

Histological Comparison of Healing Following Tooth Extraction With Ridge Preservation Using Mineralized vs. Demineralized Freeze Dried Bone Allograft

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Background: Allografts such as demineralized freeze-dried bone allograft (DFDBA) and mineralized FDBA are commonly used by clinicians for ridge preservation procedures. The primary objective of this study is to histologically evaluate and compare the healing of non-molar extraction sockets grafted with DFDBA vs. FDBA for ridge preservation. The secondary aim of this study is to compare dimensional changes in ridge height and width following grafting with these two materials.

Materials: Forty subjects were randomly divided into 2 groups of 20. Extraction sockets were filled with either FDBA or DFDBA. To minimize variables associated with the organ donor and with tissue processing, all of the graft material was procured from a single donor; the only difference in the 2 materials was the percent mineralization of the final bone graft. A 2mm diameter core biopsy was taken from each grafted site approximately 19 weeks after grafting. Histomorphometric analysis was performed to determine percentage of vital bone, residual graft particles, and connective tissue (CT)/other.

Results: There were no significant differences when comparing changes in alveolar ridge dimensions of the two groups. There was no significant difference in percentage CT/other between groups. DFDBA had a significantly greater percentage of vital bone at 38.42% versus FDBA at 24.63%. The DFDBA group also had a significantly lower mean percentage of residual graft particles at 8.88% compared to FDBA at 25.42%.

Conclusion: This study provides the first histologic and clinical evidence directly comparing ridge preservation with DFDBA versus FDBA in humans and demonstrates significantly greater new bone formation with DFDBA.

KEY WORDS

dental implants; bone transplantation; tooth extraction

Dental implants have become a predictable and reliable therapy in the replacement of missing teeth.¹⁻³ In areas where teeth have been missing for an extended period of time the alveolar ridge is often dimensionally unstable, losing height and width. Extraction sites that are not grafted for ridge preservation may lose up to 50% of their ridge width in the first year post extraction.⁴⁻⁶ Ridge preservation is a simple procedure that has proven to decrease the loss of ridge width during healing.⁷⁻⁹ Many graft materials such as autogenous bone, allografts, xenografts, and alloplasts have been used in an attempt to maintain the dimensions of the alveolar ridge following extraction.⁷⁻⁹ Allografts such as demineralized freeze-dried bone allograft (DFDBA) and mineralized freeze-dried bone allograft (FDBA) are among the most commonly used materials. While the dimensions of the ridge are crucial for subsequent implant placement, intuitively the quantity of vital bone that forms in the healing extraction socket is also important. There is currently no evidence to indicate which of these materials promotes greater bone growth in the non-molar extraction socket. There have been no direct histologic comparisons of these two allografts in ridge preservation to date. Both materials are commonly used and both

materials are accepted and effective, yet clinicians have little evidence to guide a selection of materials.

While both DFDBA and FDBA are osteoconductive, only DFDBA has been proven to be osteoinductive. In the 1960s, Urist et al.¹⁰ showed that demineralized bone has osteoinductive potential by stimulating bone formation in extraskelatal sites. The osteoinductive potential of DFDBA is related to the amount of bone morphogenetic proteins (BMP) that remain after commercial processing has been completed. Shigeyama et al.¹¹ detected BMP-2, -4, and -7 in a commercial lot of demineralized bone matrix. Schwartz et al.¹² tested commercial lots of DFDBA from 6 different bone banks and found that most of the lots were able to induce ectopic bone formation when placed in a nude mouse muscle, while other lots did not induce new bone at all. In a ridge preservation study by Becker et al.¹³ DFDBA failed to show any signs of osteoinduction while autologous bone grafts had significant new bone formation. The BMPs in DFDBA can either be active or inactive due to a number of factors; if inactive, the inductive properties are lost.^{12,14} The release of these self-contained BMPs stimulates the differentiation of mesenchymal cells to osteoblasts in a location such as muscle, even though bone does not normally form there. While the absence of BMPs from DFDBA will eliminate inductive capabilities in the nude mouse model, the addition of BMP-2 has been shown to restore the osteoinductive nature of DFDBA.¹⁵ In addition an osteopromotor such as enamel matrix derivative (EMD) will increase the osteoinductive potential of active DFDBA.¹⁶

Although FDBA has the same BMP content in its organic matrix, it does not have this same osteoinductive capability. Demineralization by osteoclasts is necessary in order to release BMPs from the mineralized matrix, and since there are no osteoclasts in extraskelatal sites, the BMPs remains trapped in the mineralized particles; thus, no ectopic bone formation is induced. There is a compromise along the spectrum from FDBA to DFDBA in removing enough mineral to facilitate the release of soluble factors such as BMPs while retaining enough calcium to facilitate crystal formation for mineralization of newly formed bone matrix. Evidence suggests that maximum osteoinduction is observed when there is approximately 2% residual calcium remaining in DFDBA, and it is believed that this small percentage of calcium acts as a nidus for hydroxyapatite crystal formation.¹⁷

FDBA may provide a better scaffold than DFDBA for space maintenance, and may also be more osteoconductive.¹⁸ When FDBA is used as a graft material, osteoclasts break down the mineral content until the FDBA is also demineralized. Osteoinductive proteins then become available to induce new bone formation. With DFDBA the initial demineralization process is not required, and the soluble osteoinductive proteins are able to function immediately after implantation. There may be a prolonged osteoclastic breakdown when FDBA is used, and therefore a beneficial prolongation of osteoinductive protein release.

The purpose of the current study was to compare mineralized freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA) materials in the preservation of alveolar bone following extraction of non-molar teeth. The primary objective was to histologically evaluate and compare the percentage of new bone formation in healing extraction sockets of non-molar teeth grafted with DFDBA versus FDBA for ridge preservation. The secondary aim was to observe clinical changes in ridge height and ridge width following grafting with these two materials.

MATERIALS AND METHODS

Subject Enrollment

The Institutional Review Board of the University of Texas Health Science Center at San Antonio (UTHSCSA) reviewed and approved the protocol for this study. A power analysis was performed to determine the minimum number of subjects needed to detect a clinically significant difference of at least one standard deviation between the mean of two groups. It was assumed that there would be a minimum of 70% compliance among subjects who were enrolled in the study using a Mann-Whitney U test at 0.05 with a power of 88.5%. It was determined that the minimum number of subjects per group was 14. Anticipating a potential 30% drop out rate, 40 total subjects were recruited, with 20 in each group.

Subjects enrolled included patients treated at UTHSCSA between February 2009 and April 2010 who required extraction of a single-rooted non-molar tooth and who were interested in receiving a dental implant. Subjects committed to have the dental implant placed 18 to 20 weeks following extraction and ridge preservation, at which time core biopsies at the grafted extraction site would be harvested. Subjects were screened, consented and enrolled in the study if the following inclusion criteria were met: adequate restorative space for implant-retained restoration; at least 10mm alveolar bone height without impingement on the maxillary sinus or inferior alveolar canal; and, root location and angulation that would be consistent with the subsequent implant placement. In order to ensure an adequate depth of socket for harvesting of a bone core biopsy without inclusion of native bone, only single-rooted teeth were included, all roots were required to have a minimum of 10mm radiographic bone support, and all roots had to have an angulation similar to the angulation of the implant to be placed at the site. Multi-rooted teeth were not included due to the potential for presence of interradicular bone that might be harvested as part of the core biopsy procedure. Subjects were excluded if they had an uncontrolled systemic disease such as uncontrolled diabetes or hypertension, displayed evidence of an acute infection or periapical lesion, or had other contraindications to surgery. Although smoking was not an exclusion criterion, no smokers were enrolled in the study. Forty subjects were enrolled, and each subject was assigned to one of the two treatment groups at the time of surgery by random selection of sealed envelopes.

To strengthen the validity of the comparison it was important to insure that the only variable in question was the mineral content of the graft particles used. To minimize variables associated with the organ donor and with tissue processing, all of the graft material was procured from a single 47-year old female donor. All bone materials were ground to a particle size ranging from 250 to 750 μ m, and the DFDBA was demineralized to 3.3% residual calcium. Thus, the only difference in the two materials was the percent mineralization of the final bone graft.

In addition, following processing the DFDBA was tested for osteoinductivity by LifeNet Health[†], *in vivo* in the athymic mouse gluteal muscle pouch model, and explants were evaluated histologically with hematoxylin and eosin (H&E). This was done to determine whether the DFDBA had high, low or no inductivity. Inductivity in this model is rated on a scale from 0 to 4, with a 0 rating indicating no inductivity. Ratings of 1 through 4 indicate progressively increasing amounts on new bone induction, with 4 being the highest quartile of new bone formation. The

processed donor DFDBA used in this study had an inductivity score of 1 out of 4, indicating that the inductivity was in the bottom quartile when compared to the inductivity of established control samples. The DFDBA had inductive potential, but it was relatively low.

Surgical Procedure

The identified tooth was extracted in a minimally traumatic manner with periostomes and either no flap or minimal flap reflection no more than 2mm beyond the alveolar crest, followed by curettage and irrigation of the socket. The socket was thoroughly examined for defects such as dehiscences or fenestrations. As previously described by Beck and Mealey¹⁹, a UNC-15 periodontal probe[‡] was used to make the following clinical measurements: the height of the buccal and lingual crest was measured by using a periodontal probe to connect the midfacial CEJs of the adjacent teeth, then measuring the vertical distance from that reference line to the crest of bone on the midfacial and midlingual. Calipers[§] were used to measure ridge width as well as buccal plate thickness 2 mm from the crest of the ridge at the midfacial aspect to the nearest 0.5mm. All measurements were performed by a single examiner (RW), who also performed the majority of the surgical procedures. All surgeries were performed by periodontal residents under the direct supervision of board certified attending faculty. While the measurements were being completed the randomly selected graft material (DFDBA or FDBA)[†] was hydrated with sterile saline. The socket was filled to or slightly coronal to the crest of bone. A resorbable collagen membrane material^{||} was placed over the graft material and was secured over the socket orifice with nonresorbable sutures. Flaps were not reflected unless a minor dehiscence was detected. In cases where there was a bony dehiscence, small flaps were reflected just beyond the extent of the dehiscence and a longer-resorbing collagen membrane material[¶] was used. Only minor dehiscences were accepted; if the depth of the dehiscence was greater than 50% of the depth of the socket the subject was excluded from the study. Post operative instructions were given and all patients were prescribed 500 mg of amoxicillin three times daily for one week unless an allergy to penicillins was present, in which case 100 mg of doxycycline once daily for 10 days was given. All patients were instructed to rinse for 30 seconds twice daily with 0.12% chlorhexidine gluconate for two weeks. Sutures were removed 2 weeks after the ridge preservation was performed.

Subjects returned approximately 3 months after the extraction for a cone beam computerized tomographic (CBCT) scan in order to evaluate the dimensions of the alveolus prior to implant placement. At the time of implant placement 18 to 20 weeks post-extraction, minimal buccal and lingual flaps were reflected and ridge width as well as buccal and lingual height measurements were made as previously described. A trephine drill[#] with a 2.0mm internal diameter was used to take a core biopsy approximately 8 mm in length, which was placed in 10% neutral buffered formalin.

Histologic Processing and Analysis

Biopsies were decalcified, embedded in paraffin, sectioned longitudinally into multiple 4- μ m thick sections, and stained with Harris Hematoxylin and counterstained with Treosin. The innermost section of each biopsy was examined whenever possible. When artifact prohibited the innermost section from being used, the next closest section was examined; these were located 4 to 12 μ m from the innermost section. Each section was examined at a minimum of 20x magnification, and the entire area of the section was evaluated. Digital images of each section

were acquired and used to trace the areas identified as vital bone, residual particle and CT/other (Figures 1 and 2). Image manipulation software** was used to create individual layers of vital bone, residual graft particles, and CT/other. These layers were then converted to a binary (black and white) form, and area by percentage of each of the three layers was digitally calculated based on number of pixels using image analysis software.††. The above-mentioned method of analysis was developed and described by Beck and Mealey.¹⁹

Statistical Analysis

Unpaired Student t-tests were performed for between-group comparisons of ridge dimensions and of percentages of newly formed vital bone, residual bone graft particles, and CT/other. Mann-Whitney U tests were performed to confirm significant findings based on t-tests. Discrete measure comparisons for treatments were performed using Fisher Exact tests. For all tests, $p < 0.05$ was considered significant. Pearson correlations were performed to assess the relationships between the histologic bone core percentages for the three tissue groups. Spearman correlations were used to evaluate relationships between the histologic percentages and the clinical ridge dimension changes in each group. Statistical analysis was performed by a statistician using PASW 17.0 software (SPSS Inc., Chicago, IL).

RESULTS

Thirty-three of 40 subjects completed the study, 13 males and 20 females with an average age of 56.7, ranging from 20 to 78. One of the subjects did not have enough ridge width for an implant upon re-entry; hence, a core biopsy was not taken and only clinical measurements were made for that subject. Of the 7 dropouts, 3 exited due to discovery of a buccal dehiscence greater than 50% of the length of the buccal socket wall at the time of extraction, and 4 subjects were not able to have their implant placed within the 18 to 20 week post-extraction time frame. Medical history and demographic information were obtained by patient interview. A total of 32 biopsies were analyzed histologically with 16 biopsies in each group. The FDBA group included 6 maxillary incisors, 1 maxillary canine, 5 maxillary premolars and 4 mandibular premolars. The DFDBA group included 5 maxillary incisors, 3 maxillary canines, 6 maxillary premolars and 2 mandibular premolars.

There was no significant difference in average healing time from ridge preservation to implant placement between groups. The DFDBA group had an average healing time of 19.81 weeks (SD=1.76), while the FDBA group had an average healing time of 19.25 weeks (SD=1.53). The majority of the sites treated were in the maxilla, with only 2 of 16 DFDBA sites and 4 of 16 FDBA sites being in the mandible. Two subjects from the DFDBA group and one from the FDBA group showed signs of potential infection at the 1-week postoperative follow-up appointment. In each case the antibiotic regimen was changed to clindamycin 150-mg 4 times a day for 10 days, and each of the sites healed without further complications. None of the sites lost graft material during healing. Small facial dehiscences requiring use of a longer lasting collagen membrane were found in 3 FDBA sites and 2 DFDBA sites. After the core biopsy was removed and the final osteotomy prepared, each of the 32 subjects received dental implants, with primary stability attained in each case.

Dimensional Changes

There were no significant differences when comparing changes in the alveolar ridge dimensions of each group at the time of implant placement. The average pre-extraction ridge width in the FDBA group was 9.97 ± 1.01 mm, compared to 9.70 ± 1.13 mm in the DFDBA group. In both groups, average loss of ridge height was less than 1mm, and loss of ridge width was approximately 2mm. The dimensional stability of the alveolar ridge following ridge preservation was clinically indistinguishable when grafted with DFDBA versus FDBA (Table 1).

Histologic Observations

Each specimen was examined under a light microscope at a minimum of 20x magnification to differentiate vital bone, residual particles and CT/other. Residual graft particles presented as generally well defined lamellar regions with lacunae absent of osteocytic nuclei. These regions of residual graft particles had a similar appearance regardless of graft type. In some instances the DFDBA particles were less well defined, making it more difficult to determine the exact point at which the residual particle ended and newly apposed vital bone began. The residual FDBA and DFDBA material was regularly surrounded by new woven bone with osteocytes which intimately contacted the lamellar graft particles. The CT/other included vasculature, loose fibrous stroma, and inflammatory cells as well as regions of amorphous material that constituted "bone dust". Such "bone dust" is commonly seen when ground bone is forced into the adjacent marrow spaces during trephine harvesting of the bone cores and when the cores themselves are sliced during tissue processing. It was counted in the CT/other component of the section and did not interfere with quantification of new bone and residual graft particles. These areas of bone dust were characterized by spaces that appeared to be densely filled with a finely ground "granite like" mineralized and unmineralized substance.

For both DFDBA and FDBA groups the mean area of CT/other was close to half of the total area, and there was no significant difference between groups (Table 2). DFDBA had a significantly greater mean percentage of newly formed vital bone ($p=0.01$) and a significantly smaller mean percentage of residual graft particles ($p=0.004$) compared to FDBA. In addition to this overall analysis of 3 tissue components, the bone tissues alone were evaluated to determine the percentage of vital bone and the percentage of residual graft particles. Newly formed vital bone constituted 81.26% of the total bone area in the DFDBA group compared to 50.63% in the FDBA group, while residual bone graft material constituted only 18.74% of the total bone area in the DFDBA group compared to 49.37% in the FDBA group. The differences between the DFDBA and FDBA groups were statistically significant for both percentage vital bone ($p=0.005$) and percentage residual graft particles ($p=0.005$).

The only significant correlations in histologic parameters were between % vital bone and % residual graft, which were negatively associated ($r= -0.837$ for DFDBA and $r= -0.761$ for FDBA). This negative correlation is especially clear because the amount of CT/other in each group was nearly equal at about 50%. As the graft particles were resorbed, new bone formed in its place, not CT/other.

DISCUSSION

The primary aim of this study was to evaluate new bone formation when comparing the use of two common allografts at a single time point for ridge preservation. All subjects in this study exhibited new bone formation, but DFDBA promoted significantly greater new bone formation and resulted in less residual graft particles at approximately 18 to 20 weeks post-ridge preservation. The 18 to 20 week post-extraction time period was selected for this and several other ongoing studies in our research group based primarily on common clinical practice and other study designs. In a recent study, Beck and Mealey¹⁹ showed no difference in percent new bone formation or residual graft particles when sockets were grafted with a mineralized bone allograft for 3 months versus 6 months prior to core biopsy. Other studies have used healing periods of 4 to 21 months prior to core harvest.²⁰⁻²³ A study with similar design to the current study examining molar sites used an identical 18 to 20 weeks healing period.²⁴

It is accepted that the quantity of new bone formation is time dependent, and that variability among reentry time points between studies or between subjects in a given study may account for variability in the data. The current study was designed with weaknesses of previous studies in mind. In this study, both types of graft material came from a single donor. This eliminated donor age, gender, or other host factors as variables in potential inductivity. In addition, the DFDBA was tested for inductivity *in vivo* and showed low, but present, inductivity. It was important to test the inductivity of the DFDBA used in the study to shed light on any differences that might have been noted between DFDBA and FDBA after healing. For example, if the FDBA had resulted in significantly greater new bone formation than DFDBA, which it did not, one explanation might be a total lack of inductivity of the DFDBA used in the study. Conversely, if the DFDBA resulted in significantly greater new bone formation than FDBA, one explanation might be very high inductivity of the DFDBA used in the study. The results of *in vivo* athymic nude mouse testing revealed that the DFDBA procured for this study had inductive potential, but that the potential was rather low on the inductivity scale used by the company† performing the testing. This strengthened the findings of the study in that this minimally inductive DFDBA resulted in a significantly greater percentage of new bone formation and a significantly lower percentage of residual graft particles compared to FDBA. How variations in inductivity of DFDBA affect new bone formation in extraction sockets is unknown. We speculate that had the DFDBA possessed even greater inductive potential, the differences between DFDBA and FDBA may have been even greater; however, research is needed to support or refute this speculation. In addition, the DFDBA was tested for residual calcium content, and had a relatively ideal 3.3% calcium content. Since all of the graft materials were ground to the same particle size of 250-750 microns, particle size was eliminated as a potential variable between the DFDBA and FDBA.

There was very little variability in recipient sites because only single-rooted non-molar teeth were extracted, sockets with severe dehiscences were excluded, and only teeth with 10 mm of radiographic bone support that were oriented similarly to ideal implant placement were included. This ensured that the core bone biopsies were taken from the grafted socket site and did not include native bone surrounding the socket. In addition, only the innermost sections of the cores were used in order to minimize possible inclusion of native bone. Finally, core biopsy samples were all harvested at a similar time point following the ridge preservation procedure. All of these

factors helped to isolate the single variable in question; namely, the mineral content of the graft particles.

Evaluation of change in ridge dimensions was a secondary aim of this study. Stents were not used to standardize pre and post grafting measurements. We therefore cannot state with certainty that pre-grafting and post-grafting measurements were made at exactly the same point on the ridge in each site, and this lack of precise measurements may explain the relatively wide standard deviations in dimensional changes seen in Table 1. This is a weakness of the study, but the method of measurement was uniform for both groups, and the results were remarkably similar. Both groups lost less than 1 mm of vertical height and approximately 2 mm of ridge width. These numbers are favorable when compared to a recently reported systematic review evaluating dimensional changes after tooth extraction without ridge preservation, which reported a loss of 1.67 mm in height and 3.87 mm in width.⁷ Within the limits of this study, it appears that there is no significant difference between the two materials when used to preserve the dimensions of the alveolar ridge for future implant placement. It is clear in the current study that the sites grafted with DFDBA had significantly greater vital bone on average at the time of implant placement.

This study raises some questions. If there is no difference in the dimensional stability when DFDBA is compared to FDBA in ridge preservation, is there a clinical benefit to placing an implant in a site with significantly more vital bone? How much vital bone is needed to support a dental implant during healing? Does drilling into a site with a greater percentage of residual graft particles present the tactile sensation of drilling into “better” quality bone or “poorer” quality bone compared to a site with less residual graft material? Will sites grafted with FDBA in time “catch up” and have a higher percentage of vital bone after the residual particles have been broken down and replaced? Further research in these areas is needed to determine the clinical significance of these findings.

CONCLUSION

The present human study is the first to directly compare healing following ridge preservation with DFDBA versus FDBA using bone obtained from a single tissue donor and processed in an identical manner, with the sole exception of the amount of calcium in the final grafted product. The results indicate that there are no statistically significant differences in the changes in ridge dimensions after ridge preservation is performed with DFDBA versus FDBA. Histologically, there is a significantly greater percentage of vital bone in sites grafted with DFDBA versus FDBA, and DFDBA sites had significantly less residual graft particles. There is no significant difference in percent area of non-bone tissue (CT/other) between the groups. Therefore the clinician may be supported in using DFDBA rather than FDBA for the purpose of ridge preservation to attain greater new bone formation at a time point 18 to 20 weeks after grafting, assuming that the particular lot of DFDBA has at least some inductive potential.

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Figure 1

DFDBA 20x Histology

VB: Vital bone with osteocytes in the lacunae

GP: Graft particle with empty lacunae

CT: Connective tissue with fibroblasts and irregularly organized collagen

Figure 2

FDBA 20x Histology

VB: Vital bone with osteocytes in the lacunae

GP: Graft particle with empty lacunae

CT: Connective tissue with fibroblasts and irregularly organized collagen

Table 1: Clinical Change in Ridge Dimensions

Bone Graft Material	Δ Ridge Height Buccal \pm SD (mm)	Δ Ridge Height Lingual \pm SD (mm)	Δ Ridge Width \pm SD (mm)	% Δ Ridge Width \pm SD (%)
DFDBA	-0.37 \pm 1.11	-0.97 \pm 1.61	-2.18 \pm 1.62	-22.8 \pm 16.2
FDBA	-0.57 \pm 1.18	-0.60 \pm 1.34	-2.09 \pm 1.71	-20.9 \pm 16.6

No statistically significant differences in any clinical parameter ($P > 0.05$)

Table 2: Histologic observations

DFDBA			
Site ID	% Vital bone	% Residual Graft	% CT
1	35.67	0.84	63.49
2	43.92	4.83	51.25
3 (d)	31.74	5.20	63.06
4	39.65	4.62	55.72
5 (d)	49.22	5.24	45.55
6	45.73	8.43	45.84
7	23.66	13.06	63.28
8	54.80	2.38	42.86
9	32.99	0.00	67.01
10	44.80	6.80	48.40
11	9.720	33.55	56.73
12	44.45	6.96	48.59
13	50.70	2.41	46.89
14	7.17	46.42	46.42
15	44.46	1.27	54.27
16	56.08	0.00	43.92
Mean + S.D.	38.42 + 14.48*	8.88 + 12.83†	52.71 + 7.96

FDBA			
Site ID	% Vital bone	% Residual Graft	% CT
1	15.07	45.34	39.60
2	44.03	0.00	55.97
3	48.74	1.73	49.53
4 (d)	30.63	22.49	46.88
5 (d)	25.50	24.70	49.80
6	22.57	3.63	73.80
7	29.53	28.25	42.21
8	8.31	52.46	39.24
9	12.98	45.26	41.76
10	50.25	0.00	49.75
11	16.76	29.86	53.38
12 (d)	18.34	30.29	51.36
13	25.21	31.60	43.18
14	0.23	27.11	72.66
15	21.90	44.30	33.80
16	24.07	19.74	56.19
Mean + S.D.	24.63 + 13.65*	25.42 + 17.01†	49.94 + 11.07

* P= .010 for DFDBA versus FDBA

† P= .004 for DFDBA versus FDBA

(d) indicates a small dehiscence at site required use of longer lasting collagen membrane

† Life Net Health, Virginia Beach, VA

‡ UNC-15 periodontal probe, G. Hartzell & Son, Concord CA

§ Castroviejo caliper, Salvin Dental Specialties, Charlotte, NC

|| Collatape® and Collaplug®, Zimmer Dental, Warsaw, IN

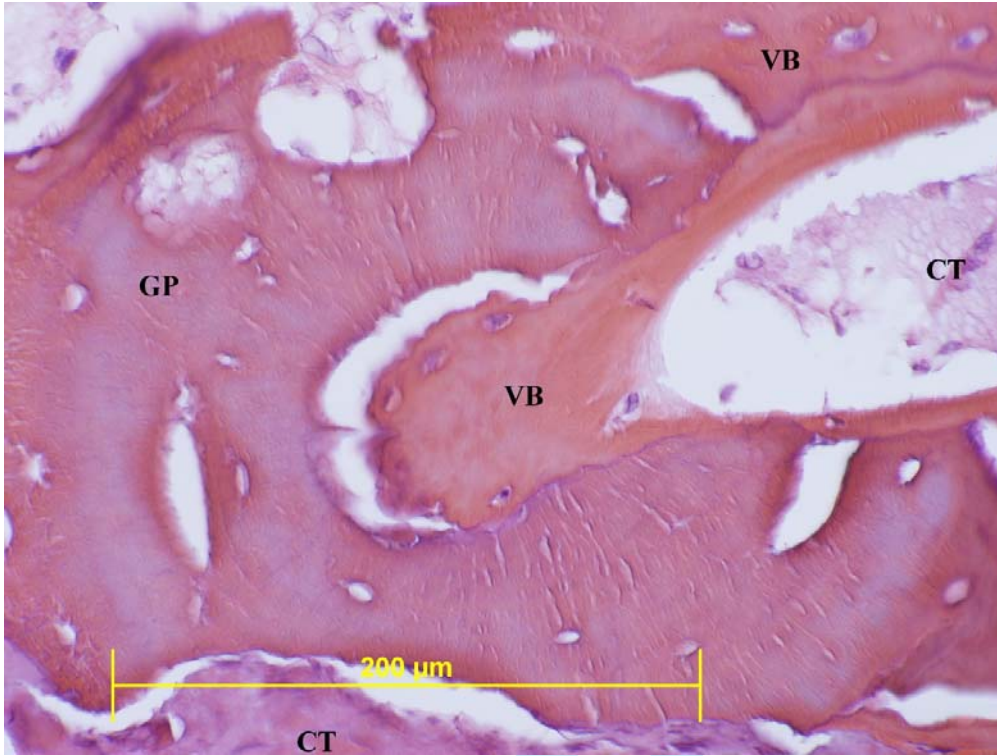
¶ Socket Repair Membrane[®], Zimmer Dental, Warsaw, IN

Salvin Dental Specialties, Charlotte, NC

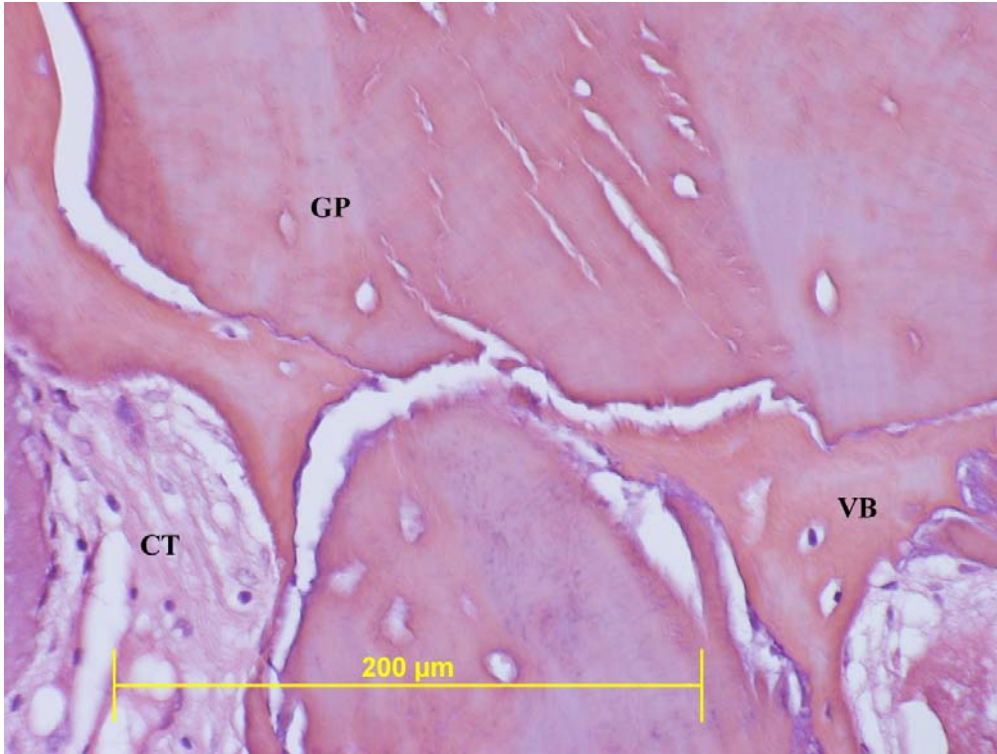
** Adobe Photoshop Elements 7, Adobe Systems, San Jose, CA

†† Image J, National Institute of Health, Bethesda, MD

unedited



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