Histologic Analysis of Healing After Tooth Extraction With Ridge Preservation Using Mineralized Human Bone Allograft

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Background: Ridge preservation was developed as a therapy to prevent severe bone resorption after tooth extraction. The purpose of this study is to determine if there is any difference in the amount of new bone formation ≈3 months after extraction and ridge preservation compared to that after ≈6 months.

Methods: Minimally traumatic extraction with ridge preservation using mineralized human bone allograft was performed at 38 single-rooted tooth sites in 33 subjects. Sixteen sites healed for an average of 14 weeks (early healing), whereas 22 sites were allowed to heal for an average of 27 weeks (delayed healing) before harvesting bone core samples. Histomorphometric analysis was performed to determine the percent of new bone formation, residual graft particles, and connective tissue/non-mineralized structures for each site.

Results: All specimens showed evidence of new bone formation, with most of the residual graft particles surrounded intimately by woven bone. No statistically significant differences in the amount of newly formed bone or residual graft particles were found between the two groups. Overall, the early healing group demonstrated a mean of 45.8% new bone, 14.6% residual graft material, and 39.6% connective tissue/non-mineralized tissue. The delayed healing group showed mean values of 45%, 13.5%, and 41.3%, respectively.

Conclusion: The results of this study suggest that waiting ≈6 months after tooth extraction and ridge preservation using mineralized bone allograft does not provide a greater amount of new bone formation or less residual bone particles compared to that after only ≈3 months. *J Periodontol 2010;81:1765-1772.*

KEY WORDS

Bone transplantation; dental implants; tooth extraction.

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Dental implants have been widely accepted as a predictable treatment option for the replacement of missing teeth.[1-4] In recent years, treatment protocols have shifted from placement of implants only into mature lamellar bone[5] to procedures that reduce overall treatment times, such as the immediate placement of dental implants into fresh extraction sites. A clinical study of bone healing after tooth extraction revealed that the average single-tooth extraction site loses 50% of its alveolar width, an average loss of 6.1 mm, during the 12 months after extraction.[6] Araújo et al.[7] reported an average loss of 2.5 mm, or about 35%, of ridge width in the 6 months after tooth extraction in a dog model. Significantly greater resorption of the facial aspect of the ridge was seen after extraction compared to the lingual aspect. In a study evaluating the morphologic changes of the alveolar ridge after extraction of maxillary anterior teeth in humans, Nevins et al.[8] found an average loss of 5.2 mm in buccal ridge height in teeth with prominent roots and intact, but thin, buccal plates. Some cases lost as much as 9 mm in buccal ridge height after extraction. Clearly, the changes in alveolar dimension after tooth extraction may greatly alter treatment decisions including the ability to place a dental implant for optimal esthetics and long-term success.

doi: 10.1902/jop.2010.100286

Ridge preservation was developed as a technique to preserve alveolar dimensions during the healing of an extraction site when immediate implant placement is contraindicated. In general, most studies show a smaller loss of ridge width when sockets are grafted compared to when they are not grafted.[8-10] Several materials have been studied for this purpose and proven successful to varying degrees, including autogenous bone, allografts, xenografts, and alloplastic materials;[8-10] however, the ideal healing time before implant placement is unclear. Studies on ridge preservation have generally provided healing times of 2 to 12 months before implant placement.[9] To date, no controlled clinical trials in humans have examined differences in new bone formation within the former tooth socket at varying time intervals after ridge preservation grafting. Therefore, the purpose of this study is to determine if there is any difference in the amount of new bone formation 3 months after tooth extraction and ridge preservation compared to that after 6 months using the same allograft material in both groups of subjects.

MATERIALS AND METHODS

Patient Selection

The Institutional Review Board of the University of Texas Health Science Center at San Antonio reviewed and approved this research protocol, and this study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.[11] A power analysis was performed to determine the number of subjects needed to detect a clinically significant mean difference of one standard deviation or more, assuming a minimum of 70% of the subjects to be fully compliant, using a Mann-Whitney *U* test at the 0.05 level with a power of 88.5%. It was determined that a minimum of 14 histologic samples were required per treatment group. To allow for an anticipated rate of 30% dropouts, a total of 20 subjects were enrolled per group, for a total of 40.

Subjects were recruited from the University of Texas Health Science Center at San Antonio dental patient pool between January 2008 and April 2009. All potential subjects were screened and written consent obtained if the eligibility criteria were met. Eligible subjects had ≥1 single-rooted tooth with a minimum of 10 mm bony support that required extraction and replacement with a dental implant. Careful attention was paid to select only sites where the tooth location and root angulation was consistent with the ideal future implant orientation and where restorative space was adequate for a dental implant. Presence of any acute infection or the presence of a periapical lesion were exclusion criteria. Subjects were not enrolled if they were pregnant or planning to become pregnant during the study period; if there was any medical contraindication to dental surgery; or if they had a medical condition or therapeutic regimen known to affect hard or soft tissue healing, such as autoimmune disease, immunosuppressive therapy, or poorly controlled diabetes (HbA_{1c}>7%). Forty subjects between the ages of 18 and 99 were sequentially allocated to one of two treatment groups. The first 20 subjects enrolled were assigned to the delayed healing group and the remaining 20 subjects were assigned to the early healing group. Multiple single-rooted teeth from the same subject were included if they met the inclusion criteria, and all sites within a subject were in the same healing group, early or delayed.

Surgical Protocol

No presurgical antibiotics were provided and all subjects received a single dose of 800 mg ibuprofen before extraction. After administration of local anesthesia, minimally traumatic extraction was performed. The sockets were thoroughly degranulated and examined for the presence of a fenestration or dehiscence. The following measurements were recorded using a periodontal probe† and rounded to the nearest millimeter: the depth of socket on facial and lingual/palatal measured from the most apical aspect of the socket to the most apical point on the corresponding alveolar crest; and the height of the buccal and lingual cortices measured at the mesio-distal midpoint of the adjacent teeth using a horizontal reference line marked with a periodontal probe connecting the midfacial cemento-enamel junction of the adjacent teeth. No stent was used during the dimensional measurements. In addition, sharp calipers‡ were used to measure the bucco-lingual ridge width at the mesio-distal midpoint between adjacent teeth at a level 2 mm apical from the ridge crest; and the buccal plate thickness 2 mm apical from the ridge crest at the mesio-distal midpoint of the socket. Because flaps were not reflected, the points of the caliper were generally pierced through the soft tissue until they contacted the bone.

After copious irrigation, hydrated particles of 250 to 1,000 μm non–freeze-dried cancellous mineralized human bone allograft§ were lightly compressed into the socket. The socket was filled to the crest of the ridge and a double layer of bioabsorbable collagen wound dressing‖ was placed on top of the graft and secured with sutures in a cross-mattress technique (Fig. 1). If a bony dehiscence or fenestration was evident, a socket repair procedure was performed by placing a bioabsorbable collagen membrane¶ inside the socket before grafting. Flap elevation was not performed at any site. If >50% of any socket wall was absent, the site

† UNC-15 periodontal probe, G. Hartzell & Son, Concord, CA.
‡ Castroviejo caliper, Salvin Dental Specialties, Charlotte, NC.
§ Puros, Zimmer Dental, Warsaw, IN.
‖ Colla Tape, Zimmer Dental.
¶ Socket Repair Membrane, Zimmer Dental.

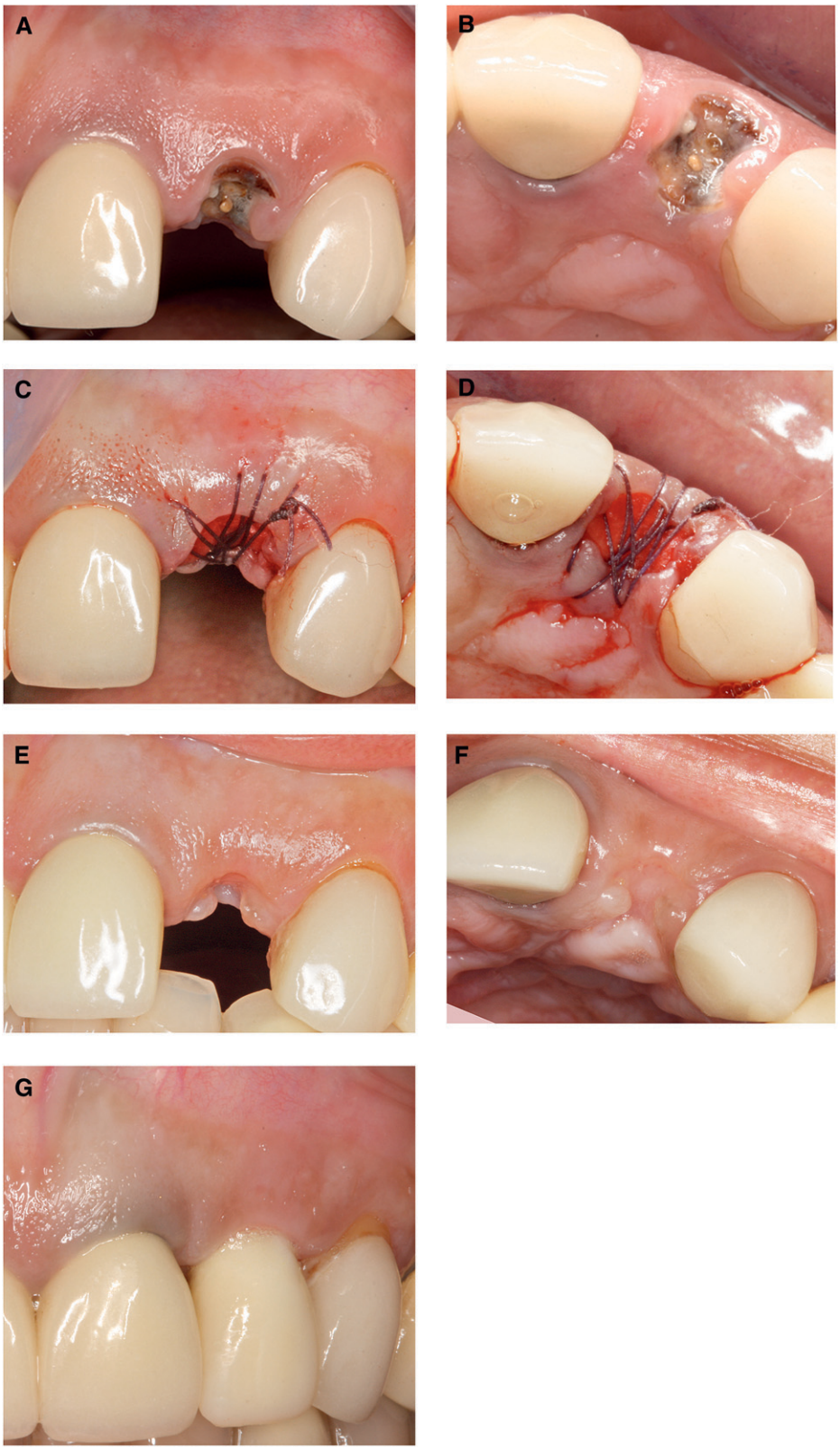

Figure 1.
Composite of clinical photographs from a subject in the early healing group. ***A)*** *Initial presentation of hopeless #10.* ***B)*** *Occlusal view #10.* ***C)*** *Minimally traumatic extraction with allograft and bioabsorbable membrane in place.* ***D)*** *Occlusal view of C.* ***E)*** *Clinical healing after 3 months.* ***F)*** *Occlusal view of E.* ***G)*** *Final restoration #10.*

was excluded from further study and lateral ridge augmentation was performed.

Customary postoperative instructions were provided and all patients were prescribed 100 mg doxycycline twice daily for 10 days and 0.12% chlorhexidine mouthrinse twice daily until the sockets were 100% epithelialized (range, 10 to 21 days). Narcotic analgesics were prescribed for some, but not all subjects, depending on the patient desires and anticipated pain levels. Sutures were removed after 2 weeks. Subjects who presented with signs and symptoms consistent with infection were prescribed 500 mg amoxicillin three times daily for 1 week.

Follow-Up

Subjects from the early healing group were recalled 2 months after extraction for a cone-beam computerized tomographic (CBCT) scan to evaluate the site for implant placement. Subjects from the delayed healing group were recalled for the CBCT 5 months after extraction. Once the CBCT had been obtained for each subject, the implant surgery was scheduled within 3 to 6 weeks. At the time of implant placement, the buccal and lingual ridge height and the ridge width were measured after flap reflection using the aforementioned techniques. A hollow trephine drill with 2-mm internal diameter was used to obtain hard tissue biopsies 8 mm in length from all implant recipient sites. The apical aspect of all biopsies was marked to identify the apico-coronal orientation during histologic analysis and then placed into 10% neutral buffered formalin.

Histologic Processing and Analysis

Biopsies were decalcified, dehydrated, embedded in paraffin, and sectioned apico-coronally

into multiple 4-μm thick sections. Sections were stained with Harris hemotoxylin and counterstained with treosin using routine procedures. The innermost two sections of each biopsy were examined at a minimum of ×20 magnification. Digital images of each section were acquired and imported into a software imaging program[#] to create individual layers of new vital bone, residual graft particles, and connective tissue/non-mineralized tissue (CT). These layers were then imported into image analysis software[**] to determine the percent composition of vital bone, residual graft particles, and CT.

Statistical Analysis

Student *t* tests and Mann-Whitney *U* tests were used to compare the area of newly formed vital bone, residual graft particles, and CT for sites with <4 months of healing (early healing) to those with >5 months healing (delayed healing). For the few subjects who had two sites included for analysis, the differences in new bone formation between the two sites within the same subject ranged from 14% to 36%, a difference that was similar to differences between sites from different subjects. Biopsies were further analyzed for the effect of performing a socket repair procedure using Mann-Whitney *U* tests. Spearman correlations were used to evaluate and relationship between clinical findings and histologic parameters. Findings were considered significant when $P < 0.05$.

RESULTS

Clinical Findings

Thirty-three of the 40 enrolled subjects completed the study. One subject was exited because of the presence of a dehiscence >50% of the buccal plate and six subjects were not compliant with the study protocol. Thirteen males and 20 females with an average age of 57.4 years (range, 39 to 76) completed the study, with no statistically significant differences between groups. Reasons for extraction included non-restorable teeth caused by fracture or decay and failed endodontic rescues. Teeth with apical lesions were excluded.

Thirty-eight sites in 33 subjects were analyzed, with five subjects contributing two sites each. When two sites were present in a single subject, both sites were in the same healing group. In the subjects with 2 sites included for analysis, sites were considered to be statistically independent because analysis determined that the range of percent new bone formation between sites within a single subject were similar to the range of percent new bone formation between sites from different subjects. The 22 sites in the delayed healing group had an average healing time of 27 weeks (±14 days), whereas the 16 sites in the early healing group had an average healing time of 14 weeks (± 11 days). Sites were evenly distributed between the two study groups, with 24 sites in the maxilla and 14 sites in the mandible.

One site in the early healing group and two sites in the delayed healing group presented with signs of potential infection at the 7- to 10-day postoperative follow-up. After administration of 500 mg amoxicillin three times daily for 7 days, all sites healed without further complications. Four sites from the delayed healing group presented with deficient fill of the socket at the 7- to 10-day follow-up and were suspected to have lost some or all of the graft material. Two of these sites were in the same subject. None of the sites in the early healing group presented with signs of lost graft material during healing.

After osteotomy preparation, 34 of the 38 study sites achieved primary implant stability, with the remaining four sites exhibiting insufficient ridge width to provide primary stability. These sites received guided bone regeneration and the implants were placed successfully 6 months later.

Histologic Observations

Light microscopic evaluation showed that 37 out of the 38 histologic specimens had well-defined organized lamellar structures with lacunae absent of osteocytic nuclei, a presentation consistent with residual allograft particles. One specimen demonstrated no residual allograft particles. Most of the lamellar structures were observed intimately surrounded by a haphazard arrangement of collagen-rich, anastomosing mineralized tissue generally lacking organized lamellar structure and canaliculi, an observation consistent with newly formed woven bone. Resorption bays with multinucleated giant cells were identified in some specimens. Scattered osteocytes and blood vessels were consistently observed throughout the woven bone. Specimens presented with variable amounts of loose, fibrous stroma filled with plump, spindle-shaped mesenchymal cells, fibroblasts, adipocytes, and few inflammatory cells. Numerous vascular structures were observed interspersed in this connective tissue matrix (Fig. 2).

Histomorphometric Analysis

The early healing group had a similar percentage of new vital bone formation, residual graft material, and non-bone CT compared to the late healing group (Table 1). There were no statistically significant differences between groups for any of the histologic parameters. When the four sites that were associated with partial or complete loss of the graft during healing were excluded, the delayed healing group had an average of 41.8% (± 19.3%) vital bone; 43.1% (± 12.3%)

[#] Adobe Photoshop Elements 7, Adobe Systems, San Jose, CA.
[**] Image J, National Institutes of Health, Bethesda, MD.

CT bone; and 15.3% (± 12%) residual graft material. No statistically significant difference between groups for any histologic parameter was identified when the seven sites that experienced either postoperative loss of graft material or signs of potential infection were excluded from analysis. In addition, no statistically significant differences were observed when comparing the composition of the biopsy (amount of vital bone, CT, and residual graft material) with maxillary versus mandibular sites, smokers versus nonsmokers, and subjects with controlled type 2 diabetes mellitus versus healthy subjects.

Overall, the percentage of vital bone was negatively correlated with the percentage of CT ($r = -0.827$) and the percentage of residual graft material ($r = -0.763$). The percentage of residual graft material was not correlated with the percentage of CT. The four sites requiring socket repair membranes had significantly higher mean percentage of CT (56.3% ± 6.6%; $P = 0.024$) and significantly lower mean percentage of vital bone (19.3% ± 8.8%; $P = 0.01$) and residual graft material (26.8% ± 1%; $P = 0.035$) compared to all other sites. The four sites that presented with deficient socket fill at the 7- to 10-day follow-up had significantly lower mean percentage of residual graft material ($P = 0.003$) compared to the 30 sites that healed without complications.

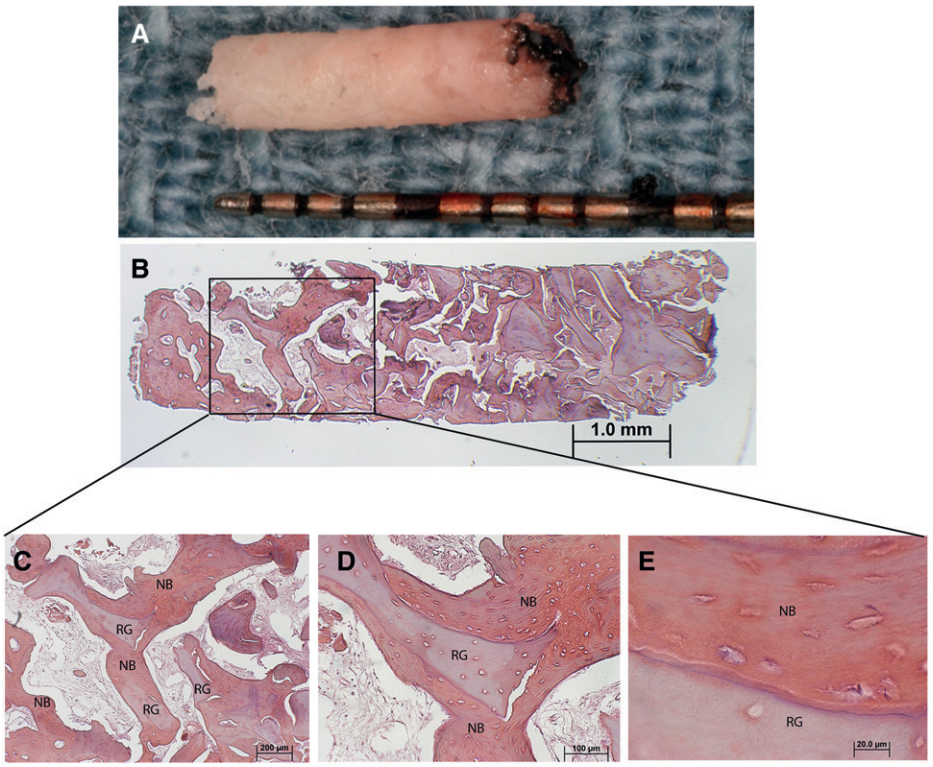

Figure 2.
Composite of one clinical photograph and four histologic slides all stained with hemotoxylin and treosin. ***A)*** *Clinical photograph of core biopsy; ink stains the apical end of the core.* ***B)*** *Magnification ×1 of core biopsy in A.* ***C)*** *Magnification ×4 of rectangle in B showing new bone formation (NB) in intimate contact with residual graft particles (RG).* ***D)*** *Magnification ×10 showing empty lacunae in RG and osteocytes in NB.* ***E)*** *Magnification ×40 showing empty lacuna in RG and osteocytes in NB.*

Dimensional Changes

From a mean initial ridge width of 8.47 mm, the early healing group had an average loss of 1.47 mm in ridge width (17.3%), whereas the delayed healing group initially had a mean width of 9.38 mm and lost an average of 1.43 mm (15.2%) (Table 2). The early healing group lost <1 mm of buccal and lingual ridge height, whereas the delayed healing group lost <1 mm of lingual ridge height and showed a slight average gain in buccal ridge height. No statistically significant or clinically relevant differences in average dimensional changes between the two healing groups were found, nor was there a correlation with biopsy composition. No correlation was found between

Table 1.

Histomorphometric Analysis

	Vital New Bone (% ± SD)	Residual Graft Material (% ± SD)	CT (% ± SD)
Early healing	45.8 ± 22.4	14.6 ± 12.9	39.6 ± 13.0
Delayed healing	45.0 ± 19.8	13.5 ± 12.2	41.3 ± 14.6

No significant differences between groups for any parameter ($P > 0.05$).

Table 2.

Changes in Ridge Dimensions

	Loss of Ridge Width (mm ± SD)	Loss of Buccal Ridge Height (mm ± SD)	Loss of Lingual Ridge Height (mm ± SD)
Early healing	1.47 ± 1.81	0.37 ± 1.46	0.87 ± 1.46
Delayed healing	1.43 ± 1.89	–0.32 ± 2.61*	0.61 ± 1.38

* Negative number indicates gain in buccal ridge height.

buccal plate thickness and changes in ridge height or width. Furthermore, buccal plate thickness and initial ridge width were not correlated with biopsy composition, regardless of healing time.

DISCUSSION

The primary aim of this study is to histologically evaluate new bone formation using a single bone allograft material at two different time points after tooth extraction and socket grafting. All sites examined in this study displayed evidence of new bone formation. No statistically significant difference in the amount of new bone formation was found between sites that healed for an average of 14 weeks compared to those that healed for an average of 27 weeks (45.8% and 45%, respectively). These findings are consistent with studies evaluating new bone formation 9 to 10 months after sinus augmentation using mineralized human bone allograft[12,13] and when used for the purpose of ridge preservation.[12] Our results demonstrating approximately 45% to 46% new vital bone formation, 14% to 15% residual graft particles, and 40% to 41% non-bone CT compare favorably with those of Fotek et al.[13] who reported a range of 27% to 32% vital new bone formation 16 weeks after ridge preservation in 18 sites, with an average of 14% to 15% residual bone graft particles and 53% to 58% non-bone CT. In a case series of seven sites examined 16 to 20 weeks after ridge preservation with mineralized human bone allograft using a similar procedure to that reported in the current study, Wang et al.[14] reported an average of 68% vital bone, 4% residual bone graft, and 38% non-bone CT. In an undisturbed socket, Trombelli et al.[15] reported an average of only 32.36% woven bone formation 6 months after tooth extraction. Together, these studies suggest that ridge preservation techniques using mineralized human bone allograft may promote new bone formation in the healing extraction socket.

A wide variability of new bone growth, residual graft material, and connective tissue has been reported using various graft materials and ridge-preservation techniques.[9] This variability may be affected by multiple factors, such as the disease status of the tooth before extraction, inclusion of both single and multi-rooted teeth within a single study, size of the extraction socket, presence of bony dehiscences or fenestrations, trauma from extraction, damage to the periodontium before extraction, or angulation of the biopsy core relative to the angulation of the former tooth and the resulting healing extraction site. In this study, only single-rooted teeth were included, all teeth were extracted with minimal trauma, sites were thoroughly examined for the presence of bony dehiscences or fenestrations, no teeth were included that had evidence of severe periodontitis, all roots were required to have a minimum of 10 mm radiographic bone support, and all roots had to have an angulation similar to the angulation of the implant to be placed at the site. This ensured adequate depth of socket and angulation of the core trephine to allow biopsies to contain only newly formed bone, as opposed to mature alveolar bone present at the root apex or along the socket wall. In addition, only the innermost aspect of the core biopsies was analyzed, presenting the area farthest from the socket wall. Only one biopsy specimen was observed without any residual graft particles but it also lacked mature lamellar bone indicating that the biopsy was indeed harvested from the previous extraction site that had healed with newly formed woven bone.

Most of the newly formed bone in the current study was woven bone, and the relative lack of newly formed lamellar bone was an interesting finding. We expected minimal lamellar bone in the early healing group, but not in the late healing group where we anticipated a higher degree of lamellar bone formation. In other studies a combination of woven and lamellar bone was often found at 5 to 6 months postgrafting.[14] Iasella et al.[10] reported an average of 54% vital new bone formation 6 months after extraction and socket grafting with mineralized freeze-dried bone, and most of the new bone was woven bone, with less lamellar bone formation. The histologic sections analyzed in our study were taken from the innermost aspect of the core biopsies, the location likely to take the longest to turn over woven bone to lamellar bone.

Several reports on the success of ridge preservation involve elevation of a mucoperiosteal flap for placement of a collagen membrane[16,17] and primary coverage of the extraction site.[8] Although these techniques have proven successful in preventing excessive loss of alveolar dimensions, they may increase postoperative discomfort for the patient and the addition of a membrane often significantly increases the overall cost of the procedure. In addition, elevation of a mucoperiosteal flap can increase the risk of recession on adjacent teeth if an envelope technique is used, scar formation may occur if vertical incisions are made, and loss of keratinized tissue width with reduction of vestibular depth may occur because of the coronal advancement of the mucogingival junction.

The current study avoided mucoperiosteal flap elevation and used sharp-tipped calipers to make ridge measurements. The dimensional measurements found an average loss of only 0.35-mm buccal ridge height and 1.46-mm loss of ridge width. These results are clearly an improvement from the 1.67-mm average loss of mid-buccal height and 3.87-mm loss of ridge width recently reported in a systematic review[18] that evaluated the changes in ridge dimensions after tooth extraction without any attempts at ridge

preservation. The findings of the current study are similar to that reported by Crespi et al.[19] using magnesium-enriched hydroxyapatite, which observed an average loss of 0.48 mm in ridge height after 3 months. Fotek et al.[13] observed an average loss of 1.11 mm in ridge height using mineralized human bone allograft with an acellular dermal matrix and 0.25 mm using mineralized human bone allograft with a polytetrafluoroethylene membrane. These findings are further supported by a systematic review that concluded that minimally traumatic extraction with ridge preservation can predictably preserve preextraction alveolar dimensions.[9] Evaluation of changes in ridge dimension was a secondary aim of the current study, and no stents were used as fixed reference points during dimensional measurements. Thus, we cannot state with certainty that pregrafting and postgrafting measurements were made at exactly the same vertical point on the ridge in each case. This may explain how a few cases showed an increase in ridge width, a result that would not normally be expected when a bone graft is contained within a socket.

Four of the 38 study sites failed to achieve primary implant stability because of insufficient ridge width. One of these sites lost only 1 mm in ridge width; however, the initial ridge width was only 5 mm. Two of the sites were areas that presented with lost graft material within 2 weeks of extraction and ridge preservation and displayed >2 mm loss in ridge width. The final site that failed to achieve primary implant stability presented with a bony dehiscence of the buccal plate at the time of extraction; this site had received a socket repair membrane at the time of ridge preservation because of some deficiency of facial ridge height. Although the buccal ridge height increased by 4 mm at this site after healing, the final ridge width was only 3.5 mm. These findings suggest that a site presenting with narrow initial ridge width, deep bony dehiscence of the buccal socket wall, or exhibiting loss of graft material within 2 weeks of extraction, may require additional augmentation techniques.

CONCLUSIONS

The present study indicates that there are no statistically significant differences in the amount of new bone growth or residual graft particles ≈6 months after extraction and ridge preservation using mineralized human bone allograft compared to that after only ≈3 months of healing. Therefore, waiting an extended period of time after extraction and ridge preservation until implant placement to allow more time for bone formation and graft resorption was not supported by this study. These results may not apply to grafting with other allograft, xenograft, or alloplast materials. Based on the observation of similar amounts of new bone growth, it may be speculated that implants placed 3 months after extraction and ridge preservation with mineralized human bone allograft will have similar success rates to those placed after longer healing periods; however, further studies are recommended to validate this assumption.

ACKNOWLEDGMENTS

The authors thank Sonja A. Bustamante at the University of Texas Health Science Center San Antonio (UTHSCSA) for her help with histologic preparation. They also thank Mr. John D. Schoolfield (UTHSCSA) for his invaluable help with statistical analyses. The current study received no financial support. Dr. Mealey has received research funding and lecture honoraria from Zimmer Dental. All other authors report no conflicts of interest related to this study.

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Submitted May 11, 2010; accepted for publication June 23, 2010.