

Histological Comparison of Healing Extraction Sockets Implanted With Bioactive Glass or Demineralized Freeze-Dried Bone Allograft: A Pilot Study

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Background: Various materials have been used immediately following tooth extraction to fill and/or cover the socket in an attempt to limit or prevent ridge resorption. The purpose of the present pilot study was to establish a reliable model to investigate the effect of various bone graft and bone replacement materials on extraction socket healing. This study also compared healing extraction sockets 6 to 8 months postimplantation of a bioactive glass (BG) or demineralized freeze-dried bone allograft (DFDBA) to an unfilled socket control (C).

Methods: Following tooth extraction, a total of 30 sockets in 19 patients were randomly divided into 3 treatment groups: 10 sockets received BG, 10 sockets DFDBA, and 10 sockets served as unfilled controls. Primary coverage was achieved by flap advancement over each socket. Six to 8 months postextraction at time of implant placement, histological cores of the treatment sites were obtained. These cores were processed, undecalcified sections prepared and stained with Stevenel blue/van Gieson's picric fuchsin, and histomorphometrically analyzed. Vital bone, connective tissue and marrow, and residual graft particles were reported as a percentage of the total core.

Results: A model system was described in humans and used to evaluate the healing response in the 3 treatment groups. Results concluded that mean vital bone present was 59.5% for BG-, 34.7% for DFDBA-, and 32.4% for C-treated sites. These differences were not statistically significant. However, the residual implant material was significantly higher in DFDBA-treated (13.5%) versus BG-treated sockets (5.5%).

Conclusions: Although the differences in percent vital bone were not statistically significant among the 3 treatment groups in this pilot study, BG material was observed to act as an osteoconductive material which had a positive effect on socket healing at 6 to 8 months postextraction. Further research following implant placement in treated and control sockets is warranted to determine if bone implant contact is improved in BG-filled versus unfilled sockets. *J Periodontol* 2002;73:94-102.

KEY WORDS

Alveolar bone loss/prevention and control; tooth extraction; wound healing; grafts, bone; glass, biologically active; follow-up studies.

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Root form implants have demonstrated a high degree of successful osseointegration in both fully and partially edentulous patients.¹⁻⁴ However, in order for these implants to be placed in a favorable prosthetic position for subsequent restoration, it is often necessary to augment existing alveolar bone.

Numerous biocompatible materials have been used in an attempt to correct hard tissue ridge deformities and thus allow ideal implant placement. These materials include intra- and extraoral autogenous bone; demineralized freeze-dried bone allografts (DFDBA); and various alloplasts, xenografts, bone substitutes, and membrane barriers.⁵⁻⁸ Success rates of implants placed in this regenerated bone are comparable to the success rates of implants in natural bone.⁹⁻¹¹ However, increased patient morbidity, cost, and time accompany these attempts to regenerate lost soft and hard tissues. In most cases a 6- to 12-month healing period is necessary following augmentation surgery before implants can be placed.

Some of the main causes of alveolar bone deformities which require augmentation procedures are the bone resorption and healing patterns that take place follow-

ing tooth extraction.^{12,13} Controlled clinical studies have documented an average of 4.0 to 4.5 mm of horizontal bone resorption following routine atraumatic tooth extraction.^{14,15} Other studies have documented significant dimensional changes in the surrounding alveolar bone following extraction procedures.¹⁶⁻¹⁸ One study documented that 31 of 34 (91%) patients who were partially edentulous in the maxillary anterior area had some ridge defect.¹⁹

In an attempt to preserve alveolar bone and avoid the necessity of ridge augmentation prior to implant placement, various materials have been used immediately following tooth extraction to fill and/or cover the socket. While many of these studies showed positive clinical results, histological evaluation of grafted sockets has shown mixed results.

Two separate histological studies reported positive socket healing responses with alloplasts²⁰ and a xenograft²¹ while others have shown poor results with DFDBA, bovine bone, and even autogenous bone when implanted into sockets following tooth extraction.^{22,23} In fact, one study concluded that the latter 3 graft materials interfered with the normal socket healing and therefore should not be used for socket treatment.²² However, many variables, including type and size of defect, type of graft, implant or barrier used, time of healing response, flap closure and lack of controls, as well as differences in host response, to name a few, make study comparisons and conclusions difficult. Moreover, these studies were, for the most part, case reports which are of interest but do not provide a reliable understanding of factors that affect the quality and quantity of bone and timing for the optimal implant placement.

It is therefore important to minimize as many variables as possible in order to better evaluate the effects of materials and techniques utilized to preserve alveolar bone following tooth extraction in a site diagnosed to receive a root form implant. The purpose of the present study was to establish a reliable model to investigate the effect of various bone graft and bone replacement materials on the healing of an extraction socket. This pilot study was designed to histologically evaluate the healing of extraction sockets 6 to 8 months following implantation of a bioactive glass or demineralized freeze-dried bone allograft compared to no graft at all.

MATERIALS AND METHODS

Study Population

Thirty (30) teeth scheduled for extraction for periodontal or prosthetic reasons were selected in 19 patients (12 males; 7 females; age range 35 to 77 years) who presented to the Ashman Department of Implant Dentistry at New York University Kraser Dental Center. The diagnosis of these teeth for extraction

was confirmed by 2 separate instructors who were not part of the present study. All patients met the previously established physical and psychological criteria for implant treatment in the Department of Implant Dentistry. In addition, patients did not have any medical conditions and were not taking any medications that were associated with a compromised bone healing response (i.e., diabetes, autoimmune dysfunction, prolonged cortisone therapy, or chemotherapy). All patients were non-smokers or previous smokers who had not smoked for at least 6 months. All patients had no known allergies to tetracycline and had not received any antibiotic over the previous 6 months. Patients were given an explanation of the nature of the study and, after expressing a wish to participate, they signed a written consent form prior to their participation. The informed consent and instruction to patient forms as well as the study protocol were approved by the University Committee on Activities Involving Human Subjects.

Participating patients were told that if they decided to discontinue their participation in the study at any time they could continue being treated at New York University Dental Center as a regular clinic patient.

Measurements

Prior to extraction radiographs, impressions, and diagnostic casts were taken. A template was then fabricated on the study model including at least one tooth anterior or posterior to the hopeless tooth. A light cured resin material[§] was used to fabricate the template. The crown of the hopeless tooth was cut off on the study model and a guide hole was drilled with a 3 × 10 drill^{||} through the template directly above the outline of the root on the model. A metal ring (Fig. 1) was placed in the hole and resin added around the ring to stabilize its position. At time of implant surgery (6 to 8 months following extraction) the template was again positioned in order to take a histological core from the identical site.

Surgical Protocol

Following administration of local anesthesia, crestal and intrasulcular incisions were made to expose the involved roots and alveolar crest. Buccal and lingual flaps were raised to adequately view the sockets and allow sufficient flap release to obtain primary closure. After extraction of the tooth the sockets were debrided, measured, and decorticated with a 1/2 round bur under copious irrigation (Fig. 2).

Following tooth extraction, those sockets with ≤2 mm of buccal plate bone loss were included in the study. Thus each of the sockets treated had a 4-wall configuration. Treatment selection was then made ran-

§ Triad, Dentsply International, York, PA.

|| ITI Institute Straumann, Waldenburg, Switzerland.



Figure 1.
The resin template constructed on the study model, containing the metal ring positioned over the central area of the extraction socket.

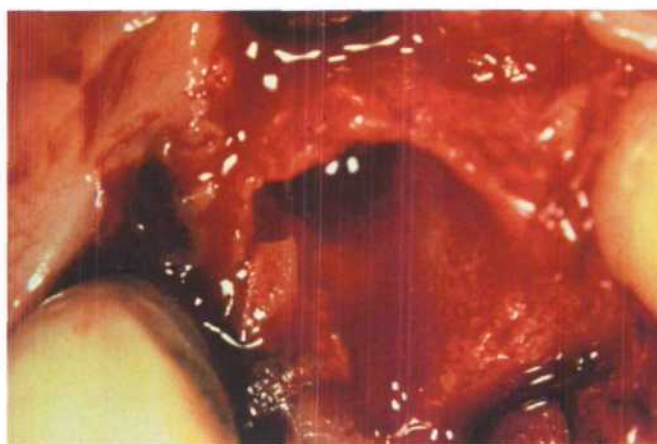


Figure 2.
The debrided and decorticated socket with 4 remaining walls including the buccal plate.

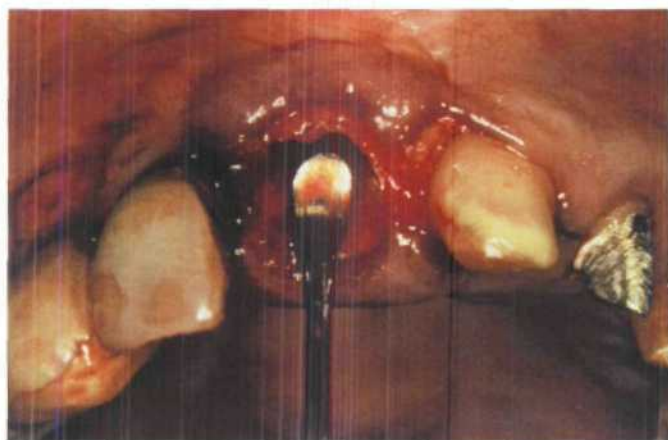


Figure 3.
Bioactive glass being compressed into the socket.



Figure 4.
The flap is sutured to obtain complete socket coverage.

domly from sealed envelopes prepared by a statistician. Of the 30 sockets treated, 10 sockets received a bioactive glass material (BG), 10 sockets received demineralized freeze-dried bone allograft (DFDBA), and 10 sockets (C) served as unfilled controls. Treatment consisted of socket debridement alone (C), debridement followed by implantation of BG, or debridement followed by implantation of DFDBA. The freeze-dried bone allograft was obtained from an accredited commercial bone bank[†] and consisted of cortical bone of 250 to 500 μ sized particles. The bioactive glass[#] had a limited particle size of 300 to 355 μ m. Both materials were hydrated with sterile saline at least 15 minutes prior to insertion in the socket and then packed into the socket (Fig. 3). Flaps were then sutured with 4-0 silk utilizing interrupted and vertical mattress sutures. In all cases tension-free primary closure was achieved and the temporary prosthesis was relieved

prior to insertion (Fig. 4). Patients were placed on doxycycline 100 mg beginning at least 1 hour prior to surgery and continuing for 13 days following surgery. Patients were also prescribed 0.12% chlorhexidine rinses^{**} twice a day beginning the day after surgery and continuing for 30 days following surgery. Sutures were removed 7 to 14 days following surgery.

Six to 8 months following extraction socket surgery, an implant of appropriate size was placed in the healed socket. At time of implant site preparation the template was again placed and a core of bone 2.0 mm \times 7.0 mm long (Figs. 5 and 6) was obtained with the same size trephine used previously. The cores were coded and sent to the Hard Tissue Research Laboratory at the University of Oklahoma College of Dentistry. The pro-

[†] University of Miami Bone Bank, Miami School of Medicine, Miami, FL.

[#] Biogran, Orthovita, Malvern, PA.

^{**} Peridex, Procter & Gamble, Cincinnati, OH.



Figure 5.

At time of implant placement (6 to 8 months following socket treatment) the template is again positioned and the trephine placed through the metal ring to obtain a core of bone from the healing socket.



Figure 6.

The 2.0 x 7.0 mm core of bone from the healing socket.

cessing and histomorphometric measurements were performed by an investigator who had no knowledge of the treatment rendered. The cores were stained with Stevenel blue/van Gieson's picric fuchsin and histomorphometrically analyzed for bone and soft tissue. Processing and analysis of the specimens using a non-decalcified technique has been described.²⁴ Values



Figure 7.

Preextraction radiograph of the hopeless maxillary right central incisor.

were then reported using a grid overlay for total bone material, percent vital bone, percent connective tissue (% CT), and percent residual implant materials (% RIM).

RESULTS

All sites healed uneventfully (Figs. 7 and 8). Mean vital bone measurements for BG, C, and DFDBA groups were as follows: 59.5%, 32.4%, and 34.7%, respectively (Figs. 9, 10, and 11) (Table 1). The range of vital bone was 22 to 88% for BG-, 17 to 53.1% for C-, and 23 to 48.1% for DFDBA-treated sites (Table 2). Connective tissue percentages averaged 35.3% for BG, 67.0% for C, and 51.6% for DFDBA. The BG-treated sockets showed 5.5% residual implant material while the DFDBA-treated sockets had 13.5% residual implant material. All bone present in control and bioactive glass-treated sockets was reported as 100% vital. Bone present at the demineralized freeze-dried bone treated sites was 100% vital in 7 sockets and 50%, 74%, and 80% vital in 3 remaining sockets. New bone and osteoid was observed surrounding the remaining BG particles (Fig. 12). New bone was not only seen at the periphery, but also in the internal pores of the BG particles (Fig. 13). In sections containing remaining DFDBA particles, non-vital bone was observed in close proximity to reossifying areas (Fig. 14). This pattern of reossification was observed to varying degrees with the remaining non-vital DFDBA particles.

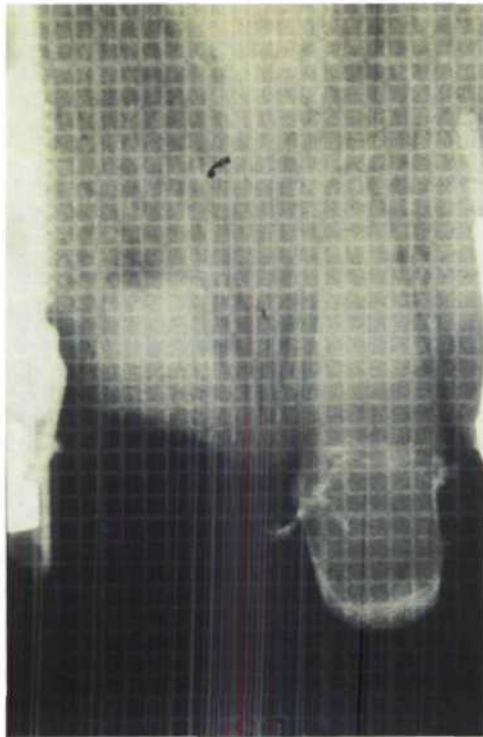


Figure 8.
Six months postsurgical radiograph of a socket filled with bioactive glass.

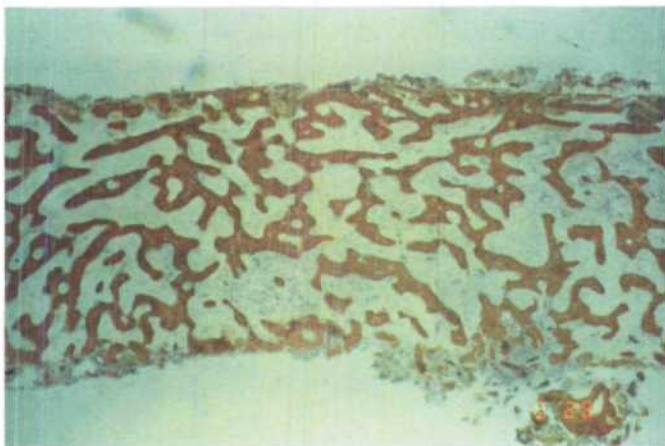


Figure 10.
A low power histological section of 6 1/2-month postextraction core of a BG-treated socket. Vital bone measured 50%. (Original magnification $\times 2.5$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

Statistical Analysis

Thirty (30) sites in 19 different patients were randomized to each of 3 treatments. The patients included 7 females and 12 males with a mean age of 54.9 years (SD = 11.9; range: 35 to 77). At the 6- to 8-month followup, data were collected for the percentage CT, the percentage RIM, and the percentage vital bone. Using the method of generalized estimating equations (GEE)



Figure 9.
Low power histological section of a core of bone obtained 8 months postgrafting with DFDBA. Vital bone measured 48%. (Original magnification $\times 2.5$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

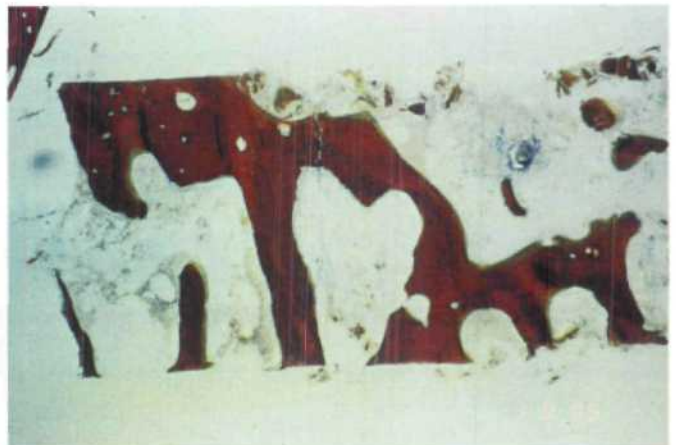


Figure 11.
A lower power histological section of an 8-month postextraction core of an ungrafted (control) socket. Vital bone measured 34%. (Original magnification $\times 2.5$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

with an exchangeable working correlation, the mean percent CT, mean percent RIM, and mean percent vital bone were compared among the 3 treatment types used. Adjusted means with corresponding confidence intervals were calculated using GEE and are given in Table 1. The mean percent CT varied by treatment type. In particular, treatment type B yielded a significantly lower percent CT compared to either type C or type D. The mean percent RIM varied by treatment type with all treatments differing significantly from each other. Although the percent vital bone for treatment types C and D appeared to be significantly different from the percent vital bone for treatment type B, the overall differences failed to achieve statistical significance ($P = 0.074$).

Table 1.
Adjusted Means by Treatment Type

Parameter	Type	Adjusted Mean (%)	95% Confidence Interval	P
% CT	B	35.3	(21.2, 49.5)	0.006
	C	97.0	(60.9, 73.2)	
	D	51.6	(43.5, 59.6)	
% RIM	B	5.5	(4.3, 6.7)	0.001
	C	0.0	(0.0)	
	D	13.5	(9.9, 17.2)	
% vital bone	B	59.5	(45.2, 73.7)	0.074
	C	32.4	(26.3, 38.4)	
	D	34.7	(26.8, 42.5)	

B = BG; C = Control; D = DFDBA.

DISCUSSION

The first goal of the present study was to establish a human model system to evaluate the healing response of various materials used to promote bone formation. It is recognized that differences in the host healing response make identical comparisons in humans impossible. However, the size and type of bone defects following tooth extraction often present similar healing environments. To further standardize our model we chose to include only those sockets with 4 remaining walls. In addition, sockets were required to have 2 mm or less of the buccal plate missing following extraction. This measurement was made in comparison with the buccal bone on the adjacent tooth or teeth at time of tooth extraction.

Since the socket healing response is mediated by the surrounding bony walls, this requirement served to standardize the model system.²⁵ Several measures were taken to ensure that the histological specimen would consist of the healing rather than the native bone. First, the template and metal ring allowed exact positioning of the trephine over the healing site. Second, a narrow trephine (2 mm internal diameter) was used and only a 7 mm core was obtained. The coronal reference point for this core was the distance between the template and buccal crest immediately following tooth extraction.

All specimens were stained with Stevenel blue/van Gieson's picric fuchsin.²⁴ This allowed a more accurate calculation of vital bone. Moreover, since the investigator performing the histomorphometric analysis had no knowledge of what procedure was performed in the socket, measurement bias was controlled. Lastly and most importantly in our human model system was the fact that a root form implant was placed into the same area from which the core was obtained. Since the implant was of larger diam-

Table 2.
Data of 30 Sockets Treated With Either Bioactive Glass, Ungrafted Control, or Demineralized Freeze-Dried Bone Allografts

Patient	Type	Gen-der	Age	Tooth	Months	% CT	% RIM	% Vital Bone
S1	B	M	66	25	8	25	3	72
S1	B	M	66	27	8	27	3	70
W	B	M	62	5	8	44	3	53
L1	B	F	61	23	6	72	6	22
B	B	M	60	8	6	28	5	67
L1	B	F	61	26	6	61	4	35
S2	B	M	46	18	6	43	7	50
M	B	F	50	20	6	11	7	82
K	B	F	47	22	8	10	2	88
F	B	F	77	8	6	66	6	28
S1	C	M	66	22	8	60	0	40
P1	C	F	55	11	6	64	0	36
L1	C	F	61	24	6	78	0	22
Y	C	M	50	15	6.5	70	0	30
L2	C	M	51	28	6	60	0	40
K	C	F	47	27	8	66	0	34
M	C	F	56	7	6	65	0	35
P2	C	F	67	20	6.5	75	0	25
B	C	F	75	4	6	83	0	17
S3	C	M	36	10	8	46.9	0	53.1
S1	D	M	66	23	8	60	8	32
S1	D	M	66	21	8	60	10	30
C	D	M	35	8	6	44	10	46
G	D	M	60	5	6	54	16	30
G	D	M	70	12	6	40	18	42
L3	D	M	47	23	6	41	17	42
B	D	M	60	9	6	42	8	50
H1	D	F	65	18	6	44.4	26	29.6
H2	D	M	42	18	8	43.2	8.7	48.1
L4	D	M	43	15	6	60	17	23

B = BG; C = Control; D = DFDBA.

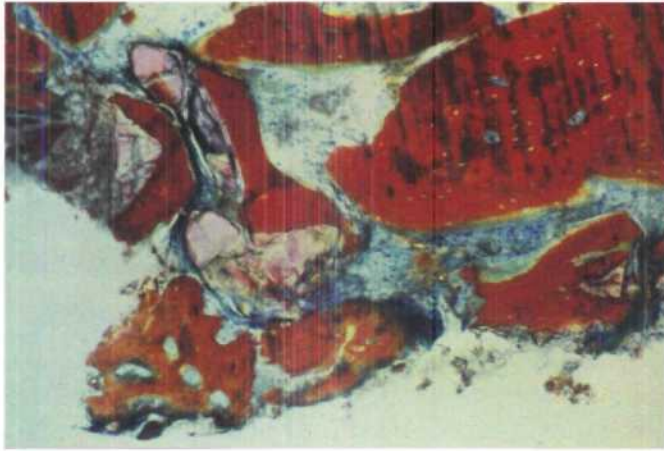


Figure 12.

A 6-month medium power view of remaining BG particles surrounded by mature vital bone and osteoid (green). (Original magnification $\times 10$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

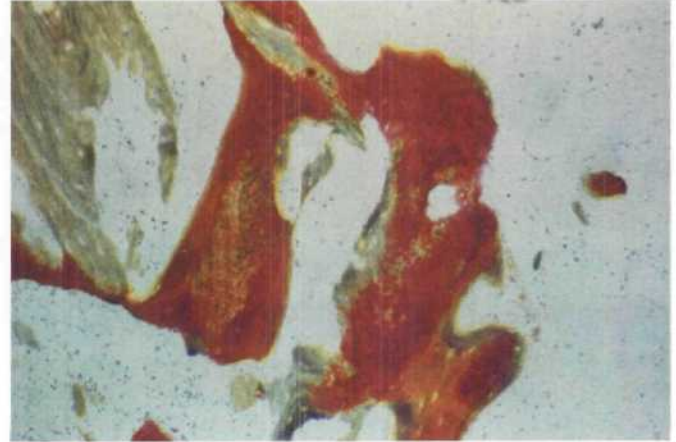


Figure 14.

A medium power histological specimen of DFDBA particles undergoing reossification. Foci of the ossifying area is seen with newly forming osteoid (green) in close proximity. (Original magnification $\times 20$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

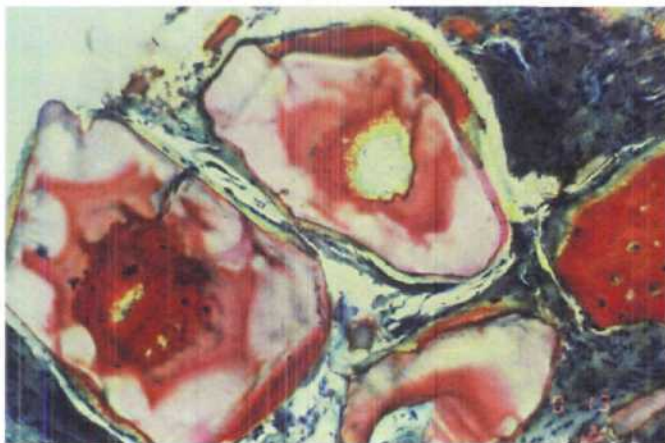


Figure 13.

A higher power histological specimen of the BG particles with areas of new bone forming on the periphery and within the pores of the particles. (Original magnification $\times 25$; Stevenel's Blue/van Gieson's picric fuchsin stain.)

eter and length that the 2.0 mm by 7.0 mm core, this avoided any additional insult to the patient.

The second goal of the study was to compare socket healing of two implanted materials, demineralized freeze-dried bone (DFDBA) and bioactive glass (BG) of limited particle size, with healing of an ungrafted socket (control). Although each of the 3 groups contained only 10 sockets, healing trends were obvious. The BG-treated sockets showed more vital bone (59.5%) at 6 to 8 months postextraction than either the DFDBA-treated sockets (34.7%) or the C sockets (32.4%). Moreover the amount of residual implanted material (RIM) was higher with DFDBA- (13.5%) than with BG-treated (5.5%) sockets.

Although the percent vital bone of the BG-treated sites was greater than either the DFDBA- or control-treated sites, this difference was not statistically significant. The small sample size in this study contributed to this statistical finding. However, the clinician may find this difference in healing response clinically relevant. While we did not calculate the differences in vital bone in the coronal versus apical part of our cores as was done by Artzi et al,²¹ the overall average vital bone in our BG cores at 6 to 8 months postextraction compared favorably with the cancellous porous bovine bone mineral (PBBM)-treated sockets at 9 months postextraction (average 46.3%) as they reported. Moreover, the residual volume of PBBM (approximately 30%) was greater than that of the remaining BG (5.5%) found in the present study. This difference may be attributed to a more rapid absorption of BG particles.

The results seen in the present study, which demonstrate more bone at 6 to 8 months in the BG-treated sockets than the ungrafted sockets, may be attributed to the osseoconductive nature of the BG particles. A direct chemical bond by BG to bone has been shown in previous studies.²⁶ Moreover, the absorbable BG material has been shown to be biocompatible and non-toxic.

The bioactive glass particles have been shown to become coated by a calcium-phosphate (Ca-P) layer in vivo.²⁶ This Ca-P rich layer is equivalent to the mineral phase of bone and is responsible for the osteoconductive properties. The particles themselves are eroded internally by phagocytic cells which absorb silica rich contents of the particles to further expose the Ca-P rich layer to interstitial fluids. New bone then forms within and external to the particles. Remodel-

ing of the particles is accompanied by replacement with bone tissue (Fig. 13).

Furusawa and Mizunuma²⁷ utilized bioactive glass in the repair of surgically created bony defects in the rat mandible and found osteoconductive bone growth around the particles by 4 weeks. Cancian et al.²⁸ compared bioactive glass, dense hydroxyapatite (HA), and an unfilled control to study the healing of surgically created cavities in the angle region of the mandible in 4 adult monkeys. At 180 days postsurgery, no bone formation was observed in the empty cavity, total bone repair of the bone defect in the bioglass-treated sites, and no bone but rather particles encapsulated by connective tissue in the HA-treated sites. Moreover, at the time of biopsy (6 to 8 months) almost all of the bioglass particles were absorbed and replaced by newly formed bone.

Although more vital bone was evident in our study in the BG-treated sockets compared to both the DFDBA-treated and ungrafted sockets, the critical question remains as to whether this bone can support an implant. Schepers et al.²⁹ studied the efficacy of using bioactive glass in the treatment of bone defects prior to implant placement. Defects were created in the mandible of 6 beagle dogs by removing the intra-alveolar septa. One side was filled with BG particles and the other side was left empty. After 4 months of healing, 3 implants 10 mm in length and 3.3 mm in diameter were placed in the BG-treated (test) area and untreated (control) areas. In 3 dogs the unloaded implants were biopsied at 3 months while in the other 3 dogs the implants were functionally loaded with fixed partial prosthesis for 7 weeks before sacrifice. Implants placed in BG-treated sites showed 52.1% more interfacial bone than those in the control sites. At a distance of 3 mm from the implant surface 134.7% more bone tissue was found in the test compared to the control sites. Thus the authors concluded that not only did BG not interfere with implant healing but actually improved the quantity of bone at the implant/bone interface. The healing response of BG-treated sockets in the above study as well as in the present paper differs from that reported by Becker et al. utilizing DFDBA, mineralized freeze-dried bone allograft (MFDBA), and autogenous bone for socket fill.²² Becker et al. utilized 4- to 13-month postsurgical biopsies and reported that the "over-riding histologic characteristic of sites implanted with DFDBA or MFDBA was retention of non-vital graft particles within fibrous connective tissue." They concluded that autologous intraoral bone chips and the allografts ". . . may serve as biologic fillers." In another study, Becker et al.²³ placed microscrews into extraction sockets treated with xenogenic bovine bone, DFDBA, or intraoral autologous bone. Biopsies from the bovine bone and DFDBA implanted sockets revealed dead particles entrapped within dense connective tissue. They concluded that neither xenogenic bovine bone, DFDBA,

nor autogenous bone "contribute to bone to micro screw contact and are not recommended for enhancement of vital bone to implant contacts."²³

Although intrastudy comparisons of materials must be approached cautiously, differences in the properties of BG, a different model system, complete soft tissue coverage of the materials, and a more standard healing time may account for the improved results seen in the present study.

Lastly, it is of interest to note that in the present study DFDBA-treated sockets presented similar levels of vital bone 6 to 8 months postgrafting compared to the ungrafted controls. The factors mentioned previously regarding the surgical technique and healing times employed may again account for the better response obtained here with DFDBA than in previous studies.^{22,23} A 1999 histomorphometric study comparing unfilled extraction sockets with those filled with DFDBA and covered with expanded polytetrafluoroethylene membranes at 8 to 23 months postgrafting found similar levels of trabecular bone in both sockets.³⁰ However, in the present study, since the vital bone values were only slightly higher than that found with the ungrafted sockets, DFDBA would have questionable value when utilized in extraction socket treatment.

CONCLUSIONS

This pilot study attempted to develop a reliable human model to histologically evaluate various materials utilized for treatment of extraction sockets. This paper describes the materials and technique utilized to accomplish that goal. In a test of 30 extraction sockets treated with a bioactive glass of limited particle size (BG), demineralized freeze-dried bone (DFDBA), or debridement only (C), BG-treated sockets yielded more vital bone (59.5%) in 6- to 8-month healing biopsies than either DFDBA or C. Similar levels of vital bone were observed with DFDBA- (34.7%) and C-treated sockets (32.4%). These differences, however, were not statistically significant. Further studies with greater numbers of sites are indicated to determine if the increased vital bone found in BG-treated sockets translates into more implant/bone contact in humans as has been shown to be the case in an animal model. However, the present study demonstrated that BG is an osteoconductive bone replacement material and, although the difference in vital bone was not statistically significant among the 3 treatment groups, BG has a positive effect on socket healing at 6 to 8 months postextraction.

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