Porous Bovine Bone Mineral in Healing of Human Extraction Sockets. Part 1: Histomorphometric Evaluations at 9 Months

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Background: Extraction socket wound healing is characterized by resorption of the alveolar bone at the extraction site. This produces a decrease in ridge volume, deformations of ridge contours, and, thus, difficulties in delayed placement of root-form implants in an ideal position. Cancellous porous bovine bone mineral (PBBM) applied to fresh extraction sockets has recently been proposed to minimize the reduction in ridge volume. The aim of this study was to investigate the influence of PBBM grafted particles on the histopathologic pattern of the intrasocket regenerated bone and to evaluate histomorphometrically the healed PBBM grafted extraction socket site at 9 months' post-extraction.

Methods: PBBM particles (250 to 1,000 µ in size) were grafted in 15 fresh human extraction sockets in 15 patients. Socket wall bone height was measured from the crestal ridge level before the mineral particles were inserted. Primary soft tissue closure was performed to protect the grafted particles via a pediculated split palatal flap. At 9 months, socket bone walls were remeasured and cylinder bone samples of the previously PBBM-grafted sites were obtained. Decalcified specimens were sectioned at a cross-horizontal plane and stained with hematoxylin and eosin for histopathologic and histomorphologic examination. Tissue area percentage of bone, PBBM, and connective tissue (CT) was calculated for each specimen from the crestal to the apical region and changes in values compared.

Results: Average clinical overall bone fill of the augmented socket sites was 82.3%. Histologically, PBBM particles were observed in all specimens. Newly formed bone was characterized by abundance of cellular woven-type bone in the coronal area, while lamellar arrangements could be identified only in the more apical region. New osseous tissue adhered to the PBBM. Histomorphometric measurements showed an increase of mean bone tissue area along the histological sections from 15.9% in the coronal part to 63.9% apically (average 46.3%). CT fraction decreased from 52.4% to 9.5% (average 22.9%) from the crestal to the apical region. PBBM area fraction varied from 26.4% to 35.1% (average 30.8%). Statistical analysis of the comparison between areas of bone, CT, and PBBM was performed in different points along the coronal-apical axis. Differences were significant ($P <0.01$) at the most crestal, middle, and apical section cut areas, but not at the cervical section cuts. Bone area fraction increased in the apical direction as much as CT correlatively decreased. Unlike CT and bone, PBBM retained constant relative volume (approximately 30%), regardless of the depth of the specimen cores.

Conclusions: PBBM particles are an appropriate biocompatible bone derivative in fresh extraction sockets for ridge preservation. The resorbability of this xenograft could not be recognized in a 9-month period. Further investigation is needed to clarify the resorptive mechanisms of PBBM. *J Periodontol 2000;71:1015-1023.*

KEY WORDS

Bone regeneration; alveolar bone loss/prevention and control; alveolar ridge augmentation; grafts, bone; dental implantation; tooth extraction/therapy; wound healing.

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Bone regeneration has become a requirement in osseous deficiencies associated with root-form fixture implantation procedures. However, alveolar ridge restoration aimed at accommodating osseointegrated implants cannot always be achieved. Prerequisites to enhance predictability of augmentation procedures include existing outlined bone topography, meticulous preservation of the biology of the wound healing process, and the quality of osseous graft materials.

Within a few weeks after extraction, the edentulous alveolar ridge resorbs and diminishes in size, especially in the anterior maxilla.[1-6] Dimensions and morphology of the alveolar ridge, especially of the labial thin plate, result in rapid resorption and adaptation of the non-stimulated alveoli. Consequently, the resorbed ridge cannot properly accommodate root-form implants.[7] Esthetic and functional implant restoration requires proper placement of oral implants. Therefore, it is essential to preserve the original contours of the edentulous ridge.

The alveolar extraction socket site has a specific pattern of wound healing cascades.[6,8-10] Generally, to preserve the height and width of the alveolar bone for future implant insertion, guided tissue regeneration (GTR) procedures are utilized.[11-13] Other techniques, such as grafting autogenous bone and bone substitute materials, i.e., allogenic, xenogenic, or alloplastic, have also been used for this purpose. Among these, the most popular are non-resorbable hydroxyapatite,[14,15] demineralized freeze-dried bone,[16,17] calcium phosphate,[18,19] HTR-polymeric composite,[20,21] coraline calcium carbonate,[22] and recombinant human osteogenic protein.[23] All are clinically effective but no histopathological-histometric analysis has been performed to evaluate the beneficial contribution at sites receiving these grafted materials.

Cancellous porous bovine bone mineral (PBBM)[‡] has been used in various types of bone deficiencies[24-35] with clinically successful results. The efficacy of PBBM particles has been tested in extraction socket sites with[35-37] or without[38-40] an occlusive GTR membrane. Controversy regarding these encouraging clinical results arose when the augmented/regenerated site was inspected histologically.[39,41-44] When compared with autologous and allogenic grafts, the advantage of this xenograft was questioned.[39] It has been claimed that PBBM does not present either osteoinductive or osteoconductive qualities, based on animal[41-43] and human[39] studies. However, others[28,45-53] report that PBBM is highly osteoconductive, and an increase in the initial rate of bone formation was observed.

PBBM has been examined histologically and histomorphometrically at augmented sinus floor sites.[31-33,45] The material was well tolerated by the host and mingled with the newly formed bone around osseointegrated titanium fixtures. However, while PBBM showed promising results and increasing percentage of vital bone formation compared to other bone derivatives,[32] autologous bone grafts still showed superiority in all biologic parameters.[32,33] When this material was tested in a critical-sized defect,[30,54] osteoconductivity was evident. In a histologic observation in a human periodontal defect, PBBM stimulated new bone and cementum formation with or without the use of GTR procedures.[55] Histomorphometrically, PBBM was evaluated only in subantral floor elevation procedures,[31-33,45] or in a critical-sized defect in animals.[30,54]

The aim of this study was to histopathologically and histomorphometrically investigate the healing of fresh extraction sockets where PBBM was used as a filler material in ridge preservation procedures. This material was applied in fresh socket sites with a partial deteriorated periodontal situation without overlying support of osteopromotive GTR barriers. Socket wall bone height from the crestal ridge level was measured before PBBM insertion and at 9 months. Cylindrical tissue core samples from the augmented socket sites were harvested for histopathological and histomorphometrical examination.

MATERIALS AND METHODS

The study comprised 15 healthy patients (9 females, 6 males), with no systemic disorders, ranging in age from 23 to 64 years. All procedures were explained and patients signed consent forms. The Ethics Committee of Tel Aviv University approved the study protocol. Maxillary single-root tooth/teeth extraction(s) (incisors, canines, and/or premolars) was scheduled, followed by restoration with titanium fixture implants at a later stage. Teeth with ongoing pathoses, i.e., periapical radiograph radiolucency and/or periodontal or periapical abscess and an alveolus with severe ridge resorption of 50% of the socket depth or more, were excluded. Although a reduced periodontium was evident, only 3- or 4-wall sockets were included in the study.

Labial and palatal local infiltration by lidocaine with 1:100,000 epinephrine was used as the local anesthetic. A conservative mucoperiosteal flap was raised buccally; a split flap was made in the palate according to the pediculated split palatal flap design.[56,57] Following careful tooth/teeth extraction(s), the bony plates, i.e., mesial, distal, buccal, and lingual, were measured. Each socket wall height was measured from the neighboring crestal ridge level with a periodontal probe. In 12 out of 15 cases, buccal plate resorption of 2 to 12 mm (average 5.64 mm) compared to the neighboring walls was measured. In case 5, distal and palatal resorption, 3 mm and 6 mm, respectively, was

‡ BioOss, Geistlich Biomaterials, Wolhusen, Switzerland.

noted. In case 15, a 4 mm palatal resorption was observed in addition to the lingual resorption. In cases 1, 6, and 7, no height differences were observed between the 4 walls (Table 1). Cancellous PBBM particles (250 to 1,000 µ in size) were grafted in the extraction socket (Figs. 1A and B). Care was taken to maintain equal proportions of particle quantities in the socket per volume unit by applying minimal compression to allow passive fill. Socket fill procedures were observed by 2 experienced clinicians (ZA and HT). No osteopromotive regenerative barrier was used. Primary soft tissue closure was conducted via a pediculated split palatal flap to protect the grafted particles (Fig. 1C). Postoperative systemic antibiotics of 500 mg amoxicillin[§] (TID) for 1 week and 275 mg naproxen[‖] (2 tablets, initial dose; thereafter 1 tablet every 6 to 8 hours as needed) for analgesia were prescribed. As an antiseptic solution, 0.2% chlorhexidine mouthwash was used for 45 seconds twice daily for 2 weeks. Sutures were removed after 10 to 14 days. Soft tissue healing over the PBBM grafted socket site was immaculate (Fig. 1D).

Table 1.

Measurement of Socket Bony Walls Before Grafting and at 9 Months

Case	Tooth*	Bony Wall Resorption	Bone Fill at 9 Months†
1	#13 (6)	NR	NR
2	#12 (7)	2 mm-buccal	2 mm
3	#11 (8)	3 mm-buccal	3 mm
4	#21 (9)	3 mm-buccal	3 mm
5	#25 (13)	3 mm-distal 6 mm-palatal	2 mm 5 mm
6	#25 (13)	NR	NR
7	#14 (5)	NR	NR
8	#24 (12)	12 mm-buccal	11 mm
9	#21 (9)	8 mm-buccal	5 mm
10	#11 (8)	10 mm-buccal	8 mm
11	#12 (7)	8 mm-buccal	6 mm
12	#15 (4)	5 mm-buccal	5 mm
13	#11(8)	7 mm-buccal	6 mm
14	#21 (9)	6 mm-buccal	5 mm
15	#11 (8)	2 mm-buccal 4 mm-palatal	2 mm 2 mm

* American classification system is in parentheses.
† Overall bone fill–82.3%.
NR = no resorption.

Radiographically, the mineral xenograft was observed constantly during follow-up (Fig. 1E). The particles filled the socket site and were dominant by their clear radiopacity. At 9 months, extraction sites were re-entered surgically for implant osteotomy (Fig. 1F). Socket bone fill and the peripheral level of the alveolar bony walls were remeasured. Osteotomy for implant insertion was performed in an axial coronal-apical direction using a 3.5 mm external diameter (2.5 mm internally) trephine bur (Fig. 1G). Cylindrical sample cores, 5 to 7 mm in length, of the newly generated intrasocket tissue were obtained. Following removal of the cores, osteotomy was completed and root-form implants were inserted. Before histological preparation, tissue samples were marked to identify the crestal and deep sides. Specimens were fixed in 10% neutral buffered formalin for 1 week and then decalcified with 5% formic acid for 2 weeks. Each core was cut horizontally (transversely) in serial sections from the coronal to apical region. Six to 8 section cuts, 5 µ in width, were mounted on every histological slide. Every seventh slide (1, 8, 15, 22, etc.) was stained with hematoxylin and eosin (H&E) for histopathologic examination (remaining slides were examined by different histochemical staining for part 2 of the study; unpublished data).

Histomorphometry

Histomorphometric measurement was performed on all H&E stained slides. Only preserved, rounded sections were submitted for these examinations. Sections that were partly torn, folded, or ruined (approximately 25%) were excluded from the study. At least 16 H&E stained slides were examined from each specimen core. The histomorphometric method was an adaptation of the point-counting procedure.[58] In practice, each section was examined using a projection microscope[¶] at ×40 magnification (Fig. 2). A 64-square (1.5 cm × 1.5 cm) graticule was superimposed on the screen. Point counting was performed on 3 components of each section: bone, connective tissue (CT), and the grafted particles (PBBM). Whenever the graticule-square center (marked by a "+") hit one of the 3 components, the specified component scored one point. The sum of the points overlying each of the specified components (Pi) was calculated. Area fraction percentage (AP) of each component in each section was evaluated as a part of the whole section area, $Pi/\Sigma i$,[59,60] where Σi represents the total number of points superimposed on each section.

§ Biogal Pharmaceutical Works Ltd., Teva Group, Debrecen, Hungary.
‖ Teva Pharmaceutical Industries Ltd., Petah-Tikva, Israel.
¶ Visopan, Reichert, Leica AG, Vienna, Austria.

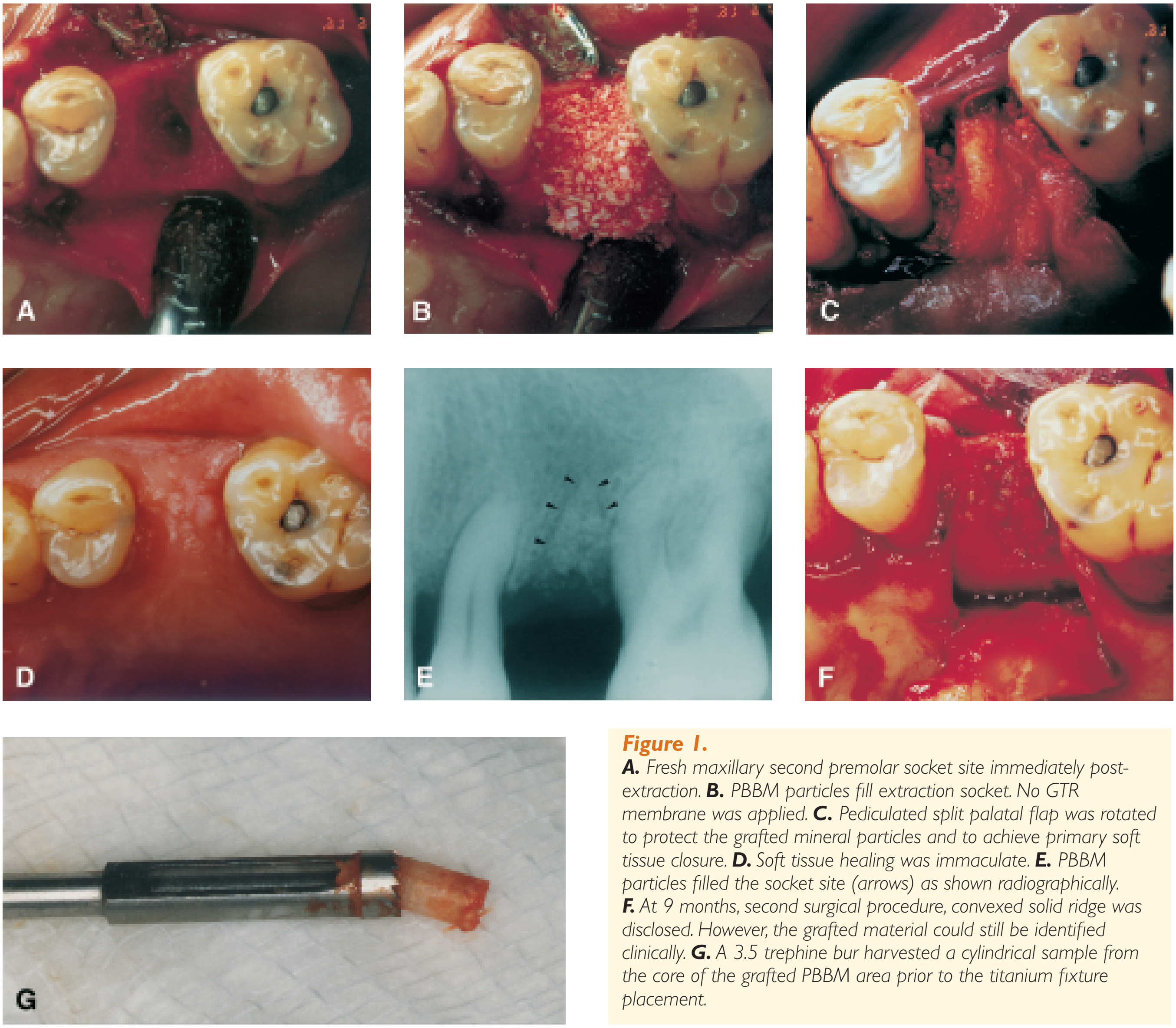

Figure 1.
A. *Fresh maxillary second premolar socket site immediately post-extraction.* ***B.*** *PBBM particles fill extraction socket. No GTR membrane was applied.* ***C.*** *Pediculated split palatal flap was rotated to protect the grafted mineral particles and to achieve primary soft tissue closure.* ***D.*** *Soft tissue healing was immaculate.* ***E.*** *PBBM particles filled the socket site (arrows) as shown radiographically.* ***F.*** *At 9 months, second surgical procedure, convexed solid ridge was disclosed. However, the grafted material could still be identified clinically.* ***G.*** *A 3.5 trephine bur harvested a cylindrical sample from the core of the grafted PBBM area prior to the titanium fixture placement.*

Statistical analysis was conducted using ANOVA with repeated measures.#

RESULTS

At 9 months, upon surgical re-entry, the augmented extraction socket sites were clinically well preserved in their volume dimension. Socket wall height was remeasured to the crestal bone level (Table 1). Bone fill was evident even in very steep buccal plate resorption. The overall fill was 82.3%. In the dehiscenced walls, mainly the buccal plate, vertical bone regeneration measured from 2 to 11 mm in height (average 4.64 mm). However, although PBBM particles were well incorporated into the generated socket osseous tissue, the augmented socket area was distinguishable from the original neighboring bony tissue. Buccolingual dimensions of the augmented alveolar ridge enabled safe insertion of the titanium fixtures for future implant reconstruction.

Histologic examination revealed an abundant amount of PBBM particles and new bone formation in all specimens. Examination of the coronal sections of the core revealed abundance of loose connective tissue and amorphous material of PBBM with small amounts of bone tissue, composed mostly of woven bone (Fig. 3A). In the examined section cuts of the deep area, newly formed bone consisted of a large population of osteocytes and osteoblasts. Between different areas of bone, cellular connective tissue with minimal foci of chronic inflammatory cells, mostly lymphocytes, was observed. At the periphery of the section, amorphous areas within the PBBM particles were

BMDP Statistical Software, Inc., Los Angeles, CA.

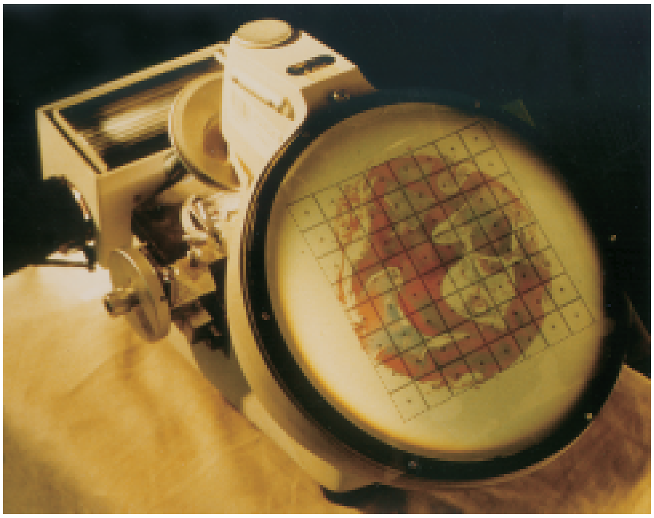

Figure 2.
A 64-square (1.5 cm × 1.5 cm) graticule superimposed to the screen of a projection microscope for histomorphometric measurements.

identified; most of the bone appeared lamellated and some were of woven type (Fig. 3B).

Morphometric Observations

Bone. Average bone area fraction was 46.3% (SD = 9.81). In the superficial cuts, i.e., crestal region, the bone area fraction was 15.9% (SD = 11.8) and in the deepest section cuts, it reached 63.9% (SD = 8.47). Bone area fraction increased gradually from the most coronal area apically as follows: 23.7%, 29.5%, 35.9%, 39.6%, 44.5%, 47.6%, 51.5%, 57.9%, and 63.9% (Table 2).

Connective tissue (CT). The CT area fraction decreased from superficial cuts (52.4%) to deeper cuts (9.5%) with an average of 22.9% (SD = 12.28). In between section cuts 7, 14, 21, 28, 35, 42, and 49, CT area fraction was 52.3%, 41.3%, 35.7%, 30.4%, 27.6%, 23.4%, and 20.5%, respectively. From cuts 49 to 77, only a moderate decrease was observed from 20.5% area fraction to 18%. A marked decrease was noted in the deep sections from cuts 84 to 112, where CT area fraction was 9.7%.

Porous bovine bone mineral. PBBM area fraction showed only a slight reduction from 35.1% at the crestal to 26.4% at the deep section cuts (average 30.8%, SD = 7.82). Between cuts 7 (31.7% area fraction) to 14 (35%), there was an initial increase of PBBM area fraction. From the coronal to the apical aspect, the grafted material relatively retained its area fraction, with an average of 30.8% (SD = 7.82).

The average tissue fraction of each tissue component, i.e., bone, CT, and PBBM, of the 15 specimens along the core depths is shown in Figure 4.

Statistical analysis of the comparison between the areas of bone, CT, and PBBM was performed in several different points along the coronal-apical axis. In the most coronal aspect (section cut 7), a significant increase in CT area was found when compared to the bone area ($P < 0.01$). In the middle of the core (section cut 56), a tendency of increase of bone area concomitant with a decrease of CT area was noted, with a significant difference between the two ($P < 0.01$). In the apical region, the bone area was significantly larger than the CT and PBBM area ($P < 0.001$).

DISCUSSION

Successful healing using PBBM has been reported in experimental animal models[45-50,61] and in compara-

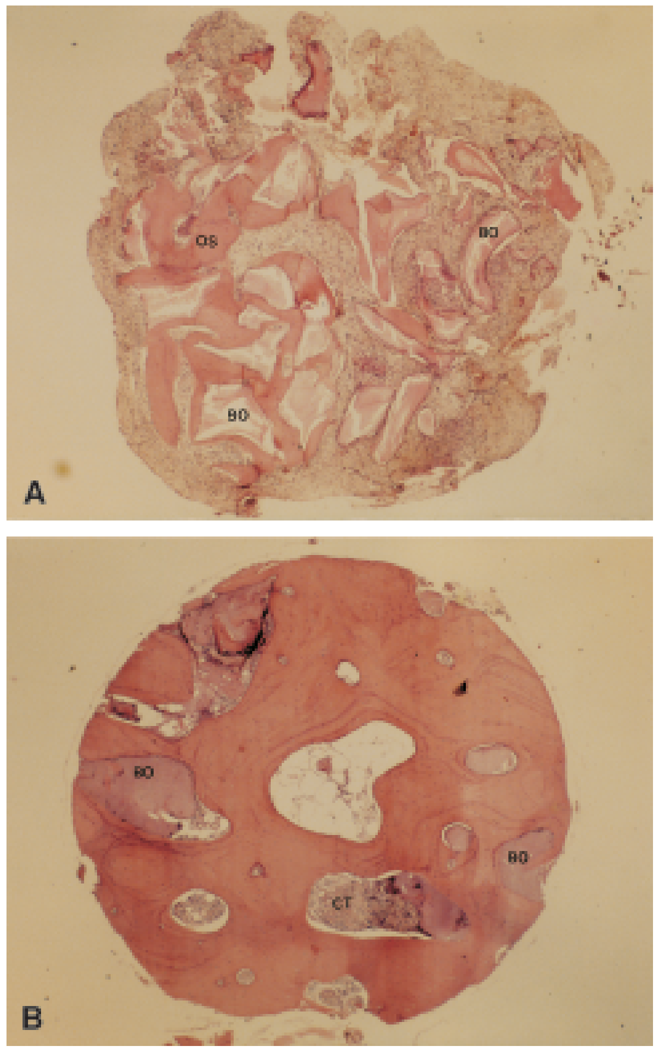

Figure 3.
A. *Case #9 specimen. Superficial section cut 7 disclosed abundant amount of CT and PBBM (BO) particles and only occasional osseous fragments (OS) (×20 magnification).* ***B.*** *Most of the area fraction of section cut 105 of case #9 specimen is occupied by osseous tissue (OS), while PBBM (BO) are well demonstrated. Only a small amount of CT could be identified (×20 magnification).*

Table 2.

Percentage of Tissue Area Fraction by Depth

Serial Cut	% of Bone Area Fraction	SD	% of PBBM Area Fraction	SD	% of CT Area Fraction	SD
7	15.9	11.86	31.7	7.86	52.4	17.55
14	23.7	11.03	35	9.34	41.3	16.5
21	29.5	11.26	34.8	8.46	35.7	14.8
28	35.9	10.76	33.7	8.09	30.4	13.76
35	39.6	9.43	32.8	8.08	27.6	12.51
42	44.5	9.99	32.1	7.89	23.4	12.15
49	47.6	10.18	31.9	8.08	20.5	10.82
56	47.9	7.75	32.1	7.13	20	9.39
63	51	6.35	30.3	8.57	18.7	8.49
70	51.5	6.04	30.6	8.33	17.9	8.37
77	52.8	6.96	29.2	8.42	18	9.49
84	55.4	7.82	28.5	6.22	16.1	9.84
91	57.9	9.49	28.7	7.7	13.4	11.13
98	61.1	10.7	26.8	5.65	12.1	11.76
105	63.3	9.16	27.2	4.32	9.5	9.07
112	63.9	8.47	26.4	3.13	9.7	8.61
Average	46.30	9.81	30.80	7.82	22.90	12.28

tive clinical studies.[62,63] The clinical efficacy of PBBM has been shown in case reports dealing with extraction socket preservation.[35,36,64] All reports involved GTR procedures. Recently, the efficacy of PBBM was observed without applying GTR occlusive membranes especially in ridge preservation procedures during the post-extraction phase.[38-40]

To determine the healing pattern of the newly formed tissues in relation to the presence of the grafted material and to evaluate the influence of the socket depth, cross-sections along tissue cores from the socket sites were examined histomorphometrically. From the most superficial section cuts to the deeper cuts, there was a consistent increase of the newly formed bone with a concomitant decrease in the CT area fraction. The proximity of the overlying soft tissue flap on the crestal surface of the graft and the difficulty in controlling the stability of the grafted particles in its orifice site (i.e., unavoidable micromovement) probably produced a relatively high fraction area percentage of CT, which decreased from cuts 7 to 14. Both tissue area fractions appeared to be diversely correlated all along the examined grafted tissue cores. The area fraction of PBBM remained fairly constant along the cores, but with only a slight change varying from 35% to 26.4%.

Natural healing of extraction socket sites at 4 months presented alveolar trabecular bone up to the crest, very little osteogenesis, and only occasional osteoblasts.[10] This same pattern was observed in the PBBM-grafted socket site. It is noteworthy to distinguish between the healing potential of the PBBM-grafted socket sites and non-grafted sites. In this study, a particular soft tissue surgical management, i.e., a rotated pediculated palatal flap,[56,57] was used to stabilize and secure the grafted particles to achieve healing by primary soft tissue closure. This is not the case in conventional non-grafted socket sites, which heal by secondary intention. The fact that both bone and CT remodeled in a manner similar to natural non-grafted sockets indicates that PBBM is not an inductive material. However, as presented in this study, osteoconductivity was evident, based on the promotion of osseous ingrowth and intimate integration with the newly formed bone. Thus, a total incorporation of the generated osseous tissue and the mineral particles was achieved. The newly generated osseous tissue, as presented within PBBM particles, indicates the micro- and macropores characteristic of this mineral. Such porosity (70% to 75%) and configuration result in a large space occupied by 25% net volume by the crystalline product. This allows for 75% new volume of the defect for regeneration and/or generation of new osseous tissue.[28] Our results clearly show that the generated tissues, the bone, and the connective tissue in the healed socket occupied approximately 70% of all examined specimens (65% to 73.6%, average 69.2%), regardless of the depth of the serial section. Therefore, based on morphometric data at 9 months, resorption of the grafted particles was not evident. Others have reached a similar conclusion, although in a short-term animal model study.[65]

Understanding the mechanism and rate of resorption, particularly in xenografts, is of special interest. The ultimate bone substitute should induce/conduct new bone formation and eventually completely resorb and be replaced by bone. While new bone formation is quite evident in all specimens in this, as well as in other studies, the resorption capability of the grafted material is not clear. The presence of PBBM particles at 6 months and up to 42 months has been documented.[40,63,66] The vast dominance of the grafted min-

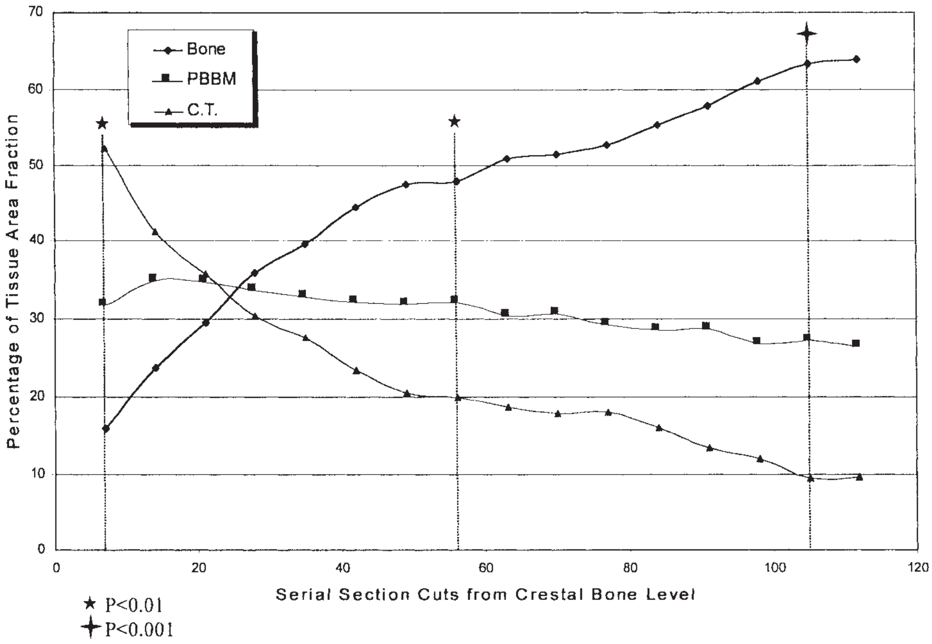

Figure 4.
Bone, PBBM, and CT area fraction curves along the histologic section cuts of the 15 specimen cores.

eral in all histologic specimens shows that the resorption rate of these particles is extremely slow. Skoglund et al.[63] postulated that the capacity of the recipient site to generate new bone formation directly onto the grafted particles prevented resorption of these particles, but there is no validated data to support this theory. Furthermore, non-resorbed particles were also evident in areas in which particles were in direct contact with connective tissue.

Clinically, the presence of these grafted particles as an integral, dominant part of the augmented site should be examined carefully as an appropriate housing to functional osseointegrated titanium fixtures. Mixed data have been reported on the quality of regenerated/generated PBBM-grafted sites and dental implant contact. Whereas human[31] and animal[51] studies report a proper osseointegration, others[39] found no contribution and therefore did not recommend the use of this bone derivative to enhance vital bone-implant contact. Long-term human data are mandatory to elucidate whether the presence of the grafted particles would eventually interfere with the longevity of functional implants in this osseous composition.

CONCLUSIONS

Measurements of tissue fraction area along 15 specimen cores available from grafted socket sites revealed a consistent pattern of newly formed bone, connective tissue, and grafted mineral from the superficial to the deeper part of the healed sockets. While PBBM showed only a minor change and generally maintained 30% of the tissue area fraction regardless of the depth of the examined socket site, bone fraction area increased from the crestal to the apical region, and connective tissue decreased at the same trend. Studies in progress will further elucidate the histochemical and morphometric nature of the bone fraction (i.e., woven bone versus lamellated bone from the crestal to the apical region), as well as assess the resorbability of this xenograft.

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