissue throughout the implant progressively replaced by bone.<sup>15,16</sup> Bioabsorbable calcium carbonate implants from the genus *Porites* appear well tolerated and have been shown to support bone regeneration in a variety of settings including posterolateral lumbar spinal fusion,<sup>17</sup> repair of long bone defects,<sup>18,19</sup> alveolar augmentation,<sup>20</sup> and periodontal regeneration.<sup>21-23</sup>

Space provision was significantly enhanced following use of the coral implant. Average defect area for cGTR defect sites was almost 3 times that of the GTR control. The increased wound space provided by the coral implant may have enhanced bone formation over that observed in the control. The significance of space provision for alveolar bone regeneration has been reported. Haney et al.<sup>4</sup> observed a statistically significant positive correlation between defect area and bone regeneration following GTR in supraalveolar periodontal defects over a 4-week healing interval. Sigurdsson et al.<sup>5,6</sup> and Wikesjö et al.,<sup>8,9</sup> also using the supraalveolar periodontal defect model, observed substantial reconstruction of periodontal architecture including alveolar bone over 8- and 24-week healing intervals using space-providing occlusive or macroporous ePTFE membranes or bioresorbable macroporous membranes. However, the possibility of an osteoconductive effect of the coral biomaterial cannot be ignored.

Comparatively limited alveolar bone regeneration was observed in the cGTR defects following the 4-week healing interval. It may be argued that the healing interval in this study was too short to adequately evaluate the potential of the coral biomaterial to enhance alveolar regeneration. This notion is supported by observations in circular ovine long bone defects.<sup>19</sup> Sixty percent of a *Porites* coral implant had been resorbed within a month concomitant with 20% bone regeneration. Two months following implantation, 90% of the implant had been resorbed concomitant with 50% bone regeneration. Bone regeneration amounted to approximately 30% of the defect area for both experimental conditions in this study. It appears entirely possible that increased bone regeneration may be expected with extended healing intervals.

In this study, implant particles embedded in newly

formed bone appeared to be simultaneously resorbing and replaced by bone. It may be argued that the resorption rate of the coral implant was too slow. Residual implant particles potentially obstructing the wound space available to bone formation such as in this study have been observed in a previous study also using the supraalveolar periodontal defect model.<sup>7</sup> It appears critical that bone biomaterials, in addition to being osteoconductive, have a resorption profile that closely matches the bone formation rate at the implant site. Coral implant materials have been reported to resorb by osteoclastic activity.<sup>15,19</sup> Osteoblastic and osteoclastic activity may concomitantly be observed at the implant edges. Moon et al.,<sup>21</sup> using the intrabony periodontal defect model, observed that the coral biomaterial was gradually replaced by bone from week 3 postsurgery when used in conjunction with GTR. These characteristics appear appropriate for a space-providing implant to support GTR and guided bone regeneration protocols.

Cementum regeneration was limited in this study closely paralleling observations in our previous studies in the supraalveolar defect model following a 4-week healing interval.<sup>4,22,23</sup> In contrast, when using an 8-week healing interval, we have observed cementum regeneration in GTR defects encompassing up to 94% of the defect height in 5 to 6 mm supraalveolar periodontal defects.<sup>5,6,8,9</sup> A longer healing interval appears necessary for appreciable cementum regeneration using a light microscopy evaluation. This concept is supported by Moon et al.<sup>21</sup> who observed cementum regeneration by light microscopy from week 6 postsurgery in an intrabony periodontal defect model.

Clinical evaluations of the coral implant in periodontal intrabony defects suggest advantages for alveolar bone regeneration compared to gingival flap surgery alone.<sup>24-27</sup> However, the coral implant does not appear to exert an appreciable clinical adjunctive effect to GTR.<sup>27</sup> This may relate to the fact that intrabony defects are space making in nature and may not necessarily benefit from an adjunctive coral implant.

## CONCLUSIONS

The coral implant significantly enhanced space provision for GTR while alveolar bone formation appeared to be enhanced by its use. Increased healing intervals are needed to fully appreciate the biologic value of the coral implant as an adjunct to GTR.

## ACKNOWLEDGMENTS

This study was supported by W.L. Gore & Associates, Inc., Flagstaff, Arizona. Biocoral 1000 was donated by Inotek, Saint-Gonny, France. Mr. Hardwick is Senior Scientist, Research and Development, W.L. Gore & Associates, Inc.

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Correspondence: Dr. Ulf M.E. Wikesjö, Laboratory for Applied Periodontal and Craniofacial Regeneration, Department of Periodontology, Temple University School of Dentistry, 3223 North Broad St., Philadelphia, PA 19140. E-mail: ulf.wikesjo@temple.edu.

Accepted for publication January 10, 2003.