Application of Periodontal Tissue Engineering Using Enamel Matrix Derivative and a Human Fibroblast-Derived Dermal Substitute to Stimulate Periodontal Wound Healing in Class III Furcation Defects

Lawrence R. Hovey,* Archie A. Jones,† Michael McGuire,‡ James T. Mellonig,§ John Schoolfield,‖ and David L. Cochran†

Background: Enamel matrix derivative (EMD) has been shown to promote several aspects of periodontal regeneration in vitro and in vivo. Recently, a bioengineered tissue (DG) was developed to promote wound healing of chronic skin ulcers. This pilot study sought to assess the effects of EMD and DG, alone or in combination, on periodontal wound healing in surgically created Class III furcation defects.

Methods: Six female baboons received bilateral ostectomy of ~10 mm around the first and second mandibular molars to achieve Class III, subclass C furcation defects. Wire ligatures and cotton pellets were left in place for 2 months to maintain the depth of the defects and promote plaque accumulation. Each furcally involved molar was then assigned to one of four treatments: open flap debridement (OFD), OFD plus EMD, OFD plus DG, or OFD plus DG and EMD. This resulted in six total sites per treatment group. Seven months after defect creation and 5 months after treatment, and after no oral hygiene, tissue blocks of the mandible were taken for blinded histometric analysis to assess parameters of periodontal regeneration adjacent to furcal root surfaces and from the mid-furcal aspect (i.e., new bone, new connective tissue attachment, new epithelial attachment, and new cementum formation).

Results: Histometric analysis demonstrated differential regenerative responses with respect to treatment within each animal. However, statistically significant differences between treatments from all six animals were not observed ($P > 0.20$, mixed-model analysis of variance). EMD-treated sites presented mildly positive regenerative results and no negative responses. Both DG only and combination therapy demonstrated similar or less than positive responses relative to OFD controls.

Conclusion: The descriptive analysis may suggest a positive effect of enamel matrix proteins and a negative effect of DG used alone or in combination with enamel matrix proteins on the regeneration of Class III furcation defects in baboons. *J Periodontol 2006;77:790-799.*

KEY WORDS

Furcation defects; histology; regeneration; tissue engineering.

* Private practice, Portland, OR.
† Department of Periodontics, University of Texas Health Science Center at San Antonio School of Dentistry, San Antonio, TX.
‡ Private practice, Houston, TX.
§ Head Specialist Division and Advanced Education Program, University of Texas Health Science Center at San Antonio School of Dentistry.
‖ Department of Academic Informatics Services, University of Texas Health Science Center at San Antonio School of Dentistry.

Much effort has been devoted to determine the specific cells, soluble mediators, and extracellular matrix components that contribute to the formation of periodontal tissues.[1-5] The information gained from this effort has given us the opportunity to begin applying the concepts of tissue engineering to develop new regenerative therapies. Tissue engineering is a field of the biomedical sciences involved in the development of techniques for the fabrication of new tissues to replace lost tissues and is based on the principles of cell biology, developmental biology, and biomaterials.[6] Engineered tissue is defined as tissue produced by cells seeded onto a bioabsorbable matrix and includes the implantation of devices that promote tissue regeneration.[7,8] An engineered tissue should provide three main components: regulatory signals

doi: 10.1902/jop.2006.030264

and mediators, progenitor cells, and an extracellular matrix or three-dimensional scaffold.[2]

Enamel matrix derivative (EMD)¶ may represent a potentially valuable component for the development of an engineered periodontal tissue construct. Multiple clinical studies and case reports have provided evidence for the ability of EMD to promote increased bone and attachment level gains, comparable to bone grafting and guided tissue regeneration (GTR) therapy, in treated intrabony defects.[9-12] Histologic analyses from human and non-human primate block sections of defects treated with EMD have also demonstrated periodontal regeneration.[13-17] EMD has been described by Gestrelius et al.[18] as providing a unique environment for cell-matrix interactions. They demonstrated that EMD enhanced the proliferation, total protein production, and mineral nodule formation by periodontal ligament fibroblasts. Boyan et al.[19] have also demonstrated EMD's ability to potentiate de novo bone formation stimulated by "active" demineralized freeze-dried bone allograft (DFDBA). Schwartz et al.[20] tested the in vitro response of osteoblastic cell lines at different stages of maturation to EMD and concluded that EMD stimulated proliferation at early stages of maturation, but as cells matured in lineage, EMD tended to enhance differentiation. Thus, in vitro and in vivo evidence has demonstrated EMD's ability to stimulate metabolic and cellular processes, contributing to the regeneration of periodontal tissues.

The recent development of DG,# a three-dimensional, allogenic, human neonatal dermal fibroblast culture grown on a bioabsorbable scaffold, has been shown to significantly accelerate the wound healing of diabetic foot ulcers.[21-23] Undifferentiated dermal fibroblasts are cultured in three dimensions, using a specially designed bioreactor, on a knitted lactate/glycollate copolymer scaffold. At harvest, when the cultures are in the stationary phase of the cell cycle, the tissue is cryopreserved and stored at –70°C. Upon thawing, the culture retains more than 50% viability. During repopulation, the fibroblasts secrete a three-dimensional extracellular matrix comprised largely of type I collagen, proteoglycans, and other proteins, including decorin and fibronectin. Upon thawing, the culture also synthesizes and releases a variety of cytokines and growth factors known to be involved in the formation of periodontal tissues.[24] These factors include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), interleukin (IL)-6, IL-8, transforming growth factor (TGF)-β1, granulocyte colony stimulating factor (G-CSF), platelet-derived growth factor A (PDGF-A), insulin growth factor-1 (IGF-1), keratinocyte growth factor (KGF), and tumor necrosis factor alpha (TNF-α).

One example of the potential beneficial effects DG may have on the regeneration of tissues is its ability to stimulate angiogenesis. A chorioallantoic membrane (CAM) angiogenesis assay demonstrated DG's ability to stimulate blood vessel production 2.8-fold over scaffold alone.[25] This has been largely attributed to its expression of VEGF, a known angiogenic factor.[26,27] DG fibroblasts demonstrate a 22-fold greater cellular content of VEGF mRNA compared to fibroblasts grown in a monolayer.[22] Other factors secreted by DG that may also have angiogenic effects include HGF, TGF-β1, IL-8, and G-CSF.[28] The actions of these factors are complex and may have an indirect effect by stimulating cell differentiation and recruitment of host inflammatory cells.

Thus, both EMD and DG demonstrate potential attributes that may contribute to the regeneration of periodontal tissues; however, whereas the benefits of EMD on periodontal regeneration have been thoroughly investigated, the effects of dermal fibroblast implantation into the periodontal wound-healing environment are unknown. It is also unknown how this environment, or exposure to EMD, might affect cell function or expression of the aforementioned growth factors by dermal fibroblasts. This pilot study sought to test the ability of EMD, DG, or a combination therapy of EMD and DG to promote periodontal regeneration of surgically created chronic Class III furcation defects in the primate model.

MATERIALS AND METHODS

Animals

These studies were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio (UTHSCSA) and in accordance with the requirements set by the National Institutes of Health for the care and use of laboratory animals. Six adult female baboons (*Papio anubis*), 15 to 18 kg in weight, were used in this investigation. Medical and research history of the animals was reviewed to exclude other research usage and/or systemic therapy within the prior year. The animals were housed individually at UTHSCSA in wire-bottom cages and maintained on a diet of commercially available animal food supplemented daily with fresh fruit and water *ad libitum*.

Anesthesia/Analgesia

For all surgical procedures, the animals were first sedated with an intramuscular injection of ketamine hydrochloride, 100 mg/ml, 10 mg/kg body weight. General anesthesia was obtained by isofluorane gas intubation, supplemented with local administration of 2% xylocaine containing epinephrine (1:100,000) to reduce hemorrhage in the surgical area.

¶ Emdogain, Institute Straumann, Basel, Sweden.
# Dermagraft, Smith and Nephew, Wound Management, Hull, U.K.

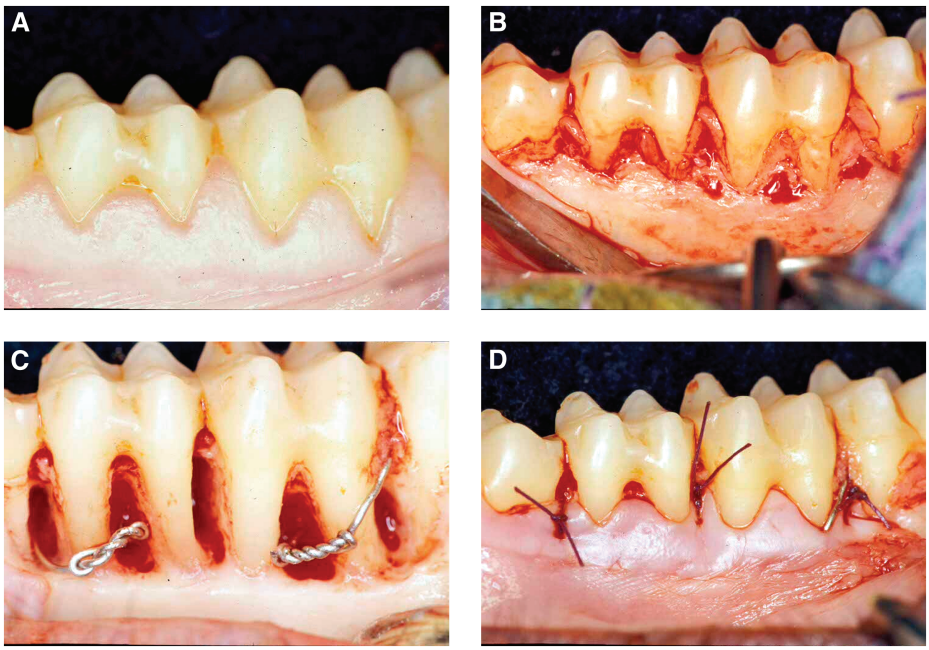

Figure 1.
Defect creation: ***A)*** *preoperative view;* ***B)*** *view upon reflection;* ***C)*** *wire ligatures placed to maintain a chronic defect phenotype over the following 2 months (cotton pellets not shown): note the lack of interproximal bone; and* ***D)*** *flaps sutured.*

Defect Creation

Experimental furcation defects were surgically created and stabilized to simulate chronic periodontal lesions that occur in humans. A modification of the protocol previously described by Vernino et al.[29] was used. After sulcular incisions and elevation of buccal and lingual mucoperiosteal flaps from the mesial of the first premolar to the distal of the second molar, circumferential ostectomy was performed around the first and second mandibular molars to achieve through-and-through furcation and interproximal defects measuring 10 mm from the fornix of the furcation to the reduced alveolar crest. The root surfaces were thoroughly instrumented to remove any remaining periodontal ligament or bone. A cotton pellet attached to a twisted orthodontic wire was placed into the base of each of the defects. The flaps were approximated and sutured. Sutures were removed after 2 weeks, and the lesions were left undisturbed until the experimental surgical phase in an additional 6 weeks (Fig. 1).

Experimental Surgical Treatment

Before flap reflection, through-and-through furcation defects were verified visually and with a Nabers probe (Fig. 2A). After flap reflection and wire ligature removal, the root surfaces were then thoroughly debrided using hand instruments and sonic furcation scalers** (Fig. 2B). A notch was created in the root at the most apical extent of the furcation defect. In accordance with the protocol for EMD application, 24% EDTA in carboxymethylcellulose gel†† was then applied to the exposed root surface for all experimental teeth, excluding sites treated with open flap debridement (OFD) alone, for 2 minutes to remove the smear layer, facilitate fibrin clot stabilization, and expose underlying collagen fibrils.[30]

The four mandibular molars were then randomly assigned to one of the following treatments: OFD serving as a control, OFD plus EMD alone, OFD plus DG alone, and OFD plus a combination of EMD and DG. A randomized treatment schedule was generated using a randomization table. EMD was mixed with the carrier polyglycolic acid (PGA) for 15 minutes before application to the root surfaces of the appropriate experimental teeth (Fig. 2C).

DG was prepared as instructed by the manufacturer.‡‡ Briefly, DG was stored at –70°C before use, transported on dry ice, and transferred to a 37°C water bath for thawing at time of use. DG was then washed with 0.01 M phosphate buffered saline (PBS), pH 7.4, at room temperature and maintained in a sterile bath of PBS until application. DG sites received a 10 × 10-mm piece of DG that was placed against the internal aspect of the furcation (Fig. 2D) and a second sheet (10 × 15 mm) to cover the facial aspect of the furcation (Fig. 2E). For the combination therapy, EMD was placed before placement of DG and secondarily injected over the DG once placed. EMD also was placed onto the root surfaces and into the defect of sites treated with EMD alone. A periosteal releasing incision was then made to allow for coronal repositioning of the buccal flap and tension-free wound closure. The buccal and lingual flaps were approximated with 4-0 polyglactin 910§§ suture (Fig. 2F). Surgeries were performed unilaterally with 1 week of healing interposed because of time constraints. Postoperative analgesia was provided as previously described.[14,15] The animals received a soft diet for 4 weeks after surgery. Sutures were removed

** KavoSonic, Kavo, Lake Zurich, IL.
†† Prefgel, Biora.
‡‡ Advanced Tissue Sciences, La Jolla, CA.
§§ Vicryl, Ethicon, Somerville, NJ.

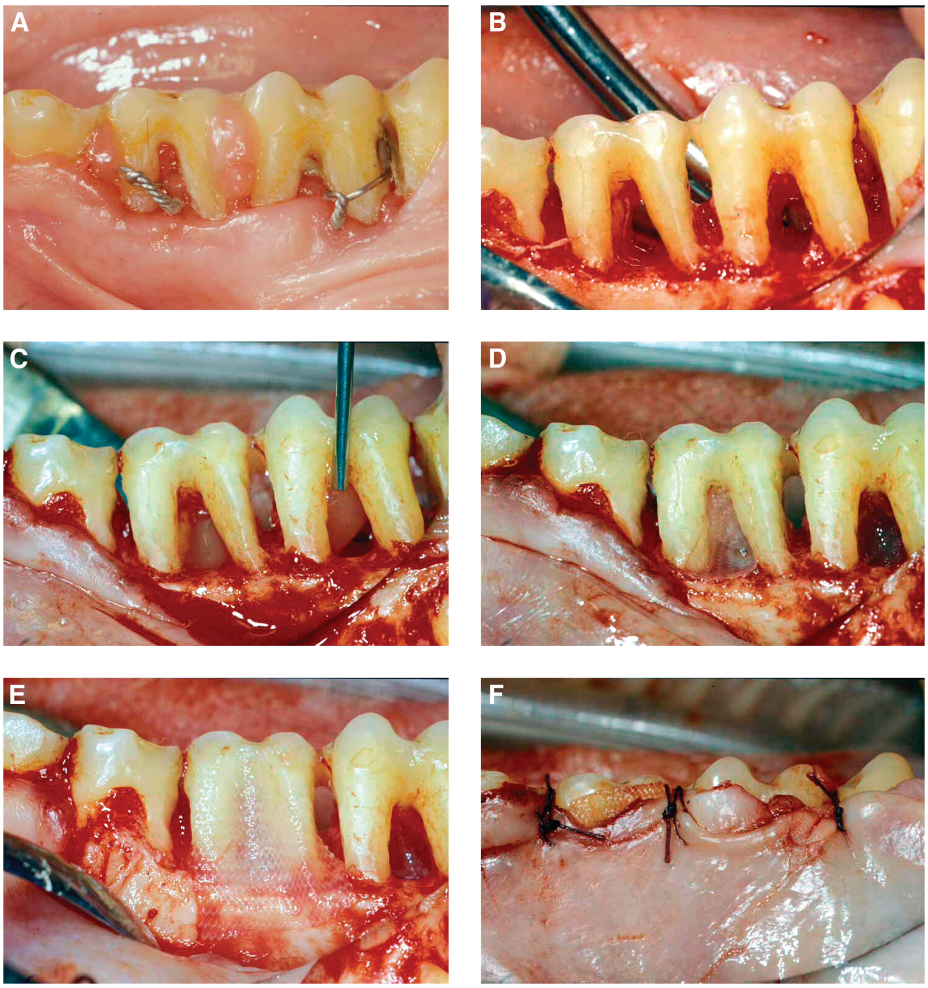

Figure 2.
Treatment phase: ***A)*** *preoperative view;* ***B)*** *postdebridement view demonstrates through-and-through furcation involvement and loss of interproximal bone;* ***C)*** *EMD is applied to EDTA-treated root surfaces free of hematogenous contamination;* ***D)*** *first sheet of DG is placed to cover the intraradicular root surfaces;* ***E)*** *a second sheet of DG is placed over the buccal root surfaces and mid-furcal region; and* ***F)*** *flaps sutured interproximally with 4-0 polyglactin 910 suture.*

between 2 and 3 weeks. No oral hygiene measures were used during the entire healing phase.

Histologic Analysis

The animals were euthanized after a 5-month healing period with a dose of pentobarbital (65 mg/kg intravenously, to effect). Block sections of the mandibular segments were obtained and immediately fixed in 4% formaldehyde solution. After fixation, each molar was separated from the mandibular segment by making mesial and distal interproximal cuts to create individual blocks. Each block was then cut in the mid-sagittal plane with reference to the center of the tooth to obtain equal facial and lingual portions. The blocks were then decalcified and subsequently dehydrated in step gradients of alcohol and embedded in paraffin. The tissue blocks were sectioned in the sagittal plane. Serial sections, 4 μm thick, were obtained from the central region of the furcation in the mesial-distal plane to compare each treatment group equally and allow reference to all necessary structures visible within the section for measuring, i.e., complete and continuous root form and fornix with reference to the surrounding attachment. Sections were stained using hematoxylin basic and eosin counterstains (H&E). Histometric analysis was performed using a light microscope connected to a high-resolution video camera interfaced to a monitor and personal computer. The histometric software package with digital image-capturing capabilities‖ was used for data collection. Using the root notch or the endpoint of root planing as references, linear measurements were made at ×10 and ×40 magnifications at the mesial, middle, and distal aspects of the furcation. Slides were examined by a single blinded examiner. The examiner was also calibrated with reference to recognition of tissue landmarks by two other experienced clinicians. Prestudy calibration trials revealed the intraexaminer calibration error to be <5% for all parameters measured. The tissue components analyzed from the mid-furcal region include new bone height (NB), new connective tissue (NCT), new epithelium (NE), and unfilled portion or "free space" (FS) (Fig. 3). The total length of the defect was then calculated by adding all four aforementioned linear measurements to obtain the histometric defect depth (HDD) for each section.

The intraradicular root-associated measurements were obtained from the mesial and distal aspects of the root, with reference to the base of the defect identified by a root notch or endpoint of root planing (N). The complete new attachment apparatus (CNAA), or regenerative component, was measured from point N, the most coronal aspect of tissues maintaining new bone, maintaining new cementum (NC), and inserting periodontal ligament fibers, to point A (height of

‖ Image-Pro Plus, Media Cybernetics, Silver Spring, MD.

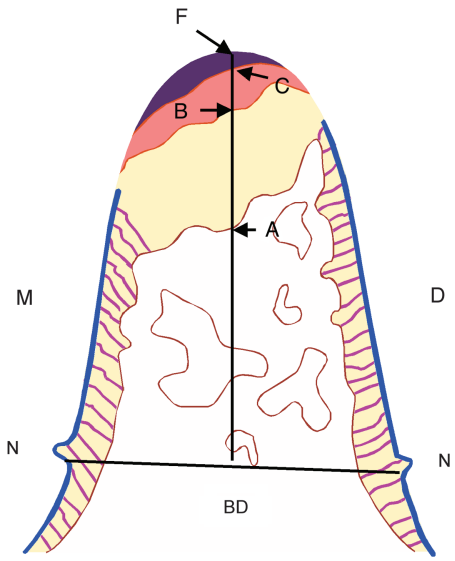

Figure 3.
Schematic representation of landmarks and tissue components measured (mid-furcation measurements). Relative base of the defect (BD) at the mid-furcation determined by the intersection of line connecting F to center of a line connecting the notches (N) identified on the mesial and distal root surfaces; NB determined by measuring point A (height of NB along line F-BD) to BD; NCT determined by measuring point B (height of NCT along line F-BD) to point A; NE determined by measuring point C (height of NE along line F-BD) to point B; unfilled or FS determined by measuring point F to point C; and HDD determined by length of line F-BD (M = mesial aspect; D = distal aspect).

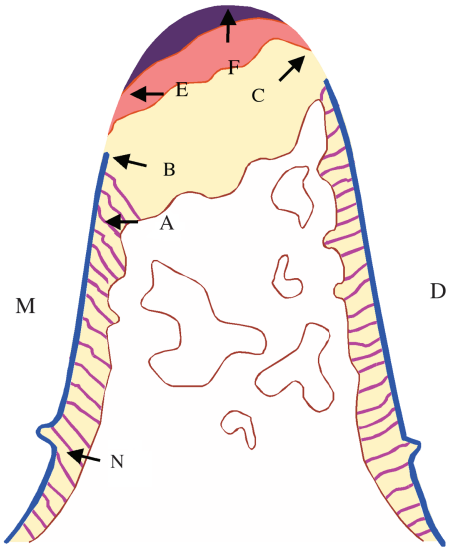

Figure 4.
Schematic representation of landmarks and tissue components measured (intraradicular, root-associated measurements). CNAA determined by measuring from point A (height of CNAA) to point N (notch or endpoint of scaling); NC determined by measuring from point B (height of NC) to point N; NCTA determined by measuring from point C (height of NCTA) to point N; NEA determined by measuring from point E (height of NEA) to point C; RS determined by measuring point F (midpoint of fornix) to point E; and HDD determined by length of line N-F (M = mesial aspect; D = distal aspect).

CNAA). NC was measured from N to the coronal extent of NC. New connective tissue attachment (NCTA) was measured from the apical extent of the CNAA to the base of the junctional epithelium (JE). New epithelial attachment (NEA) was measured from the base of the junctional epithelium to the base of the sulcus. Finally, the remaining uncovered root surface (RS) was measured to the midpoint of the fornix (F). The root surface-associated histometric defect depth (HDD-RS) was determined by adding the CNAA, NCTA, NEA, and RS (Fig. 4).

Data Management

All histometric measurements were transferred to a spreadsheet¶¶ using a numbering system that maintained blinding throughout data collection and statistical analysis. The values were recorded for up to three sections per molar from each animal.

Data Analysis

Statistical analysis was performed by a statistician in the Department of Academic Informatics Services at the University of Texas Health Science Center at San Antonio. A mixed-model analysis of variance (MANOVA) was used to determine statistical significance (set at $P < 0.05$) between treatment groups among the six animals. Statistical analysis between sites within each animal was not possible because of the lack of at least three histologically assessable sections on every tooth.

A power analysis was not performed before experimentation; however, a retrospective power analysis was performed to determine the sample size that would have been necessary to detect significant differences among treatment groups with reference to CNAA.

RESULTS

Pretreatment Clinical Measurements

The vertical dimension for all furcation defects at the time of treatment ranged from 6 to 7 mm. No significant differences were observed among treatment groups. Probing depths were recorded but were not included because of the inaccuracy of measurements caused by interference with the underlying wire ligatures. Additionally, the DG block biopsy from animal 3 was fractured during sectioning and was dropped from the analysis.

¶¶ Excel, Microsoft, Redmond, WA.

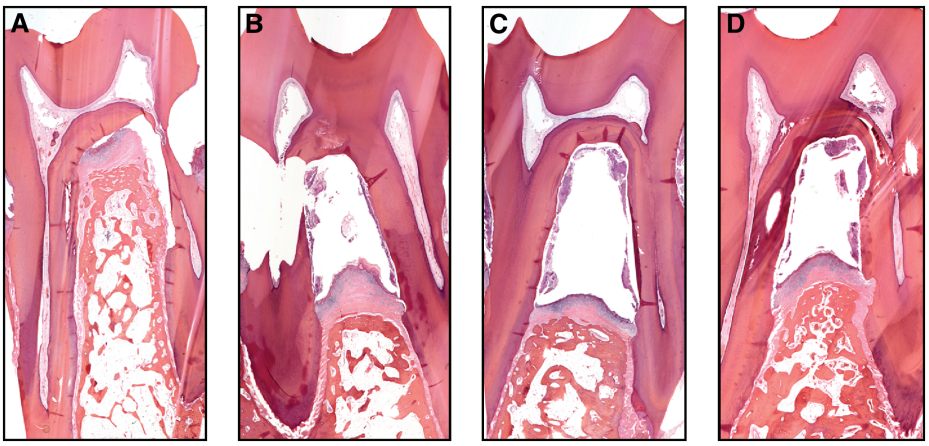

Figure 5.
*Histologic representation from each treatment site obtained from animal 6. The differential responses observed between sites within the same animal are exemplified in this series of sections obtained from each site in animal 6 treated by **A)** EMD; **B)** DG; **C)** EMD plus DG; and **D)** OFD (control) (original magnification ×10).*

Histometric Assessment (mid-furcation)

Because of variability in the plane of sectioning, resulting in possible foreshortening or elongation of the section, it was necessary to express each tissue component as a percentage of the histometric defect depth. Differential tissue gains were observed within each animal with reference to experimental sites compared to the OFD controls (Fig. 5). However, significant variability in response was observed among the animals examined. This variability contributed to the lack of statistical significance observed when the mean treatment outcomes were compared using MANOVA. Although insignificant, when examining the tissue responses among treatment groups, certain trends could be observed. EMD demonstrated a greater mean percent NB (46%), followed by OFD (33.5%), EMD plus DG (23.3%), and DG (22.1%). Similar amounts of percent NCTA were observed between EMD (21.4%), DG (21.5%), and OFD (20.3%), with EMD plus DG demonstrating a slightly greater mean of 27.3%. Similar results were also found for mean percent NE (Fig. 6).

Histometric Assessment (intraradicular root surfaces)

Similar to measurements from the mid-furcal region, each tissue component was divided by the HDD-RS to obtain a percentage to account for the distortion that might have occurred during sectioning. Mean values for each tissue component measured (CNAA, NC, NCTA, and NEA) were not significant when means were calculated and compared to OFD and among treatment types. Although not statistically significant, EMD-treated sites demonstrated a greater mean %CNAA and %NC formation compared to the OFD control sites. DG-treated sites, alone or in combination with EMD, however, demonstrated a lesser mean percent CNAA and NC formation than the OFD controls (Fig. 7).

Retrospective Power Analysis

A treatment effect size of 0.47 was observed for %CNAA in the study sample of 23 defects within six baboons. If this effect size is assumed to be representative of the population from which the experimental baboons were obtained, then a sample size of 72 defects within 18 baboons would be sufficient to detect a treatment effect by F test with MANOVA at the 0.05 probability level with a power of 80%.

DISCUSSION

The specific aim of this study was to evaluate the regenerative response obtained with the use of EMD, DG, or a combination of both compared to a control of OFD in the treatment of surgically induced, chronically maintained Class III, subclass C furcation defects using histometric analysis. Histometric analysis of newly formed tissue components, located at the mid-furcal and adjacent intraradicular root surfaces of the furcation, demonstrated differential regenerative responses with respect to treatment within each animal; however, significance with respect to each treatment examined from all six animals was not observed ($P>0.20$, MANOVA). This was attributable, in part, to a considerable degree of variability in the healing response between animals, a small number of experimental animals (six), a high number of specific treatments assigned (four per animal), and a relatively limited number of sites available for treatment (four per animal). A power analysis was not performed before experimentation because this experiment was designed as a pilot study; however, a retrospective power analysis demonstrated that significance would have been achieved, with respect to %CNAA, if an additional 12 animals were added to the experiment.

Although statistical significance was not achieved, the analysis demonstrated that EMD-treated sites presented mildly positive regenerative results and no negative responses. This represents a remarkable response when factors such as the severity of the defect, the lack of interproximal bone, the plaque-infected environment, no oral hygiene performed after treatment, and possible postoperative recession of tissues are considered. Sites treated with DG, with or without

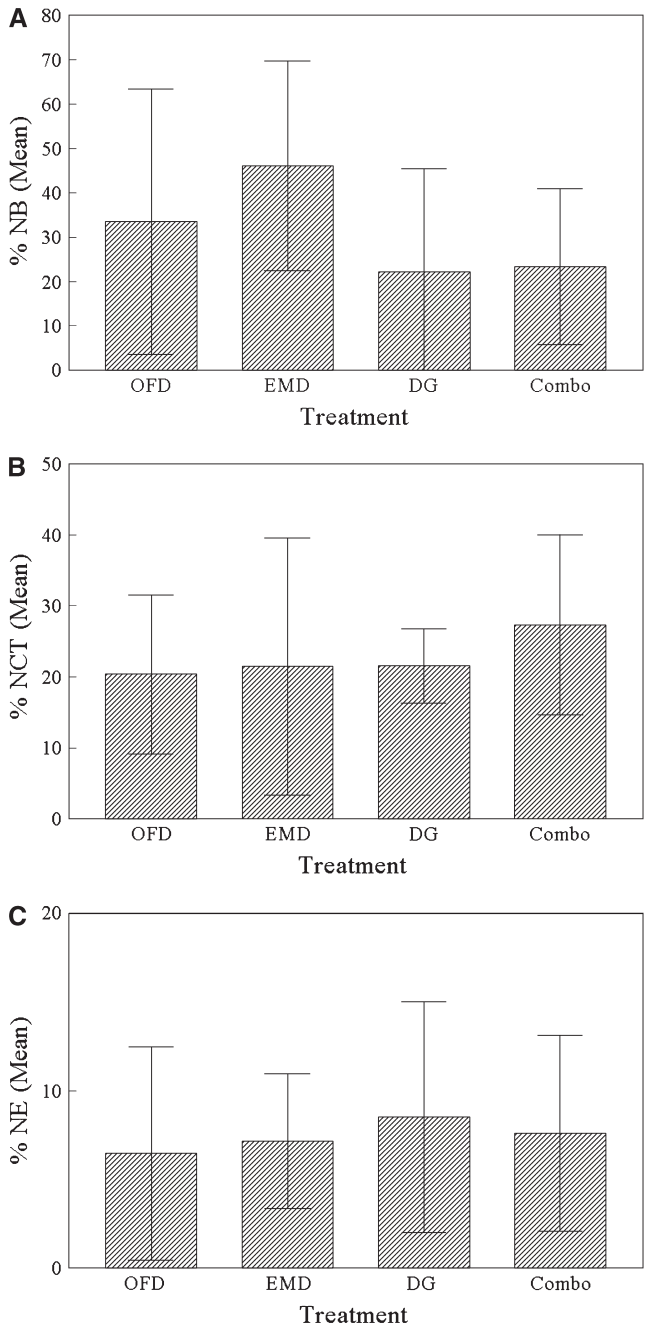

Figure 6.
Mean percent new tissue formation (mid-furcation): ***A)*** *%NB;* ***B)*** *%NCT; and* ***C)*** *%NE (combo = EMD plus DG). No significant differences among treatments were observed by MANOVA (P >0.20). Error bars represent ± 2 SEM.*

EMD, demonstrated less favorable responses and a greater number of negative responses compared to OFD controls.

The combined treatment of EMD and DG tended to negate the more positive results achieved with EMD alone. Assessment of sites treated with DG actually demonstrated a less successful outcome compared to OFD controls. Interestingly, the combination demonstrated greater mean percent NCT and NCTA compared to OFD and other treatment groups. EMD has been shown to stimulate proliferation, migration, and activation of fibroblasts in vitro.[16,31] It is possible that the addition of EMD promoted the proliferation and activation of the donor fibroblasts to achieve this result. Although no significance was demonstrated among treatment groups, the trends suggest a possible positive regenerative effect of EMD and a negative effect of DG on the regeneration of Class III, subclass C furcation defects in baboons. Reasons for this effect may be a consequence of a plaque-infected wound environment, possible host response to the tissue-engineered product, viability of the donated cells in DG, effect of the DG scaffold, and, possibly, the phenotype of the DG fibroblasts.

Oral hygiene was not performed after treatment; however, despite the presence of plaque, differential responses were observed, with EMD demonstrating mildly positive regenerative results. Traditionally, a plaque-infected environment has demonstrated significantly negative effects on the results achieved in traditional surgical therapies and regenerative surgical procedures.[32-34] The previously demonstrated antibacterial effect of EMD on subgingival plaque may have provided a benefit to EMD-treated sites.[35] However, the DG scaffold, without postoperative maintenance, may have trapped oral contaminants and contributed to plaque accumulation within the defect.

A lack of supporting interproximal bone height may have contributed to possible postoperative recession because interproximal bone height was reduced to the same level as the furcation defect created. Miller and Binkley[36] demonstrated the negative impact of a lack of interproximal bone to coronally positioned tissues associated with the involved tooth. Tarnow et al.[37] have also shown that a distance >5 mm from the interproximal contact to the height of interproximal bone is associated with a high probability of papillary tissue recession. Although no postoperative follow-up was performed to verify this finding, it is possible that recession and exposure of the furcation to the oral cavity may have contributed to plaque accumulation and loss of regenerative materials.

A host immune response to the donor fibroblasts or the bioabsorbable lactate/glycollate copolymer scaffold material may have also played a role in the effect of DG on the outcome of the regenerative results observed. The implantation of DG into baboons represents a form of xenotransplantation. It is conceivable that a certain level of graft rejection may have occurred. This host response depends in large part on the recognition of Class I and, specifically, Class II antigens by host cytotoxic and helper T cell receptors. The fibroblasts developed for and implanted in DG,

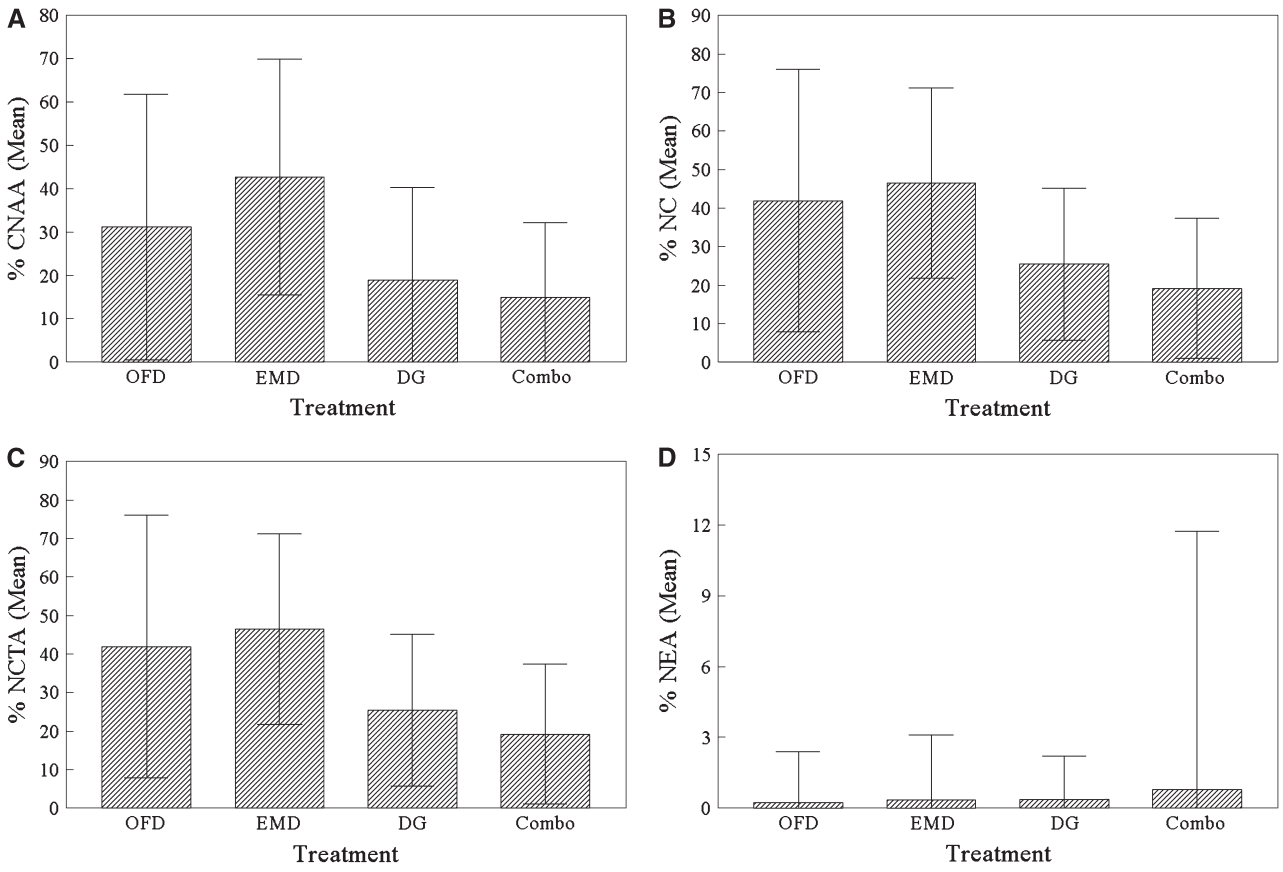

Figure 7.
*Mean percent tissue gains (intraradicular, root-associated measurements): **A)** %CNAA; **B)** %NC; **C)** %NCTA; and **D)** %NEA (combo = EMD plus DG). No significant differences among treatments were observed by MANOVA (P >0.20). Error bars represent ± 2 SEM.*

however, do express a low level of type II MHC proteins but have demonstrated no detectable host response in human studies.[21] Because of the necessity to provide surgical therapies unilaterally and 1 week apart, host cytotoxic T cells may have been presensitized to the antigens present within DG, resulting in an accelerated host response to the second side treated. Polyglactin 910, similar in composition to the scaffold used in DG, has also been shown to elicit a host immune response when used as a barrier for GTR.[38,39]

Another possible negative effect of DG may be represented in the dermal fibroblast phenotype of the donor cells. These fibroblasts may have supported a more reparative or scarring process rather than a regenerative process.[40,41] A high turnover rate has been observed in gingival connective tissue, as described by Melcher.[40] The effect of the differences in turnover rates between gingival and dermal tissues on the particular application studied is unknown. In addition, although it has been demonstrated that various compartments or tissue components adjacent to the periodontal apparatus may contribute cells that support periodontal regeneration, dermal-derived fibroblasts have never been shown to demonstrate this activity.[42] Thus, it is possible that the phenotype of the DG fibroblasts may not be ideal for promoting periodontal regeneration. The development of a DG-like product incorporating cells known to contribute to the formation of periodontal tissues, such as periodontal ligament fibroblasts or pluripotent stem cells, may provide a better outcome in the treatment of furcation defects. Also, cell survival or viability was not confirmed in this study, and it is not known if the DG-originated fibroblasts survived the furcation wound environment.

Space maintenance was also a problem due to the defect chosen, the characteristics of the bioabsorbable scaffold, and the lack of interproximal bone height. DG was designed for assistance in healing dermal ulcers and chronic wounds. Periodontal regeneration is thought to require space maintenance. Thus, DG may be more useful for the treatment of lost periodontal soft tissues that do not require space maintenance, such as recession or mucogingival defects. Additionally, the lack of interproximal bone height provided difficulty in attempting to coronally position the tissue for coverage of the furcation.

Unknown endodontic status of the experimental teeth was also a potential concern and may have contributed to the variability observed. The endodontic status of a tooth has been shown to negatively affect the clinical outcomes achieved in regenerative procedures.[43] The incidence of lateral canals within the furcation has also been reported in humans and may be similar in the baboon.[44,45] Thus, regenerative effects may have been influenced by the pulpal status of the tooth examined.

Knowledge of the mobility status of each tooth also may have been useful. Although limited evidence has been reported demonstrating significant benefits of splinting on the results obtained in response to periodontal surgery, wound stabilization remains a tenet of wound healing, and mobility may have contributed to negative effects observed in the final results.

CONCLUSIONS

No statistical significance was observed among treatment groups with reference to all tissue components measured versus the OFD controls. Despite the severity of the defect and plaque-contaminated environment, the analysis suggests that EMD provided a mildly positive regenerative result in chronically infected severe Class III furcation lesions. This is particularly noteworthy because most previous attempts to regenerate these lesions have not been successful. DG, however, used alone or in combination with EMD, demonstrated equal or lesser regenerative responses compared to the OFD controls. The only observable effect of EMD on DG seemed to be an increase in %NCT and %NCTA formation compared to all other treatment groups (Figs. 6B and 7C). This may be attributed to EMD's known stimulatory effects on fibroblast proliferation, activation, and migration.

Further studies are necessary to determine the immunogenicity of DG in this animal model, the in vitro responses of different cells resident in the periodontal wound environment to the growth factors released by DG, and the survival of DG donor fibroblasts within the wound environment characteristic of periodontal intraosseous defects. Overall, our results demonstrate no clinical or histologic closure of the severe furcation defects used in this study, no statistical differences between treatments, a slight trend in regeneration of the furcation defects using EMD, and no influence of the addition of DG, with some indication that this rationale, under the conditions used in these experiments, appeared detrimental.

REFERENCES

1. Hamp SE, Nyman S, Lindhe J. Periodontal treatment of multirooted teeth: Results after 5 years. *J Clin Periodontol* 1975;2:126-135.
2. Tarnow D, Fletcher P. Classification of the vertical component of furcation involvement. *J Periodontol* 1984; 55:283-284.
3. Cochran DL, Wozney JM. Biological mediators for periodontal regeneration. *Periodontol 2000* 1999;19: 40-58.
4. Bartold PM, McCulloch CAG, Narayanan AS, et al. Tissue engineering: A new paradigm for periodontal regeneration based on molecular and cell biology. *Periodontol 2000* 2000;24:253-269.
5. Gräber HG, Conrads G, Wilharm J, et al. Role of interactions between integrins and extracellular matrix components in healthy epithelial tissue and establishment of a long junctional epithelium during periodontal wound healing: A review. *J Periodontol* 1999;70: 1511-1522.
6. Nerem R, Sambanis A. Tissue engineering: From biology to biological substitutes. *Tissue Eng* 1995;1:3-13.
7. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920-926.
8. Giannobile WV. What does the future hold for periodontal tissue engineering? *Int J Periodontics Restorative Dent* 2002;22:6-7.
9. Heijl L, Heden G, Svardstrom G, et al. Enamel matrix derivative (Emdogain) in the treatment of intrabony periodontal defects. *J Clin Periodontol* 1997;24: 705-714.
10. Heden G, Wennstrom J, Lindhe J. Periodontal tissue alterations following Emdogain treatment of periodontal sites with angular bone defects: A series of case reports. *J Clin Periodontol* 1999;26:855-860.
11. Sculean A, Dent M, Reich E, et al. Treatment of intrabony periodontal defects with enamel matrix protein derivative: A report of 32 cases. *Int J Periodontics Restorative Dent* 1999;19:157-163.
12. Froum SJ, Weinberg MA, Rosenberg E, et al. A comparative study utilizing open flap debridement with and without enamel matrix derivative in the treatment of periodontal intrabony defects: A 12-month re-entry study. *J Periodontol* 2001;72:25-34.
13. Mellonig JT. Enamel matrix derivative for periodontal reconstructive surgery: Technique and clinical and histologic case report. *Int J Periodontics Restorative Dent* 1999;19:8-19.
14. Heijl L. Periodontal regeneration with enamel matrix derivative in one human experimental defect: A case report. *J Clin Periodontol* 1997;24:693-696.
15. Hammarstrom L, Heijl L, Gestrelius S. Periodontal regeneration in buccal dehiscence model in monkeys after application of enamel matrix proteins. *J Clin Periodontol* 1997;24:669-677.
16. Cochran DL, King GN, Schoolfield J, et al. The effect of enamel matrix proteins on periodontal regeneration as determined by histological analyses. *J Periodontol* 2003;74:1043-1055.
17. Cochran DL, Jones A, Heijl L, et al. Periodontal regeneration with a combination of enamel matrix proteins and autogenous bone grafting. *J Periodontol* 2003;74:1269-1281.
18. Gestrelius S, Andersson C, Lidstrom D, et al. In vitro studies on the periodontal ligament cells and enamel matrix derivative. *J Clin Periodontol* 1997;24:685-692.
19. Boyan BD, Weesner TC, Lohmann CH, et al. Porcine fetal enamel matrix derivative enhances bone formation induced by demineralized freeze dried bone

allograft in vivo. *J Periodontol* 2000;71:1278-1286.
20. Schwartz Z, Carnes DL, Pulliam R, et al. Porcine fetal enamel matrix derivative stimulates proliferation but not differentiation of pre-osteoblastic 2T9 cells, inhibits proliferation and stimulates differentiation of osteoblast-like MG63 cells, and increases proliferation and differentiation of normal human osteoblast NHOst cells. *J Periodontol* 2000;71:1287-1296.
21. Mansbridge J, Liu K, Patch R, et al. Three-dimensional fibroblast culture implant for the treatment of diabetic foot ulcers: Metabolic activity and therapeutic range. *Tissue Eng* 1998;4:403-414.
22. Naughton G, Mansbridge J, Gentzkow G. A metabolically active human dermal replacement for the treatment of diabetic foot ulcers. *Artif Organs* 1997;21:1203-1210.
23. Pollak RA, Edington H, Jensen JL, et al. A dermal replacement for the treatment of diabetic foot ulcers. *Wounds* 1997;9:175-183.
24. Mansbridge JN, Liu K, Pinney E, et al. Growth factors secreted by fibroblasts: Role in healing diabetic foot ulcers. *Diabetes Obes Metab* 1999;1:265-279.
25. Pinney E, Liu K, Sheeman B, et al. Human three-dimensional fibroblast cultures express angiogenic activity. *J Cell Physiol* 2000;183:74-82.
26. Folkman J, Damore PA. Blood vessel formation: What is its molecular basis? *Cell* 1996;87:1153-1155.
27. Nissen NN, Polverini PJ, Koch AE, et al. Vascular endothelial growth factor mediated angiogenic activity during the proliferation phase of wound healing. *Am J Pathol* 1998;152:1445-1452.
28. Pepper MS, Mandriota SJ, Vassalli JD, et al. Angiogenesis-regulating cytokines: Activities and interactions. *Curr Top Microbiol Immunol* 1996;213:31-67.
29. Vernino AR, Jones FL, Holt RA, et al. Evaluation of the potential of a polylactic acid barrier for correction of periodontal defects in baboons: A clinical and histologic study. *Int J Periodontics Restorative Dent* 1995;15:85-101.
30. Blomlöf J, Blomlöf L, Lindskog SF. Smear removal and collagen exposure after non-surgical root planing followed by etching with an EDTA gel preparation. *J Periodontol* 1996;67:841-845.
31. van der Pauw MT, van den Bos T, Everts V, et al. Enamel matrix-derived protein stimulates attachment of periodontal ligament fibroblasts and enhances alkaline phosphatase activity and transforming growth factor β1 release of periodontal ligament and gingival fibroblasts. *J Periodontol* 2000;71:31-43.
32. Rosling B, Nyman S, Lindhe J, et al. The healing potential of the periodontal tissues following different techniques of periodontal surgery in plaque-free dentitions: A 2-year clinical study. *J Clin Periodontol* 1976;2:233-250.
33. Nyman S, Lindhe J, Rosling B. Periodontal surgery in plaque-infected dentitions. *J Clin Periodontol* 1977;4:240-249.
34. Selvig KA, Kersten BG, Chamberlain AD, et al. Regenerative surgery of intrabony periodontal defects using ePTFE barrier membranes: Scanning electron microscopic evaluation of retrieved membranes versus clinical healing. *J Periodontol* 1992;63:974-978.
35. Sculean A, Auschill TM, Donod N, et al. Effects of an enamel matrix protein derivative (Emdogain) on ex vivo dental plaque vitality. *J Clin Periodontol* 2001;28:1074-1078.
36. Miller PD Jr., Binkley LH Jr. Root coverage and ridge augmentation in class IV recession using a coronally positioned free gingival graft. *J Periodontol* 1986;57:360-363.
37. Tarnow DP, Magner AW, Fletcher P. The effect of the distance from the contact point of bone on the presence or absence of the interproximal dental papilla. *J Periodontol* 1992;63:995-996.
38. Iglhaut J, Suggs C, Borjesson B, Aukhil I. Apical migration of oral epithelium in experimental dehiscence wounds. *J Clin Periodontol* 1987;14:508-514.
39. Dahlin C, Lindhe J, Gottlow J, et al. Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg* 1988;81:672-676.
40. Melcher AH. On the repair potential of periodontal tissues. *J Periodontol* 1976;47:256-260.
41. Tonna EA, Stahl SS, Asiedu S. A study of the reformation of several gingival fibers in aging mice using 3H-proline autoradiography. *J Periodontal Res* 1980;15:43-52.
42. Carnes DL, Maeder CL, Graves DT. Cells with osteoblastic phenotypes can be explanted from human gingival and periodontal ligament. *J Periodontol* 1997;68:701-707.
43. Pontoriero R, Nyman S, Lindhe J, et al. Guided tissue regeneration in the treatment of furcation defects in man. *J Clin Periodontol* 1987;14:618-620.
44. Lowman JV, Burke RS, Pelleu GB. Patent accessory canals: Incidence in molar furcation region. *Oral Surg Oral Med Oral Pathol* 1973;36:580-584.
45. Gutmann JL. Prevalence, location, and patency of accessory canals in the furcation region of permanent molars. *J Periodontol* 1978;49:21-26.

Correspondence: Dr. David L. Cochran, Department of Periodontics, University of Texas Health Science Center at San Antonio School of Dentistry, 7703 Floyd Curl Dr., San Antonio, TX 78229. Fax: 210/567-3643; e-mail: cochran@uthscsa.edu.

Accepted for publication November 9, 2005.