Defect-Determined Regenerative Options for Treating Periodontal Intrabony Defects in Baboons

Neil M. Blumenthal,* Mario E.A.F. Alves,* Salah Al-Huwais,* Ann Marie Hofbauer,* and Rebecca D. Koperski†

Background: In an effort to regenerate periodontal intrabony defects, the healing potential of the defect should determine what therapeutic modalities and materials are employed. The purpose of this study was to compare regenerative outcomes in baboon intrabony defects that were contained versus non-contained, using various regenerative therapies.

Methods: Nine adult baboons (Papio anubis) in good health were treated. Eighty-six interproximal, intrabony defects were surgically created: 43 contained by 3 walls of bone; 43 non-contained with a missing buccal wall. Chronicity and plaque accumulation were encouraged with wire ligature placement for 8 weeks. After ligature removal, scaling, and a 2- to 4-week healing period, the defects were treated with the following therapies: collagen membrane (GTR), human demineralized freeze-dried bone (DFDB) grafting (BG), combined therapy (GTR + BG) and a DFDB-glycoprotein sponge matrix (MAT). Clinical healing responses were evaluated in 58 sites by changes in soft tissue (recession, probing, clinical attachment) and hard tissue (resorption, defect fill) parameters 6 months post-treatment. Histologic evaluation (defect regeneration, connective tissue attachment, epithelial migration) was done on 26 sites.

Results: For contained defects, no real significant clinical (ANOVA) or histologic differences existed among treatments. However, for non-contained defects, combined therapy (GTR + BG) demonstrated clinically significant ($P \leq 0.05$, ANOVA) and histologically superior healing results over the other therapies tested.


KEY WORDS
Animal studies; bone regeneration; comparison studies; grafts, bone; guided tissue regeneration.

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can be used to develop an evidence-based rationale of defect regenerative potential.

The purpose of the present study was to compare regenerative outcomes in higher potential, contained defects to lower potential, non-contained defects using various regenerative therapies. The results obtained may help provide the clinician with a defect-based rationale for the most appropriate therapy. Defects were treated with guided tissue regeneration (GTR) using collagen membranes; demineralized, freeze-dried bone grafts (DFDB); a DFDB-glycoprotein matrix graft; and combined GTR-DFDB therapy. Treatment was carried out in non-human primates in order to standardize defect characteristics and obtain block sections for histologic analysis. Results were evaluated by hard and soft tissue measurements as well as histologic measurements of supracrestal new attachment and intrabony regeneration.

MATERIALS AND METHODS

Animals

Nine adult baboons (Papio anubis) in good health were utilized in this study. All guidelines regarding the care of animal research subjects were strictly followed, as well as the guidelines set forth by the Animal Research Care Committee at the University of Illinois Health Science Center. Screening procedures included all appropriate physical examinations and all necessary laboratory and radiographic evaluations. Bacteriology and virology were conducted to establish the absence of any infectious disease agents which might pose a risk to other non-human primates or human research workers.

Surgical Preparation of the Intrabony Defects

Osseous defects were prepared under aseptic conditions with injectable intramuscular anesthesia of xylazine (3 to 5 mg/kg) and ketamine (35 mg/kg). Following injection of local anesthesia with 2% lidocaine, mucoperiosteal flaps were reflected from the lateral incisor to the second molar. Only one side of the arch (mandible and maxilla) was treated at a surgical session so as not to compromise nutrition of the animal. Interproximal, intrabony 3-wall contained defects were created surgically distal to the lateral incisor, and in at least one other site where proximal space would accommodate defects 3 mm wide by 5 mm deep. In the opposing arch, 5 mm of facial bone was additionally removed from the initial 3-wall defects interproximally. These served as the non-contained, 2-wall defects. All defects were created with a high-speed handpiece with sterile saline irrigation using #700 fissure burs, assorted surgical round burs, and chisels. All the intrabony defects were made according to the same dimensions bilaterally in each animal. To prevent reattachment of the tissues in the created defects, wire ligatures were wrapped around the teeth and placed directly into each of the defects (Fig. 1a). After defect preparation, mucoperiosteal flaps were replaced and sutured, insuring primary closure using 4-0 absorbable sutures. Eighty-six interproximal, intrabony defects were surgically created: 43 contained by 3 walls of bone, 43 non-contained (with a missing buccal wall). The animals received a soft diet of fruit and bread with marmoset marmalade to enhance plaque formation during ligature placement. Eight weeks later, the ligatures were removed and the areas allowed to heal (Fig. 1b). When the ligatures were removed, the animals were placed on their normal diet of hard chow and biscuits supplemented with fruit.

At least 4 weeks after ligature removal, the animals were scaled supragingivally and polished. The defects were then ready for surgical treatment 2 to 4 weeks later (Fig. 1c). Soft tissue measurements were first made to the nearest mm using a calibrated periodon-

Figure 1.
Defect preparation. a. Defect preparation with wire ligatures in place. b. Chronic defect probing at 8 weeks at the time of ligature removal. c. Preoperative defect site 4 weeks after scaling.
Defect-Determined Regenerative Options for Intrabony Defects

Figure 2.

tal Williams probe. Measurements were taken to evaluate preoperative recession (cemento-enamel junction [CEJ] to gingival margin), clinical attachment level (CAL) (CEJ to base of pocket), and probing depth (PD) (gingival margin to base of pocket). Following surgical exposure, defects were debrided of granulomatous tissue (Fig. 2a-5a). Root surfaces were planed and notches for future histologic evaluation placed at the apical extent of calculus or defect base and adjacent to the clinical alveolar crest level. Bony defect measurements were made from the CEJ to the base of the defect at the deepest point (CEJ to BD), and from the CEJ to the alveolar crest (crest) to determine crestal bone resorption on reentry. Notches were made at this CEJ site as a reference point for postoperative reentry measurements. The debrided defects in each quadrant were treated with the following therapies.

Treatment Methods
Each baboon received all 4 treatment modalities, one in each quadrant. Two or more defects in each quadrant received the same type of treatment. Treatment was balanced and randomized as to right and left, maxilla and mandible, and contained versus non-contained defects. No controls were used to determine expected degree of healing in this model due to the large number of variables studied and the limited number of animals and sites available. However, a similar study by two of the current authors used the same animal model to study control (debridement only) healing in 2-wall intrabony defects with the same dimensions (5 x 5 mm) and morphology (no buccal wall) as the non-contained defects in this study. The soft and hard tissue measurements obtained can serve as control comparisons of the untreated healing capacity of this model.

Human Demineralized Freeze-Dried Bone (DFDB)
Cortical human bone with a particle size of 200 to 300 μm was hydrated with sterile saline and blood from the surgical site. The graft was appositionally placed into the defects to a level above the osseous crest. Flaps were then repositioned to cover the grafted defects and closed with 4-0 resorbable sutures for 2 to 3 weeks (Figs. 2a-c).

Collagen Membrane
Commercially prepared collagen membranes from bovine achilles tendon type 1 collagen were appropriately shaped to cover and overlap the defects and adjacent root surfaces by 2 to 3 mm. Following saturation to a pliable state with blood from the surgical site, the membranes were positioned and sutured as necessary to insure stability. The flaps were then repositioned as coronally as possible to ensure membrane coverage. Primary closure was accomplished with 4-0 resorbable sutures and retained for 2 to 3 weeks (Figs. 3a-d).

Human DFDB-Glycoprotein Matrix
DFDB-glycoprotein matrix, commercially prepared in a sponge form, was hydrated with blood from the surgical site. The sponge was then gently layered to fill the defect to the crest and the flaps coronally closed primarily with 4-0 resorbable sutures for 2 to 3 weeks (Figs. 4a-d).

Combined DFDB-Collagen Membranes
Hydrated DFDB was placed and covered appropriately by collagen membranes as previously described. Flaps were positioned coronally over the membranes and closed with 4-0 resorbable sutures for 2 to 3 weeks (Figs. 5a-d).

Postoperative Care
Animals were monitored on a daily basis to check for untoward reactions and healing complications. Postoperative diet consisted of monkey chow following the initial 2-week postsurgical healing period in which a soft diet was administered. Seeds and nuts were

† LifeNet, Virginia Beach, VA.
§ Biomet Periodontal Membrane, Sulzer-Calcitek, Carlsbad, CA.
|| Dynagraft, Gensci Ortho Biologics, Inc., Irvine, CA.
Methods of Evaluation: Clinical and Histologic Evaluation

Soft tissue changes reflected by recession, decreased probing depth, and clinical attachment level gain were determined by changes in clinical measurements taken at initial treatment and at the 6-month reentry. Clinical defect fill and change in crestal bone height were determined by clinical measurements made at the time of initial surgical treatment and at 6-month reentry on the 58 defect sites in all 9 animals. All measurements were made by the same examiner (NB) who was not calibrated for this study.

Modified block sections of implanted defects were taken at 6 months (on 26 separate, unentered defects) in all 9 animals. An additional 2 specimens were excluded because they were not processed correctly or the blocks were not large enough for analysis. Biopsy specimens were placed in 10% formalin solution, decalcified in 20% sodium citrate and 50% formic acid, washed, and then embedded in paraffin. Serial mesial-distal sections 5 to 7 μ in thickness were cut close to the median plane from the facial to the lingual. Every fifth section was stained with hematoxylin and eosin. Histologic analyses of wound healing events at 6 months were done on representative sections taken from the middle of the defect. Histologic healing parameters of junctional epithelial migration, new connective tissue attachment, and intrabony periodontal regeneration were evaluated for each treatment in contained and non-contained defects. Images were captured using a digital camera on a microscope. Images were processed and mea-

Figure 3.

Figure 4.
Defect-Determined Regenerative Options for Intrabony Defects

Measurements made using special software** at 50% zoom. Each section was coded so as not to reveal the treatment or defect type to the evaluator (NB).

Data Management and Statistical Methods

The clinical data were analyzed by averaging all of the same type of site measurements for each defect type and treatment among the 9 animals. For clinical measurements, comparisons were done using 9 animals with 7 to 8 sites studied for each treatment. The outcomes of interest for soft tissue response included changes in recession, probing depth, and clinical attachment. The outcomes of interest for hard tissue response included changes in crestal resorption and defect fill. Change was observed between baseline and 6 months post-treatment and in both contained and non-contained defects. A mixed effect generalized linear model using least squares estimation was applied to the data, assuming a randomized block design. Each baboon was considered a block. Analyses were run to answer 3 questions: 1) Within contained defects, was there a difference between treatments? 2) Within non-contained defects, was there a difference between treatments? 3) Was there a difference in outcome between defect types? The histologic measurements were analyzed by averaging site measurements for each defect type and treatment among the 9 animals.

RESULTS

Clinical Measurements-Soft Tissue Responses

Contained defects (Table 1). For change in recession, there was no statistically significant overall treatment effect ($P = 0.4469$). Values ranged from 1.17 to 1.78 mm. There was a statistically significant overall difference among treatments ($P = 0.0091$) for PD reduction. Specifically, collagen membrane treatment was significantly better than combined and DFDB-glycoprotein matrix treatments were 4.63, 3.56, 4.12, and 4.14, respectively. For change in clinical attachment, there was a statistically significant overall difference among treatments ($P = 0.0182$). In particular, collagen membrane treatment was significantly better than combined and DFDB-glycoprotein treatments ($P = 0.0069$ and $P = 0.0195$). The least squares estimates of the average change in clinical attachment for collagen membrane, DFDB, combined, and DFDB-glycoprotein matrix treatments were $5.46, 5.68, 5.68,$ and $5.57$, respectively.

Non-contained defects (Table 1). For recession change, there was a statistically significant overall difference among treatments ($P = 0.0034$). More specifically, collagen membrane and DFDB treatment were significantly better than DFDB-glycoprotein matrix therapy ($P = 0.0150$ and 0.0004, respectively).

For PD change, there was a statistically significant overall difference among treatments ($P = 0.0037$). Specifically, it was seen that collagen membrane and DFDB treatments were significantly superior to DFDB-glycoprotein matrix therapy ($P = 0.0018$ for both). The least squares estimates of the average change in PD for collagen membrane, DFDB, combined, and DFDB-glycoprotein treatments were $2.89, 2.89, 3.45,$ and $3.98$, respectively.

For change in clinical attachment, there was a statistically significant overall difference among treatments ($P = 0.0273$). In particular, collagen membrane treatment was significantly better than combined and DFDB-glycoprotein matrix therapy ($P = 0.0118$ and $P = 0.0087$). The least squares estimates for the average change in clinical attachment for collagen membrane, DFDB, combined, and DFDB glycoprotein matrix treatments were $4.79, 5.21, 5.43,$ and $5.46$, respectively.

** Image ProPlus, version 4.5, Media Cybernetics, Carlsbad, CA.
Table 1.
Soft Tissue Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Mean* (SD)</th>
<th>Statistically Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Collagen Membrane (1)</td>
<td>1.78 (0.160)</td>
<td>1 vs. 4 (P = 0.0150)</td>
</tr>
<tr>
<td></td>
<td>DFDB (2)</td>
<td>1.63 (0.146)</td>
<td></td>
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<tr>
<td></td>
<td>Combined (3)</td>
<td>1.52 (0.146)</td>
<td></td>
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<tr>
<td></td>
<td>DFDB-Glycoprotein Matrix (4)</td>
<td>1.17 (0.153)</td>
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<tr>
<td>Recession change</td>
<td></td>
<td></td>
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<tr>
<td>Contained</td>
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<td></td>
</tr>
<tr>
<td>Non-contained</td>
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<tr>
<td>Combined vs. non-contained</td>
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<tr>
<td>Probing depth change</td>
<td></td>
<td></td>
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<tr>
<td>Contained</td>
<td></td>
<td>4.63 (0.212)</td>
<td>1 vs. 2 (P = 0.0015)</td>
</tr>
<tr>
<td>Non-contained</td>
<td></td>
<td>3.56 (0.206)</td>
<td>1 vs. 2 (P = 0.0015)</td>
</tr>
<tr>
<td>Combined vs. non-contained</td>
<td></td>
<td></td>
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<tr>
<td>Clinical attachment change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contained</td>
<td></td>
<td>6.18 (0.173)</td>
<td>l vs. 2 (P = 0.0069)</td>
</tr>
<tr>
<td>Non-contained</td>
<td></td>
<td>5.46 (0.171)</td>
<td>l vs. 2 (P = 0.0015)</td>
</tr>
<tr>
<td>Combined vs. non-contained</td>
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</tbody>
</table>

* The least squares estimate of the average adjusted for baboon and baseline response.
† The overall treatment effect was statistically significant (P < 0.05).
Histological regeneration paralleled clinical defect fill in terms of superiority for contained defects over non-contained defects for all treatment types considered individually and averaged together (42.3% versus 35.6%). Combined treatment demonstrated greater defect fill than all other treatments. However, the differences were only statistically significant compared to collagen membrane and DFDB (P = 0.0044). The least squares estimate of the average change in defect was 4.58, 5.22, 5.99, and 5.65, respectively.

Defect type effect on soft tissue healing. For all 3 outcomes of interest, (recession, probing depth, and attachment level changes), defect type made a significant difference in response. Non-contained defects had a significantly greater change in recession versus non-contained defects (P = 0.0038). For change in probing depth and change in clinical attachment, contained defects fared statistically significantly better (P < 0.0001 and P = 0.0004).

Hard Tissue Responses

**Contained defects (Table 2).** For change in crestal resorption, there were no significant treatment effects (P = 0.5029). For change in defect fill, there was a statistically significant overall difference among treatments (P < 0.0001). Specifically, it was seen that collagen membrane and DFDB alone were each significantly inferior to both combined and DFDB-glycoprotein matrix treatment. While combined treatment resulted in the best defect fill of all treatments, it was only statistically significant over collagen membrane (P = 0.0002) and DFDB (P = 0.0006), not DFDB-glycoprotein. The least squares estimates of the average change in defect fill for collagen membrane, DFDB, combined, and DFDB-glycoprotein treatments were 5.35, 5.46, 6.30, and 6.23, respectively.

**Non-contained defects** (Table 2). For change in crestal resorption, there were no significant treatment effects (P = 0.6890). For change in defect fill, there was a statistically significant overall difference among treatments (P <0.0001). Specifically, it was seen that collagen membrane was significantly inferior to other treatments (versus DFDB, P = 0.0153; versus combined, P <0.0001; versus DFDB-glycoprotein matrix, P = 0.0002). Combined treatment demonstrated greater defect fill than all other treatments. However, the differences were only statistically significant compared to collagen membrane and DFDB (P = 0.0044). The least squares estimate of the average change in defect was 4.58, 5.22, 5.99, and 5.65, respectively.

**Defect type effect on hard tissue healing.** For change in crestal resorption, there was no significant defect type effect (P = 0.2490). For change in defect fill, defect type made a significant difference in response. Contained defects responded significantly better than non-contained defects (P = 0.0002).

**Histology**

Quantitative measurements—infrabony healing. Table 3 represents a comparison of histological healing parameters for contained versus non-contained defects evaluated for each treatment modality. Histological regeneration paralleled clinical defect fill in terms of superiority for contained defects over non-contained defects for all treatment types considered individually and averaged together (42.3% versus 35.6%). Combined treatment (DFDB and membrane) demonstrated superior histologic regeneration for contained and non-contained defects compared to other treatments and the average of all treatments (47.8% versus 38.9%). These histo-
Table 2.
Hard Tissue Results

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Collagen Membrane (1) Mean* (SD)</th>
<th>DFDB (2) Mean* (SD)</th>
<th>Combined (3) Mean* (SD)</th>
<th>DFDB-Glycoprotein Matrix (4) Mean* (SD)</th>
<th>Statistically Significant Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crestal resorption change</td>
<td>0.66 (0.219) 0.33 (0.186) 0.44 (0.175) 0.33 (0.186)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-contained</td>
<td>0.67 (0.151) 0.56 (0.151) 0.44 (0.151) 0.67 (0.151)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contained vs. non-contained</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Defect fill change</td>
<td>Contained‡ 5.35 (0.150) 5.46 (0.150) 6.30 (0.150) 6.23 (0.150) 1 vs. 3 (P = 0.0002) 1 vs. 4 (P = 0.0003) 2 vs. 3 (P = 0.0006) 2 vs. 4 (P = 0.0013)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-contained‡ 4.58 (0.174) 5.22 (0.171) 5.99 (0.172) 5.65 (0.172) 1 vs. 2 (P = 0.0153) 1 vs. 3 (P &lt;0.0001) 1 vs. 4 (P = 0.0002) 2 vs. 3 (P = 0.0044)</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Contained vs. non-contained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P = 0.0002</td>
</tr>
</tbody>
</table>

* The least squares estimate of the average adjusted for baboon and baseline response.
‡ The overall treatment effect was statistically significant (P < 0.0001).
NS = Non-significant.

logic results are consistent with the clinical reentry findings.

For non-contained defects, grafting resulted in greater histologic regeneration than collagen membrane treatment alone. Combined therapy (graft and membrane) demonstrated superior histologic healing to either grafting material used alone or collagen membrane alone. For contained defects, little difference in histologic regeneration was seen between the grafts and collagen membrane.

**Supracrestal healing.** Total histologic new attachment (JE and CT) did not appear to be influenced by infrabony defect morphology (2.83 mm contained versus 2.64 mm non-contained). New connective tissue attachment, however, was greater in contained defects (1.53 versus 1.25 mm), particularly when a collagen membrane was used (2.20 versus 1.50 mm).

The type of treatment seemed to have an effect on the relative JE/CT proportions making up the new attachment. When membrane therapy was used alone or combined with DFDB, the trend was toward a longer connective tissue and shorter junctional epithelial attachment than in grafted-only defects. In addition, sites treated with DFDB alone resulted in a longer junctional epithelial attachment than the other therapies individually or averaged together (1.83 versus 1.35 mm).

wall appositional and crestal bone growth than non-contained sites, with marrow spaces present in the regenerated alveolus (Fig. 8a). A mature supracrestal connective tissue attachment to new cementum was found in both defect types (Fig. 8b, 9a). Subcrestally, the notch areas demonstrated the thickest cementum formation with a dense ligamental fiber attachment (Fig. 9b). Coronal extent of new cementum exceeded coronal new bone formation in all specimens examined. DFDB graft particles were found either sequestered (Fig. 8b), in connective tissue (Fig. 9a), or incorporated in new alveolar bone (Fig. 10). In addition, mineralization-osteoid formation indicative of osteoinduction was also associated with graft particles (Fig. 11).

**DFDB-glycoprotein matrix graft.** Specimens demonstrated infrabony appositional bone growth and periodontal regeneration in both contained (Fig. 12b) and non-contained sites. Junctional epithelial migration was limited by a mature connective tissue attachment to new cementum (Fig. 12a). Remnants of glycoprotein matrix and encapsulated graft particles in the connective tissue formed an "osseous cap" over the defect (Fig. 12c).

**Combined DFDB-collagen membrane treatment.** Both defect types demonstrated substantial regeneration of new bone, ligament, and cementum in the spec-

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**Qualitative Histologic Observations: Methods of Treatment**

Collagen membrane. In the histological sections examined, apical migration of junctional epithelium did not occur. There was no difference between contained versus non-contained in this regard. However, contained sites generally demonstrated a thicker, continuous layer of new cementum and a denser, more mature connective tissue and periodontal ligament fiber attachment (Fig. 6). Non-contained sites generally demonstrated a thinner, non-continuous cemental layer with connective tissue and ligament fiber attachment (to cementum) interspersed with areas of connective tissue "adhesion" (Fig. 7). Infrabony periodontal regeneration was evident in both defect types.

Deminerlized freeze-dried bone. Contained defects demonstrated greater defect filling overall. For non-contained defects, grafting resulted in greater histologic regeneration than collagen membrane treatment alone. Combined therapy (graft and membrane) demonstrated superior histologic healing to either grafting material used alone or collagen membrane alone. For contained defects, little difference in histologic regeneration was seen between the grafts and collagen membrane.
Table 3.
Comparison of Histologic Measurements for Each Treatment Type (contained versus non-contained defects in 9 animals) (mean ± SD)

<table>
<thead>
<tr>
<th>Treatment Parameters</th>
<th>Collagen Membrane</th>
<th>DFDB</th>
<th>Combined</th>
<th>DFDB-Glycoprotein Matrix</th>
<th>Average of All Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont</td>
<td>Non</td>
<td>Cont</td>
<td>Non</td>
<td>Cont</td>
</tr>
<tr>
<td>Defect Infrabony Healing</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Initial hist defect (Def)</td>
<td>4.30 ± 0.07</td>
<td>4.60 ± 1.00</td>
<td>4.40 ± 0.10</td>
<td>4.50 ± 0.75</td>
<td>4.20 ± 0.70</td>
</tr>
<tr>
<td>Average</td>
<td>4.45 ± 0.53</td>
<td>4.45 ± 0.43</td>
<td>4.30 ± 0.75</td>
<td>4.45 ± 0.23</td>
<td>4.42 ± 0.74</td>
</tr>
<tr>
<td>Histo. regen.</td>
<td>1.95 ± 0.80</td>
<td>1.30 ± 0.70</td>
<td>1.85 ± 0.60</td>
<td>1.70 ± 1.00</td>
<td>2.20 ± 0.60</td>
</tr>
<tr>
<td>Percent regen.</td>
<td>45.3 ± 28.3</td>
<td>42.0 ± 37.8</td>
<td>52.4 ± 43.2</td>
<td>45.5 ± 40.0</td>
<td>42.3 ± 35.6</td>
</tr>
<tr>
<td>Average %</td>
<td>36.8 ± 39.9</td>
<td>47.8 ± 42.8</td>
<td>38.95 ± 42.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supracrestal Healing</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Connective tissue (CT)</td>
<td>2.20 ± 1.00</td>
<td>1.50 ± 0.40</td>
<td>1.00 ± 0.40</td>
<td>0.80 ± 0.40</td>
<td>1.90 ± 0.90</td>
</tr>
<tr>
<td>Average</td>
<td>1.85 ± 0.70</td>
<td>0.90 ± 0.40</td>
<td>1.80 ± 0.75</td>
<td>1.00 ± 0.70</td>
<td>1.39 ± 0.64</td>
</tr>
<tr>
<td>Junctional epithelium (JE)</td>
<td>0.90 ± 0.30</td>
<td>1.00 ± 0.40</td>
<td>1.80 ± 0.90</td>
<td>1.85 ± 1.00</td>
<td>1.00 ± 0.70</td>
</tr>
<tr>
<td>Average</td>
<td>0.95 ± 0.35</td>
<td>1.83 ± 1.00</td>
<td>1.15 ± 0.80</td>
<td>1.45 ± 0.90</td>
<td>1.35 ± 0.89</td>
</tr>
<tr>
<td>Total new attachment</td>
<td>3.10 ± 1.30</td>
<td>2.50 ± 0.40</td>
<td>2.80 ± 1.30</td>
<td>2.65 ± 1.40</td>
<td>2.90 ± 1.60</td>
</tr>
<tr>
<td>Average</td>
<td>2.80 ± 1.05</td>
<td>2.73 ± 1.40</td>
<td>2.45 ± 1.60</td>
<td>2.45 ± 1.60</td>
<td>2.74 ± 1.23</td>
</tr>
</tbody>
</table>

Cont = contained defect; Non = non-contained defect.
Def = calculus notch (CN) to alveolar crest notch.
Histo regen. = CN to coronal extent of new bone, ligament, and cementum.

DISCUSSION
Animal Model System
This study would have had more validity if done in human patients. However, the ability to provide matched defects with standardized depths and morphologies would have been very difficult. In addition, being able to obtain sufficient block sections of teeth and surrounding tissues in the magnitude presented also would have presented major obstacles. For these reasons, the non-human primate model was chosen. The anatomy of this model is closest to the human periodontum with the ability to create infrabony defects with similar defect depths, morphology, plaque colonization, and healing mechanism as in humans.24-28 Several clinical regeneration studies have confirmed the use of non-human primates and baboons as suitable models for regenerative therapy.29-31

Bone regeneration is commonly assessed through reentry procedures and measurements which rely on direct visualization of new bone formation compared to initial pretreatment levels. However, although this can give an accurate measure of "clinical fill," it does not distinguish connective tissue attachment levels or bone that is attached to the root surface by a periodontal ligament or with an intervening long junctional epithelial attachment. In the current study, in order to assess if clinical healing measurements were the result of true regeneration or repair, histological measurements from the most apical calculus notch as well as qualitative healing observations were included in order to provide a more complete evaluation.

In all specimens examined, plaque-induced inflammation was confined to the connective tissue subjacent to the sulcular and junctional epithelium (Figs. 7a, 8b, 13b). No evidence of inflammation was associated with any of the graft materials remaining at the 6-month histologic observation period. Sequential evaluations at critical time intervals would better reveal biologic differences in wound healing between defect types and among treatment modalities. However, the scope of this study was to evaluate therapeutic results at the "end point" of healing.

No control lesions were included in the present study. While comparisons were made with control lesion healing in a similar study, ideally each animal studied should also have its own control sites. This is a lim-
Itation of the present study. The values presented must be considered as absolute. However, when comparing the 33.6% defect fill found in control lesions to the 44% to 56% found in the current study, clinically relevant conclusions may be drawn. In addition, the statistical power of the sample size was probably not large enough to determine true statistical differences in the data presented. The clinical results and wound healing histology should also be viewed with this limitation in mind.

**Regenerative Materials**

**Collagen membrane.** The effectiveness of collagen membrane to support regeneration in infrabony defects has been substantiated in several animal and human studies. Its ability to be resorbed with a minimal inflammatory response made it a barrier of choice for the conditions of the current study. The positive infrabony healing potential of GTR procedures in this animal model has been demonstrated previously and was confirmed in the present study.

**Figure 6.**
Collagen membrane-treated contained defect demonstrating a mature dense connective tissue attachment (CT), new cementum (C), periodontal ligament (PDL), and alveolar bone (B) (hematoxylin and eosin; 10x original magnification).

**Figure 7.**
- **a.** Collagen membrane-treated non-contained defect demonstrating subepithelial inflammation (I), connective tissue adhesion (A), new cementum (C), and separation artifact (ART) at defect base (hematoxylin and eosin; 10x original magnification).
- **b.** Enlarged view of Figure 7a showing thick new cementum formation at the defect base (hematoxylin and eosin; 40x original magnification).

- **Figure 7a.**
  - CT (Connective Tissue Attachment)
  - C (Cementum)
  - PDL (Periodontal Ligament)
  - B (Bone)

- **Figure 7b.**
  - B (Bone)
  - C (Cementum)
  - PDL (Periodontal Ligament)
Demineralized freeze-dried bone (DFDB). Demineralized freeze-dried bone (DFDB) has been used successfully as a periodontal graft material because of its ability to enhance regeneration of a new periodontal attachment. In addition to its osteoinductive properties, DFDB has been shown to be osteoinductive in several studies. However, the osteoinductive capacity of commercially prepared material has been extremely variable due to differences in methods of harvesting, processing, and sterilizing. In addition, physical characteristics of the graft as a result of processing, such as degree of mineralization, residual calcium levels, particle size, and donor age may also affect osteoinduction. Addition of osteogenin (bone morphogenetic protein [BMP]-3) and rhBMP-2 to DFDB has been shown to enhance periodontal regeneration in clinical studies. In the future, this technology may be used to insure consistent and predictable osteoinductivity with DFDB grafts. At present, the advantage of DFDB grafts may lie in their ability to stabilize the initial clot and activate platelet degranulation and release of initial growth factors (platelet-derived, insulin-like, and transforming-B) and attachment proteins (fibronectin), which are early determinants for regeneration.

In the present study, human DFDB acted as a xenograft in the baboon model and its osteoinductive effect may have been compromised by species limitations. Ideally, baboon donor bone would have made a more suitable graft material. However, bovine xenografts have been shown to induce new bone formation in periodontal infrabony defects in dogs and monkeys. In the present study, the highly purified human DFDB grafts did not appear to elicit clinical or histologic inflammatory responses or interfere with normal wound healing. The clinical measurements and histologic observations were similar to previously reported results using DFDB allografts in human studies.

DFDB-glycoprotein matrix graft. Human DFDB combined in a glycoprotein matrix sponge is the result of an effort to enhance the regenerative capacity of DFDB with the thrombogenic (fibroblast), chemotactic properties of collagen. In addition, the collagen matrix can serve as a scaffold for ingrowth of regenerative cells and blood vessels, as well as provide physical spacing between graft particles in this process. The matrix can also serve as an excellent carrier for delivering and concentrating graft particles in the defect. A recent study in baboon periodontal infrabony defects using this xenograft material demonstrated 25% greater bone fill than control sites, with 7 of 9 animals histologically showing greater than 50% regeneration. In the present study, the DFDB matrix graft demonstrated superior clinical defect fill and histologic regeneration than particulate DFDB. In addition, the glycoprotein constituent of the graft may have provided a GTR effect by reducing epithelial migration compared to DFDB alone (Table 3).
Defect Determinants of Regenerative Therapy

Defect characteristics have been related to regenerative results in several studies. Deeper defects exceeding 4 mm were associated with better outcomes using bone grafts and membranes. Defect width and radiographic defect wall angulation have also been correlated with clinical healing responses. In addition, when grafting procedures were used, the number of bony walls remaining (defect containment) positively correlated with regeneration potential. However, mixed results have been reported when relating clinical success to defect containment using membrane therapy. Becker and Becker and Cortellini et al. found positive correlations; Tonetti et al., Selvig, and Rengert found no significant associations.

In the present study, defect containment by 3 osseous walls was correlated with superior clinical defect fill and histologic regeneration for both grafted and guided tissue regeneration sites. The biologic rationale relates to providing a protected environment by the defect walls for clot formation, wound stability, granulation tissue formation, and organization. In addition, 3-wall contained defects provide the best spatial relationship for defect bridging by vascular and cellular elements from the periodontal ligament and adjacent osseous walls. Space maintenance is provided by the defect walls to minimize membrane collapse and/or provides for protection and retention of the graft. Under these “ideal defect conditions,” significant clinical differences among therapies were minimal. Histologically, GTR and combined therapy resulted in a trend toward shorter junctional epithelial migration and longer connective tissue attachments. Histologic regeneration values were similar among therapies.

Non-contained defects missing a buccal wall provided a less than optimum environment for regeneration. Membrane therapy alone may be compromised due to collapse of the membrane into the defect and loss of potential "regenerative space." Graft materials alone may provide better space maintenance for granulation tissue influx by supporting the flap better. In addition, grafting may offer a matrix for clot protection, stability, and maturation in an unprotected environment. In the current study, non-contained defects that were grafted demonstrated superior regeneration than those receiving membrane therapy.
**Figure 10.**
DFDB graft particles (G) incorporated in marrow spaces within new regenerated alveolar bone (NB) (hematoxylin and eosin; 40x original magnification).

**Figure 11.**

a. DFDB graft (G) material within the defect of Fig. 9a with associated osteoid formation (O) and increased collagen density (C) (hematoxylin and eosin; 10x original magnification).

b. Enlarged view of smaller graft particle (G) in 11a undergoing resorption with associated osteoid formation (O) and collagen (C) (hematoxylin and eosin; 40x original magnification).

c. Enlarged view of supracrestal area of 12a showing glycoprotein remnants in the connective tissue (GP) and encapsulated DFDB graft particles (G) (hematoxylin and eosin; 40x original magnification).

**Figure 12.**

a. DFDB-glycoprotein matrix graft in a contained defect demonstrating limited epithelial migration (E), a mature supracrestal fiber attachment (F) to new cementum (C), and regeneration (R) within the defect (hematoxylin and eosin; 4x original magnification).

b. Enlarged view of infrabony component of 12a showing thick new cementum formation (C), oppositional alveolar bone growth (B), and new periodontal ligament (PDL) (hematoxylin and eosin; 40x original magnification).

c. Enlarged view of supracrestal area of 12a showing glycoprotein remnants in the connective tissue (GP) and encapsulated DFDB graft particles (G) (hematoxylin and eosin; 40x original magnification).
bran treatment resulted in 31% defect fill; deminer-
alized bone, 48% fill.\textsuperscript{10}

Biologically, combined graft-membrane therapy addressed many of the regenerative challenges offered by non-contained defects. In addition to a potential osteoinduction-conduction effect, the graft provides a scaffold for clot and granulation tissue maturation and also supports the membrane and flap from collapsing into the defect. The membrane potentially isolates the intrabony healing compartment from intrusion from epithelium and gingival connective tissue. In addition, the membrane may also act as a buffer against the early tensile forces of flap margin mobility. This mobility may disrupt the fibrin clot-root surface interface both supracrestally and within the defect. Compromise of this interface may lead to disruption of the initial forming connective tissue attachment, resulting in migration of junctional epithelium.\textsuperscript{64,65} These concepts are supported in the current investigation by the histologic and clinical regenerative results.

Superior defect fill with combined therapy is supported in the literature in several clinical studies comparing it to membrane therapy alone\textsuperscript{10,12,66,67} or DFDB alone.\textsuperscript{10,68} Long-term case reports by Schallhorn and McClain\textsuperscript{6,9} validate the enhancement of GTR with root conditioning and grafting. However, other human studies have found the same result\textsuperscript{69} or insignificant differences\textsuperscript{70} when combining DFDB grafting with ePTFE membranes. Caffese et al.\textsuperscript{67} did not find the addition of xenografts enhanced GTR treatment of furcation defects in dogs and suggested the graft may inhibit cell migration. These variations are to be expected when comparing several different studies. In addition, most regenerative studies treated contained 2- and 3-wall defects, which have a higher potential for regeneration. The current study showed similar healing results for this type of defect, independent of therapy employed. Clinical studies on lower potential, non-contained or combination defects would provide a better test of treatment efficacy more relevant to the clinical situations most frequently encountered.

In clinical practice, pure contained 2- and 3-wall defects are uncommon. Chodroff and Ammons\textsuperscript{70} found only 16 of 130 defects had pure 3-wall morphology. The rest were combinations. Selvig et al.\textsuperscript{17} found in combination defects, the 3-wall component consisted of 53% or less of the total defect. The more occlusal component was a combination of 1- and 2-wall configurations. The regeneration potential of this more superficial component is compromised due to the biologic limitations of non-contained defects and/or the increased susceptibility to oral environmental factors leading to incomplete bone fill.\textsuperscript{18} In order to provide the most predictive therapy (and avoid a secondary postoperative procedure), it has been suggested to eliminate the coronal 1- and/or

alone. These results are in agreement with a con-
trolled human study comparing regenerative thera-
pies in infrabony defects in which collagen mem-

\textbf{Figure 13.}

\textbf{a.} Combined DFDB-collagen membrane treated non-contained specimen revealing thick new cementum formation (C) in the notch area (N) and coronally with oppositional and coronal new bone (NB) formation and new periodontal ligament (PDL). Note communication of marrow space (M) with PDL (hematoxylin and eosin; 10x original magnification). \textbf{b.} Supracrestal area of 13a showing a mature connective tissue fiber (CT) attachment to new cementum (C) with no migration of junctional epithelium (JE). Note subrosulcular inflammation (I) (hematoxylin and eosin; 10x original magnification).
2-wall portion of the defect with ostectomy-osteoplasty. The remaining 3-wall component can then successfully and predictably be regenerated with GTR or other procedures.  

However, the present study showed the regenerative capacity of the non-contained 1-2 wall component may be enhanced with a combined graft-membrane procedure. While not as definitive as a resective procedure, the increase in potential new periodontal support may be worth the minor compromise in predictability.

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