Histomorphometric Evaluation of the Extraction Sockets Treated with Different Graft Materials

Gamal M. Moutamed

Department of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine, Cairo University, Cairo, Egypt
Corresponding author: gamalmoutamed@yahoo.com

ABSTRACT: Various grafting materials have been used immediately in the extraction socket following tooth extraction for ridge preservation in an attempt to limit or prevent ridge resorption. The purpose of the present study was to investigate histologically and histomorphometrically, in mongrel dogs, the influence of bioglass (BG), demineralized freeze-dried bone allograft (DFDBA) and Grafton demineralized bone matrix (DBM) putty implanted in extraction sockets for ridge preservation compared to no graft at all. Following lower third premolar tooth extraction of both right and left side, a total of 48 sockets in 24 mongrel dogs were randomly divided into two groups. Each group comprised 12 dogs and split-mouth design was applied. In the first group the right sided-socket of 12 dogs was grafted with BG and the left sided- socket of the same dogs was grafted DFDBA. In the second group the right sided-socket of 12 dogs was grafted with Grafton DBM putty and the left sided- socket of the same dogs was un-grafted served as unfilled control. Primary coverage was achieved by flap advancement over each socket. Three animals from each group were euthanized and sacrificed at one, 3, 6, and 12 weeks post-operatively. The right and left mandibular segments, including the implanted sockets, were obtained. The specimens were processed, decalcified and stained with haematoxylin and eosin for histological examination and histomorphometrically analyzed. The count of new bone trabeculae and the average size as well as the total area of new bone trabeculae was calculated. Moreover, changes of alveolar ridge height were evaluated on postoperative radiographs. At one, 3, 6 and 12 weeks post-extraction, a statistical significant difference (P value < 0.05) was obtained in the count of bone new trabeculae, the average size of new bone trabeculae and the total area of new bone trabeculae among treated sites. Grafton DBM putty showed statistically significant highest values amongst treated sites. However the was no statistical significant difference when comparing these variables in the BG treated sockets with the DFDBA treated sockets. Furthermore, there was no statistically significant difference (P > 0.05) in the alveolar ridge height changes between the grafted and un-grafted sockets throughout all time intervals. Conclusion, the implantation of BG, DFDBA as well as DBM putty in extraction sockets was accompanied by varying degrees of bone formation as well as a host response. All used graft materials were biocompatible and biodegradable. DBM putty seems to be an ideal graft material in extraction sockets as it was simple to use, effective, providing scaffold for new bone to build up for healing process. Efficacy of Grafton DBM putty may relate to methods of demineralization, the concentration of graft material per unit volume, or the nature of the carrier. DBM putty, when used for extraction sockets grafting, resulted in replacement of most of the graft material with bone.
Nature and Science 2011; 9(9):132-146] (ISSN:1545-0740). http://www.sciencepub.net.

1. INTRODUCTION

Tooth loss is responsible for alveolar bone atrophy leading to decrease in both bone height and width. The resultant ridges present functional and aesthetic problems to prosthodontists, as well as, implantologists. Un-aesthetic dental restoration and over closure are among the problems that are accelerated by loss of alveolar bone, Weitraub and Burt (1985). These problems could be prevented by ridge preservation or replacement therapy, Fiorellini and Nevins (2003). Ridge preservation is the immediate replacement of the removed tooth with bone grafting material. It is based on the theory that, when you take something out, you should put something back, Ashman et al., (1994); Christensen (1996). This provides a better site for the placement of dental implants with greater implant to bone contact by allowing the placement of longer, wider implants and improved aesthetics of the final restoration with better emergence profiles and gingival architecture, Douglass (2005).

Different types of bone grafting materials accomplish this issue in different ways. Osteoinductive grafts contain bone morphogenetic proteins (BMPs) that can stimulate bone growth through the differentiation of cells into osteoblasts. Osteoconductive grafts provide a scaffold for bone regeneration on or within the surface of the graft material, Wozneyet al., (1990); Byrne et al., (1991); Landesberg et al., (2000); Babush et al., (2003). Graft materials are generally classified into three groups. Bone taken from a donor site in the patient to be grafted at another site is known as auto grafts. Allografts refer to bone harvested from another person (living or deceased) and then processed. Bone grafts composed of a wide variety of synthetic materials fall into the alloplast category, Babush (2003). Although auto grafts have traditionally been

considered the gold standard of bone-grafting materials, donor-site morbidity and the invasive nature of harvesting at a second site have somewhat limited their use in oral and maxillofacial surgery. These have led to the development of allografts and alloplasts as alternative grafting materials, Rummelhart et al., (1989); Andre et al., (1995).

Allograft bone currently is available, Takikawa et al., (2003) as either mineralized bone tissue or demineralised bone matrix (DBM). DBM is cadaveric bone that has been processed to remove the mineral component, leaving a scaffold of collagen and various growth-factor proteins that have been shown to induce bone formation Coulson et al., (1999). Demineralisation is necessary to maximize the osteoinductive properties of bone allograft. DBM provides an osteoconductive matrix and, if properly processed from a suitable host, can be osteoinductive, Harakas (1984); Ripamonti et al., (1991); Zambonin and Grano (1995); Becerra et al., (1996); Torricelli et al., (1998). DBM has been used successfully in many clinical situations, including craniofacial defects, Mulliken and Glowacki(1980); Glowacki et al.,(1981); Sonis et al.,(1983) and tumour surgery, Pals and Wilkins(1992); Tiedeman et al.,(1991). The enhanced osteoinductive capability of DBM is afforded mostly by BMPs, Christopher et al., (1995); Kusumoto et al., (2000).

Currently DBM is available as demineralised freeze-dried bone (DFDB) as a powder, crushed granules, or chips. Problems with handling and containing DBM particles have limited the exclusive use of this material. Maintenance of the graft material within the defect site is of paramount importance. Any migration of particles from the area could compromise the graft success because of inadequate regeneration of the defect and potential ectopic bone formation, Krauser and Garg (2001). To address this problem, DBM graft materials have been developed that use a carrier to keep the particles together at the graft site in a putty-like formula as Grafton DBM putty (Osteotech, Inc., Eatontown, NJ) which is a combination of DBM fibers in a glycerol carrier, Babbush(1998); Takikawa et al.,(2003).

Callon et al .,(2000) grafted extraction sockets of 8 patients with DBM putty (Grafton DBM putty) or flexible sheets (Grafton DBM flex) and both showed excellent bone height and width for placement of dental implants 4 months postoperatively. Mellonig (2006) evaluated the potential of allogenic DBM (Grafton) to regenerate new bone, cementum and periodontal ligaments around teeth previously contaminated by bacterial plaque. After six months of healing the teeth was removed unblock and evaluated histologically. Results showed regeneration of new bone, cementum, and periodontal ligament.

Furthermore, Lindsey et al., (2006) evaluated the effectiveness of Grafton putty and aspirated bone marrow for treating long bone fractures. Results demonstrated 90% of DBM patients achieved full bone formation compared to 75% of autograft patients. These findings suggested that DBM putty enriched with bone marrow may be comparable to autograft for treating long bone fractures. Hass et al., (2007) studied Grafton for treatment of bone cyst in children. The results showed significant decrease in bone transparency and simultaneous cortical remodeling was radiographically detected.

Alloplastic materials have been widely used in dento-alveolar surgery. Members of this family include; hydroxyapatite (HA), glass ceramics (bioactive glass) and tri-calcium phosphate (TCP), Urist et al., (1984); Hollinger et al., (1989); Tamimi et al., (2006). Bioactive glass (BG) is a bioactive material because they bond and enhance bone tissue formation. It is considered as three-dimensional silica (SiO_2) network, which is modified by incorporating oxides such as sodium oxide (Na_2O), calcium oxide (CaO) and phosphorous pent-oxide (P_2O_5). Bioactive glass or Bioglass is composed of 45% SiO_2, 24.5% CaO, 24.5% Na_2O and 6% P_2O_5., Stanley et al.,(1987); Wilson and Low (1992); Bendall(1995); Vallet (2004). Bioactive glass demonstrated both osteoconduction and osteoinduction by not only having a biocompatible interface for bone migration, but a bioactive surface that is colonized by osteogenic stem cells free in the surgical environment, Hench and LaTorre (1991); Oonishi et al.,(1995); Low et al.,(1997). BG has been used for grafting purposes including the repair of osseous defect, grafting for sinus lift procedure and around load bearing dental implants, Stanley et al.,(1987); Wilson et al.,(1993). In an animal study on BG, it was provide that BG has enhanced repair by the osteoconductive and osteoinduction properties which lead to the differentiation of osteoprogenitor cells to osteoblasts, Johnson et al., (1997).

The aim of the present study was to investigate histologically and histomorphometrically, in mongrel dogs, the influence of bioglass (BG), demineralized freeze-dried bone allograft (DFDBA) and Grafton demineralized bone matrix (DBM) putty implanted in extraction sockets for ridge preservation compared to no graft at all.

2. MATERIALS AND METHODS
2.1. Materials:
2.1.1. Animal model and grafting
The current study comprised 24 healthy adult male mongrel dogs of comparable age (9-12 months), with a complete set of permanent dentition and their weights averaged between 15 – 19 kilograms. All

dogs were examined by a veterinarian to rule out the presence of any disease. The lower right and left third premolars of each animal was a traumatically extracted.

The animals were randomly divided into: group 1 (G-1) and group 2 (G-2), each group comprising 12 dogs; 24 sockets. In each group, a split-mouth design was followed where right or left mandibular third premolar sockets in 12 animals were randomly assigned to receive one type of bone graft material. In G-1 , the socket of the lower right third premolar (G-1R) was grafted with bioglass (BG) material *(of 300 to 360 µm sized particles*; *Glass research department, National Research Centre; NRC , Cairo , Egypt)*, while the socket of the lower left third premolar (G-1L) of the same animal was grafted with demineralised freeze–dried bone allograft (DFDBA) particles *(consisted of cortical bone of 250 to 500 µm sized particles*; *Cera MED Dental , L.L.C , Mile High Transplant Bank , 8085)*.In G-2, the socket of the lower right third premolar (G-2R) was grafted with Grafton demineralised bone matrix putty (*Grafton DBM putty is a combination of DBM fibers with a glycerol carrier produced by Osteotech, Inc., Eatontown, NJ)*, while the socket of the lower left third premolar (G-2L) of the same animal was left un-grafted served as a unfilled controls.

2.2. Methods:
2.2.1. Premedication and anaesthesia
For eradication of any septic foci, all animals intra-muscularly injected with a single dose of Procaine Penicillin 300.000 u, Penicillin G 100.000 u and Streptomycin 0.5 gm *(Streptopencid: CID laboratories, A.R.E)* for three days preoperatively. Animals were fasted twelve hours before the operation to avoid aspiration of gastric contents during general anesthesia. Immediately preoperatively, a dose of Atropine sulphate *(1mg / 1ml, Misr Laboratories)* 0.05 mg/kg body weight in combination with Diazepam *(Valium 10 mg/2 ml, Roche)* 1 mg/kg bodyweight was injected intravenously via the cephalic or recurrent tarsal vein, using intravenous canula. Dexamethasone sodium phosphate *(4mg/ml, Egyptian International pharmaceutical industries Co., A.R.E.)* 2 ml was also given intravenously as anti-inflammatory drug to avoid postoperative edema.

Anesthesia was induced by administration of a combination of Ketamine hydrochloride *(Ketalar 5 % sol – Park Davis USA)* 7mg/kg bodyweight and Xylazine hydrochloride *(Rompun 2% sol-Bayer Leverkusen, Germany)* 1mg/kg bodyweight. These mixtures were slowly injected via intravenous canula. Anesthesia was maintained by solution of 2.5 %

Thiopental Sodium *(Thiopental 500mg, Epico, Egypt)* 20-30 mg/kg bodyweight. Depth and maintenance of anesthesia were confirmed by loss of eye blinking reflex and relaxation of skeletal muscles at surgical site.

2.2.2. Surgical extraction and implantation
All the experimental procedures were performed aseptically (Figure 1).The animals were fixed on surgical table and draped. The mouth was opened using canine mouth gag. Antiseptic solution was used to disinfect the operative field. Intra-sulcular buccal and lingual incisions were made around the lower right and left third premolars using bard parker blade number 15. Then, buccal and lingual flaps were raised to adequately view the sockets, facilitate the atraumatic tooth extraction without soft tissue injury and allow sufficient flap release to obtain primary closure after implantation of the bone-substitute graft materials. The lower third premolar was then atraumatically extracted using lower anterior forceps. Releasing incision was made in the periosteum at the base of flap to help in approximation of wound edges after implantation of the graft material. Following tooth extraction, the socket was grafted as mentioned earlier in animal model and grafting. Out of the 48 sockets treated, 12 sockets in G-1R grafted with bioactive glass material (BG), 12 sockets in G-1L grafted with demineralized freeze-dried bone allograft (DFDBA), 12 sockets in G-2R grafted with Grafton demineralized bone matrix putty (DBM) and 12 sockets in G-2L served as unfilled controls (C). The bioactive glass (BG) and the demineralized freeze-dried bone allograft (DFDBA) were hydrated with sterile saline at least 15 minutes prior to insertion in the socket and then packed into the socket. Flaps were then sutured with 2-0 Vicryl *(Ethicon, Ltd)* utilizing interrupted and mattress sutures. In all cases tension-free primary closure was achieved. Animals were caged individually and fed soft dog chow and water throughout the postoperative time interval.

The same antibiotics given pre-operatively were given for 3 days post-surgically. In addition, the animals were given 1 ml of methyl prednisolone acetate *(Depomedrol 40 mg/ml, Egyptian International pharmaceutical)* and Diclofenac Sodium *(Voltaren 75 mg/3ml, Novartis)* intramuscularly to reduce postoperative edema and pain. Animals were euthanized with a lethal overdose of pentobarbital (50 mg/Kg) through rapid intravenous injection at different postoperative intervals. Three animals from each group were euthanized and sacrificed at one, three, six, and 12 weeks post-operatively.
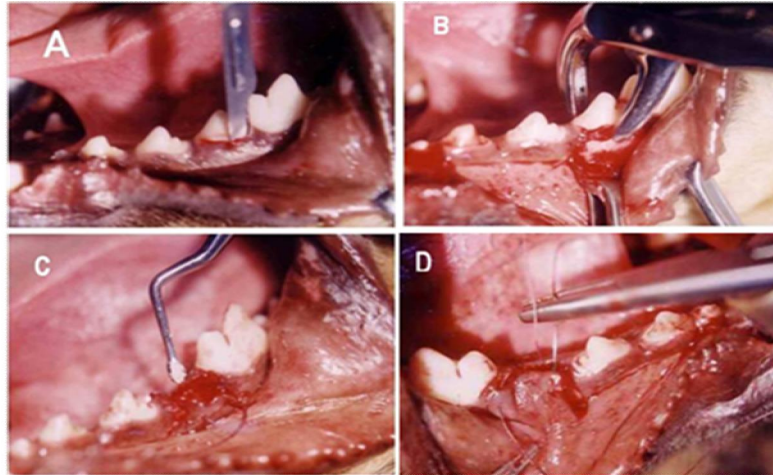
Figure (1): Photographs showing surgical extraction and grafting procedures; A) intra-sulcular incision around lower third premolar, B) atraumatic extraction of that tooth, C) extraction socket during implantation of the graft material and D) adequate closure of the socket after grafting

2.2.3. Processing and sectioning of tissues

Each mandible was dissected free, and soft tissue was immediately removed. The right and left mandibular segments, including the implanted sockets, were dissected and the specimens were processed. Specimens were fixed in neutral buffered formaldehyde solution for 72 hours. The blocks were then decalcified by immersing the specimen in decalcifying working solution of EDTA for 10 days. The decalcified specimen was then dehydrated with graded ethyl alcohol washes (50%, 70%, 85% then 100%) and imbedded in paraffin wax with an orientation that provide sectioning along the sagittal plane of the jaw. Sections of 4μm thickness were cut sequentially from the lateral to the medial side till the socket center was reached. The sections were stained with hematoxylin and eosin for routine examination.

2.2.4. Histomorphometric image analysis

For each histological section, a photomicrograph was taken by CCD digital camera *(Olympus – Japan)* attached to zoom stereo microscope *(Olympus SZ-PT – Japan)*. The number of bone trabeculae as well as the mean surface area of bone trabeculae of each photomicrograph was automatically calculated using the image analysis software *(Image ware, Image 1.3-1b, USA)*. Images were processed for colour enhancement and brightness adjustment. The processed images were then converted into 8-bit gray scale images (Figure 2-A and B). The newly formed bone trabeculae inside the extraction socket were automatically colour-coded after selection of the appropriate threshold that ensures count of this structure specifically and exclude other non- desired tissues (colour-code threshold of new bone trabeculae was automatically calculated as a perimeter of trabeculae) that range between 150 - 185 pixels and stand at 76 gray level (Figure 2-C and D). At the same colour code threshold, the average size and the total area of new bone formation was also calculated. Then data were collected and tabulated for statistical analysis.

2.2.5. Changes of alveolar ridge height

Post-operative radiographs (Figure 3) were done immediately, one, three, six, and 12 weeks post-surgically to estimate the changes in the alveolar ridge height. Three parallel lines were drawn on the radiograph starting from the crest of the alveolar ridge at the grafted socket area to the inferior border of the mandible to estimate the ridge height and the mean of these measurements was recorded at each time interval for each group. Statistical analysis of these measurements was done.
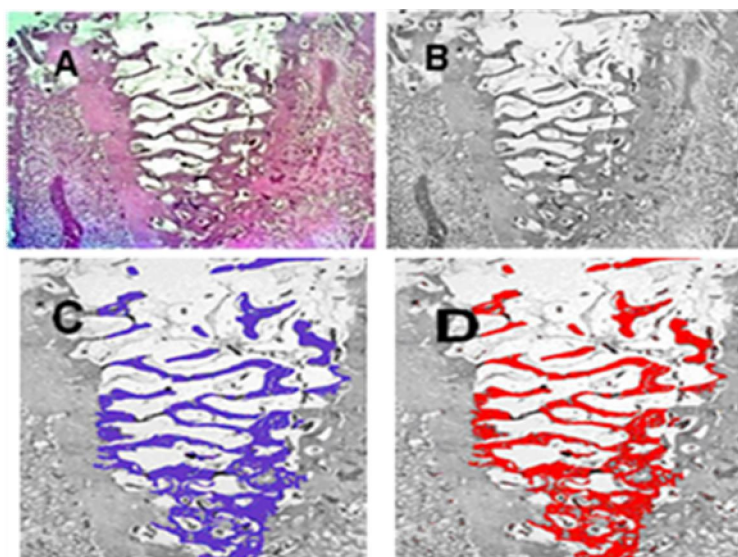
Figure (2): Photomicrographs showing histomorphometric image analysis; A) image was processed for color enhancement and brightness adjustment; B) the processed image was converted into 8-bit gray scale image, C) color coding of the newly formed bone trabeculae inside the extraction socket D) the previous photomicrograph after thresholding of the desired structure and prior to calculation
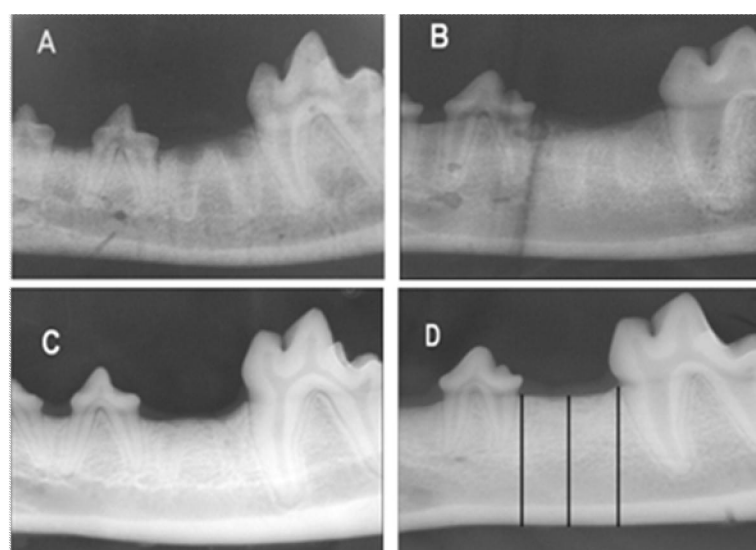
. (Stereo X 200)

Figure (3): Postoperative radiographs showing BG grafted sockets A) at 1 week, B) at 3 weeks, C) at 6 weeks and d) three parallel lines drawn on the radiograph starting from the crest of the alveolar ridge at the grafted socket area to the inferior border of the mandible to estimate postoperative changes in the alveolar ridge height

2.2.6. Statistical analysis
ANOVA (analysis of variance) was used to examine the difference between the continuous numerical values of the number, average size, as well as, the total area of new bone trabeculae in BG-grafted sockets, DFDBA-grafted sockets, Grafton DBM putty-grafted sockets as well as un-grafted sockets. Tukey test was used to determine significant

difference between means when ANOVA test is significant. All statistical analysis were performed using SPSS 10.0 for windows (SPSS Inc) and the significance level was set at (P <0.05). Data of the alveolar bone height in all groups over time intervals were presented as means and standard deviation (SD) values to study changes by time.

3. RESULTS

The extraction sockets of all animals were healed within one week postsurgical and covered with healthy mucosa. None of the animals enrolled in this study showed abscess, swelling, wound dehiscence, or allergic reactions during the course of the study. Suture material was lost seven to ten days post surgically.

3.1. Histological findings

Histological analysis of the extraction sockets grafted with BG particles (G-1R) at one-week postoperative, showed new bone trabeculae especially at the base and periphery of the socket. The central area was devoid of any bony trabeculae (Figure 4-A). At three-weeks, horizontally oriented bone trabeculae were observed forming bridging from one side to the other. The number of bone trabeculae was increased. The bone trabeculae at the base of the socket were thickened and showed union with the original bone. At six-weeks, the whole socket was filled with mature bony trabeculae with minimal marrow spaces in between. At twelve-weeks, newly formed bony plates increased in size and coalesced with each other enclosing narrow marrow spaces. Few Haversian systems were observed. Union between the new bone and old one was noted (Figure 4-B).

Histological analysis of extraction sockets grafted with DFDB particles (G-1L) at one-week postoperative, showed fibrous tissue formation with appearance of few new bony trabeculae especially at the base of the socket (Figure 4-C). At three-weeks, numerous bony trabeculae were laid down. Union of most of these trabeculae was noted especially at the base and periphery of the socket while the central area showed few bony trabeculae. At six-weeks thickening of the newly formed trabeculae was noted and bridging of most of these trabeculae was observed. At twelve-weeks, the newly formed bony plates increased in size enclosing marrow spaces. Blood capillaries were seen inside the endosteum.

Few Haversian systems were evident. Union between the newly formed bone and the old one was noted especially at the base of the socket (Figure 4-D)

Histological analysis of the sockets grafted with DBM Grafton putty (G-2R) at one-week postoperative, showed formation of new bone trabeculae especially at the base and most of these trabeculae were thick and discrete (Figure 5-A). At three-weeks, union of newly formed trabeculae was noted. Bony plates showed minimal marrow spaces. Few Haversian systems were observed. At six-weeks, most of the socket was filled with new bony plates with minimal marrow spaces and many Haversian systems. Union between the newly formed bone and original bone was also observed. At twelve-weeks, the socket was completely filled with new bone that showed union with the old one. Many Haversian systems were also observed. Islands of woven bone and osteoblasts were observed suggesting that the mechanism of bone formation was similar to that of natural bone remodeling process. The marrow spaces were minimal but highly vascular (Figure 5-B). few remnants of the graft material were observed incorporated inside the regenerated bone with direct bone apposition onto its surface which indicates the high porosity of the material and that the rate of bone regeneration matches the rate of the DBM putty resorption which leaves a space for the attached bone cells to deposit new bone.

Histological analysis of the un-grafted sockets (G-2L) that act as a control sockets, at one-week postoperative, showed a fibro cellular tissue originating from the peripheries and dilated blood capillaries extended between the formed tissue .The intercellular spaces in the mid-portion of the socket did not show signs of bone formation. Areas of new osteoid tissue were evident in the peripheries (Figure 5-C). At three-weeks, very fine bone trabeculae extending from the socket wall and funds were formed between the fibro cellular tissues seen previously. Some entrapped osteocytes could be observed. At six-weeks, the formed bony trabeculae became thickened and filled the whole socket. Union of these trabeculae was noted. At twelve-weeks, as bone was continuously laid down, narrowing of the bone marrow spaces occurred. Bridging of most bone trabeculae was observed (Figure 5-D).
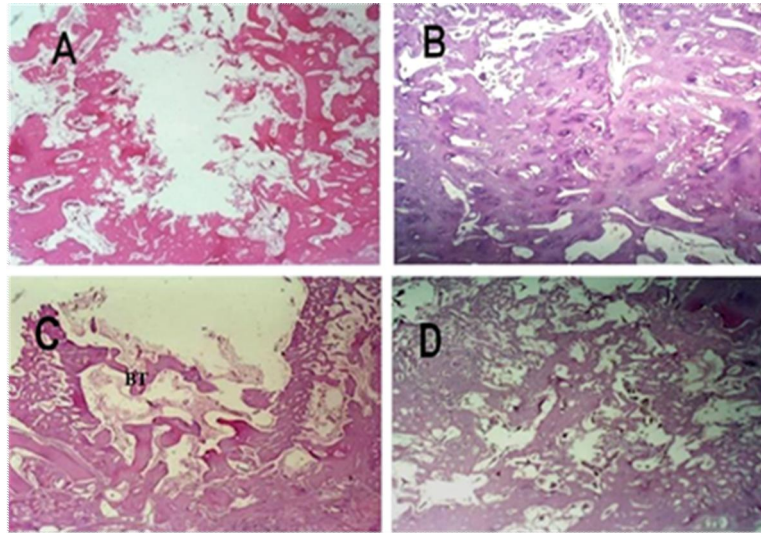
Figure (4): Photomicrographs of BG-grafted and DFDBA-grafted sockets, A) at one-week BG-grafted sockets showing new bone trabeculae at the base and periphery of the socket, B) at 12-week BG-grafted sockets showing maturation of bone trabeculae and formation of Haversian system, C) at one-week in DFDBA-grafted sockets showing fibrous tissue formation with few new bony trabeculae (BT) at the base of the socket, and D) at 12-week in DFDBA-grafted sockets showing thickened bony plates filled most of the socket. . H&E stain; magnification X 20

Figure (5): Photomicrographs of Grafton DBM putty-grafted and unfilled control sockets, A) at one-week Grafton DBM putty-grafted sockets showing new bone trabeculae at the base and periphery of the socket, B) at twelve-week Grafton DBM putty-grafted sockets showing maturation of bone trabeculae and formation of Haversian system, C) at one-week un-grafted sockets showing fibrous tissue formation with few new bony trabeculae (BT) at the base of the socket, and D) at twelve-week un-grafted sockets showing thickened bony plates filled most of the socket. H&E stain; magnification X 20
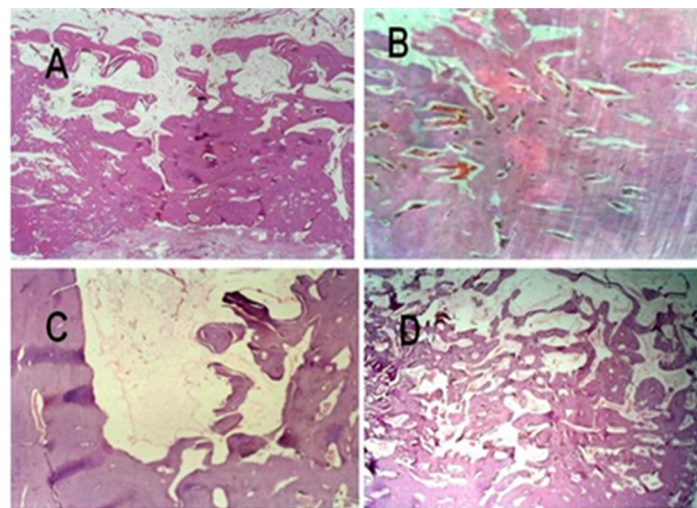
3.2. Statistical results

At one, three, six and 12 weeks post-operatively, a statistical significant difference (P value < 0.05) was

obtained in the count of bone new trabeculae (Table 1), the average size of new bone trabeculae (Table 2) and the total area of new bone trabeculae (Table 3) among different grafts. Grafton DBM showed statistically significant (P value <0.05) highest values amongst treated groups. However the was no statistical significant difference when comparing the count of bone new trabeculae (at one week P = 0.072 and at six weeks P= 0.120), average size of new bone trabeculae (at three weeks P= 0.979 and at 12 weeks P= 0.117) and total area of new bone trabeculae (at one week P = 0.092, at six weeks P= 0.94 and at 12 weeks P=0.193) in the bioglass treated sockets (G-1R) with the DFDBA treated sockets (G-1L).

Regarding the changes in the alveolar ridge height, there was no statistically significant difference (P > 0.05) in the alveolar bone height changes between the grafted sockets and un-grafted socket throughout all time intervals (Figure 6).

4. DISCUSSION

Many different augmentation methods of ridge preservation were identified. The most commonly used method was a graft that was placed in the extraction socket, covered by a membrane followed by flap advancement to achieve complete or partial primary closure, Iasella et al.,(2003) Carmagnola et al.,(2003);

Vance et al.,(2004); Froum et al.,(2004); Pinho et al.,(2006); Molly et al.,(2008). The second most commonly employed technique was covering a graft by the flap, but without a membrane, Froum et al.,(2002); Vasilic et al.,(2003); Guarnieri et al.,(2004); Nevins et al.,(2006); Wang and Tsao (2006), in agreement with the current study where the soft tissue was used to fully cover the grafted extraction socket.

Various types bone graft materials for ridge preservation techniques were used. Demineralised freeze-dried bone allograft (DFDBA) has been used extensively, Babush (2003); Iasella et al., (2003); Froum et al., (2002). Other graft materials include autologous bone, Becker et al.,(1994); Becker et al.,(1996) Pinho et al.,(2006) bioactive glass and hydroxyapatite, Yilmaz et al.,(1998); Camargo et al.,(2000); Froum et al.,(2002);Norton and Wilson(2002). The goal of the current study was to compare histologically and histomorphometrically socket healing and osteoinductive and/or osteoconductive properties of the three different implanted materials which was bioactive glass (BG), demineralised freeze-dried bone (DFDBA) and Grafton DBM putty, with healing of an un-grafted control sockets.

Table (1): Statistical difference between grafted sockets in the count of new bone trabeculae at one, 3, 6 and 12 weeks post-extraction

Time interval	(I) material	(J) material	Mean Difference (I-J)	Sig.
One week	BG	DFDBA	11.27	0.072
		DBM	-34.34*	0.000
		Control	31.55*	0.000
	DFDBA	BG	-11.27	0.072
		DBM	-43.82*	0.000
		Control	17.30*	0.010
	DBM	BG	34.34*	0.000
		DFDBA	43.82*	0.000
		Control	66.92*	0.000
Three weeks	BG	DFDBA	11.37*	0.025
		DBM	-40.35*	0.000
		Control	53.30*	0.000
	DFDBA	BG	-11.37*	0.025
		DBM	-52.71*	0.000
		Control	37.91*	0.000
	DBM	BG	40.35*	0.000
		DFDBA	52.71*	0.000
		Control	92.81*	0.000
Six weeks	BG	DFDBA	17.71	0.120
		DBM	-33.53*	0.011
		Control	55.20*	0.000

	DFDBA	BG	-17.71	0.120
		DBM	-51.31*	0.001
		Control	36.43*	0.005
	DBM	BG	33.53*	0.011
		DFDBA	51.31*	0.001
		Control	88.75*	0.000
Twelve weeks	BG	DFDBA	25.06*	0.011
		DBM	-45.33*	0.000
		Control	75.88*	0.000
	DFDBA	BG	-25.06*	0.011
		DBM	-70.30*	0.000
		Control	51.90*	0.000
	DBM	BG	45.33*	0.000
		DFDBA	70.30*	0.000
		Control	123.30*	0.000

The mean difference is significant at the 0.05 level.
BG; bioglass, DFDBA; demineralized freeze-dried bone allograft, DBM; demineralized bone matrix Grafton putt

Table (2): Statistical difference between grafted sockets in the average size (mm^2) of new bone trabeculae at one, 3, 6 and 12 weeks post-extraction

Time interval	(I) material	(J) material	Mean Difference (I-J)	Sig.
One week	BG	DFDBA	2.0031*	0.015
		DBM	-1.7221*	0.032
		Control	4.3512*	0.000
	DFDBA	BG	-2.0031*	0.015
		DBM	-3.7211*	0.000
		Control	2.3446*	0.006
	DBM	BG	1.7221*	0.032
		DFDBA	3.7211*	0.000
		Control	6.0711*	0.000
Three weeks	BG	DFDBA	-6.12	0.979
		DBM	-1.9221*	0.000
		Control	3.0446*	0.000
	DFDBA	BG	6.12	0.979
		DBM	-1.7311*	0.000
		Control	3.1046*	0.000
	DBM	BG	1.9221*	0.000
		DFDBA	1.7311*	0.000
		Control	4.9410*	0.979
Six weeks	BG	DFDBA	1.9662*	0.000
		DBM	-2.1311*	0.000
		Control	8.9321*	0.000
	DFDBA	BG	-1.9662*	0.000
		DBM	-4.2112*	0.000
		Control	6.9431*	0.000
	DBM	BG	2.1311*	0.000
		DFDBA	4.2112*	0.000
		Control	11.0711*	0.000
Twelve	BG	DFDBA	2.8123	0.117

weeks		DBM	-5.0811*	0.008
		Control	16.7011*	0.000
	DFDBA	BG	-2.8123	0.117
		DBM	-7.9667*	0.000
		Control	14.8111*	0.000
	DBM	BG	5.0811*	0.008
		DFDBA	-7.9667*	0.000
		Control	21.8000*	0.000

* The mean difference is significant at the 0.05 level.
BG; bioglass, DFDBA; demineralized freeze-dried bone allograft, DBM; demineralized bone matrix Grafton putty

Table (3): Statistical difference between grafted sockets in the total area (mm^2) of new bone trabeculae at one, 3, 6 and 12 weeks post-extraction

Time interval	(I) material	(J) material	Mean Difference (I-J)	Sig.
One week	BG	DFDBA	226.321	0.092
		DBM	-341.651*	0.092
		Control	581.321*	0.009
	DFDBA	BG	-226.321	0.092
		DBM	-619.321*	0.000
		Control	364.000*	0.015
	DBM	BG	341.651*	0.009
		DFDBA	619.000*	0.000
		Control	964.342*	0.000
Three weeks	BG	DFDBA	339.321	0.488
		DBM	-501.000*	0.000
		Control	699.321*	0.000
	DFDBA	BG	-339.321	0.488
		DBM	-431.233*	0.000
		Control	813.667*	0.000
	DBM	BG	501.000*	0.000
		DFDBA	431.233*	0.000
		Control	1244.000*	0.000
Six weeks	BG	DFDBA	380.133	0.094
		DBM	-476.000*	0.035
		Control	2070.000*	0.000
	DFDBA	BG	-380.133	0.094
		DBM	-821.000*	0.001
		Control	1688.000*	0.000
	DBM	BG	476.000*	0.035
		DFDBA	821.000*	0.001
		Control	2542.123*	0.000
Twelve weeks	BG	DFDBA	699.000	0.193
		DBM	-1513.000*	0.005
		Control	5299.000*	0.000
	DFDBA	BG	-699.000	0.193
		DBM	-2341.300*	0.000
		Control	4588.123*	0.000
	DBM	BG	1513.000*	0.005
		DFDBA	2341.300*	0.000
		Control	6822.222*	0.000

* The mean difference is significant at the 0.05 level.
BG; bioglass, DFDBA; demineralized freeze-dried bone allograft, DBM; demineralized bone matrix Grafton putty
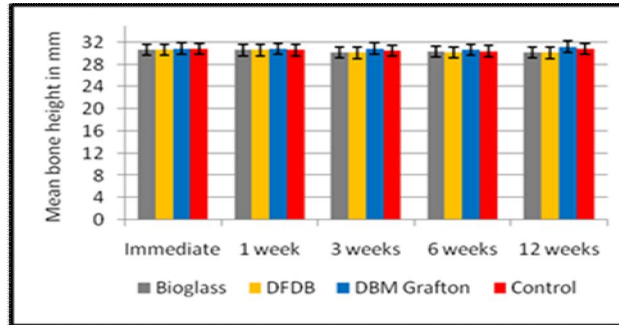
Figure (6): Mean bone height (mm) at different time intervals in grafted and un-grafted groups

A split-mouth design was followed where right or left mandibular third premolar sockets in both study animals groups were randomly assigned to receive bone graft material. In G-1 animals, right mandibular third premolar socket was grafted with BG and the left mandibular third premolar socket of the same animal was grafted with DFDBA. In G-2, right mandibular third premolar socket was grafted with Grafton DBM putty and the left mandibular third premolar socket of the same animal was left un-grafted served as control. To measure the osteoconductive and/or osteoinductive potential of BG, DFDBA and DBM, the amount of bone produced at an implantation site can be quantified by histomorphometry.

Grafton DBM putty-treated sockets showed statistically increased count of new bone trabeculae, as well as, increased size and total area of new bone trabeculae at one, 3, 6 and 12 weeks post-extraction than the BG-treated sockets, the DFDBA-treated sockets, or the control sockets $(P < 0.05)$. Although the count of new bone trabeculae (at one and 6 weeks post-extraction), as well as, average size of new bone trabeculae (at 3 and 12 weeks post-extraction) and total area of new bone trabeculae (at one, 6 and 12 weeks post-extraction) of the DFDBA-treated sites was greater than the BG - treated sites, this difference was not statistically significant. Unfilled sockets served as controls showed the statistically significant lowest count of new bone trabeculae, as well as, average size and total area of new bone trabeculae at one, 3, 6 and 12 weeks post-extraction

In the current study, although the mean alveolar bone height (measured radiographically from the crest of the alveolar ridge at the grafted sockets to the inferior border of the mandible) of the Grafton DBM putty-treated sockets was greater than the DFDBA - treated sites, BG - treated sites, or the control sockets throughout all time intervals, this difference was not statistically significant (P > 0.05). The result of the present study was in agreement with Darby et

al.,(2009) who reviewed the techniques and outcomes of post-extraction ridge preservation and the efficacy of these procedures in relation to subsequent implant placement. They concluded that despite the heterogeneity of the studies, ridge preservation procedures are effective in limiting horizontal and vertical ridge alterations in post-extraction sites. There is no evidence to support the superiority of one grafting material over another.

Results of the present study demonstrated an increased count of new bone trabeculae, as well as, average size of new bone trabeculae and total area of new bone trabeculae bone throughout all time intervals in the BG-treated sockets than the un-grafted sockets. This might be attributed to the osteoconductive nature of BG particles as a direct chemical bond by BG to bone has been shown in previous studies, Schepers et al., (1991). Moreover, the absorbable BG material has been shown to be biocompatible and non toxic. It has been postulated that BG particles implanted into organic tissues were transformed by a specific ion exchange process responsible for their osteoconductive, osteointegrative, and osteostimulatory properties. Initially, a silica-rich gel layer was formed, upon which an in situ calcium phosphate layer was gradually precipitated. Subsequently, organic species were incorporated into this bio-actively developing layer, and osteoblasts were attracted to form new bone attached to the particles' surface. At the same time, fissures and lacunae forming in the particles enable osteoprogenitor cells to enter within this protected space and differentiate into osteoblasts, which form new bone without any connection with bone tissue outside the particles; this unique response of BG to biological tissues and fluids has been called osteostimulatory property, Low et al., (1997); Shapoff et al., (1997); Froum et al., (1998).

Clinical and radiographic investigations have suggested the efficacy of BG particles in the treatment of human bone defects resulting from

periodontal disease, cyst resection, or apicectomy, as well as, in the maintenance of alveolar bone ridge and recuperation of atrophic alveolar processes for placement of osseointegrated implants, Low et al.,(1997); Han et al.,(2002). Furusawa and Mizunuma (1997) utilized BG in the repairing of surgically created bony defects in the rat mandible and found osteoconductive bone growth around the particles by 4 weeks. They reported that BG materials of small particle size distribution were claimed to be osteoconductive and resorbable. Cancian et al., (1999) compared BG, 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 post-surgery, 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 almost all of the BG particles were absorbed and replaced by newly formed bone.

In the current study the count of new bone trabeculae, as well as, average size of new bone trabeculae and total area of new bone trabeculae of the DFDBA-treated sockets was greater than the BG -treated sockets. This might be attributed to the osteoconductive and osteoinductive nature of DFDBA particles. DFDBA was believed to induce bone formation due to the influence of bone-inductive proteins called BMPs exposed during the demineralization process. Thirteen proteins have been identified (BMP1-BMP13) as osteoinductive compounds and encourage new bone formation, Hoexter (2002). DFDBA is therefore thought to be osteoinductive and osteoconductive, Boyanet al., (2006). The amount of BMPs in any single allograft has shown dramatic variability, Schwartz et al., (1998); Minichetti et al., (2004). Schwartz and colleagues (1996) have shown that there is a wide variety of DFDBA products on the market which have different inductive capabilities. These differences might be related to the origin and methods of preparation of DFDBA and if the preparation methods were the same in different bone banks, this would be due to individual donors' ages and sexes, disease and injury, medical treatment or genetic differences. There were many differences in size and the surface shape of DFDBA particles that may affect their inductive ability. Bone cells distinguished different surface shapes and roughness and this would lead to differences in phenotypic diversity, Martin et al., (1995).

DBM-based formulations became available for clinical use in 1991. Several factors are expected to influence the osteoinductive properties of a DBM,

including the concentration of osteoinductive proteins in the bone matrix of the individual donor, the intrinsic osteoinductive potential of the individual donor, and the nature of the host response and implantation site. Processing procedures also are known to play an important role in the osteoinductive properties of a DBM. Pre-process handling, varying demineralization times and final particle sizes are among the many factors that may affect osteoinductivity, Syftestad et al.,(1979); Guo et al.,(1991). Bae et al (2006) conducted a study to evaluate and compare the quantity of BMPs among several different DBM formulations (inter product variability), as well as, examine the variability of these proteins in different production lots within the same DBM formulation (intra-product variability). They reported that it was essential to relate osteoinductivity of a commercial compound to the methods of sterilization and processing, and to the relative proportion of BMPs remaining in the DBM product.

In the current study, Grafton DBM putty offered many advantages over BG and DFDBA. It was found to be superior in terms of enhancement of bone generation and graft material resorption. These findings was confirmed by statistically increased count of new bone trabeculae, as well as, increased size and total area of new bone trabeculae compared with BG and DFDBA at one, 3, 6 and 12 weeks post-extraction. The histological analysis of the sockets grafted with Grafton DBM putty showed concentric lamellar structures (Haversian system) in the bone tissue formed around and in place of the of the graft. Islands of woven bone and osteoblasts were observed suggesting that the mechanism of bone formation is similar to that of natural bone remodeling process. The results of the current study was in agreement with Babush (2003) study which indicated that demineralised bone matrix putty, when used in patients for dental augmentation in either mandibular or maxillary sites, resulted in replacement of the graft material with bone by as early as 4 months, there upon enabling implant placement and subsequent prosthetic reconstruction.

In our study we succeeded to overcome problems with handling and containing DBM particles which have limited the exclusive use of this material, as our material had a putty form. Maintenance of the graft material within the defect site was of paramount importance. Any migration of particles from the area could compromise the graft success because of inadequate regeneration of the defect and potential ectopic bone formation.

In conclusion, the implantation of BG, DFDBA, and Grafton DBM putty as graft materials in extraction sockets for ridge preservation was

accompanied by varying degrees of bone formation in the extraction socket as well as a host response. This response was dependent on the morphology and chemical composition of the biomaterial. All used graft materials were biocompatible and biodegradable. Grafton DBM putty seemed to be an ideal graft material in extraction sockets as it was simple to use, effective, superior osteoconductivity, providing scaffold for new bone to build up for healing process. Efficacy of Grafton DBM putty might relate to methods of demineralization or to some other factor, such as the concentration of graft material per unit volume or the nature of the carrier. Demineralised bone matrix putty, when used for extraction sockets grafting, resulted in replacement of most of the graft material with bone.

5. REFERENCES

1. Andre RG, Josepb ML, David G and Robert AF. Alternatives to autogenous bone graft: Efficacy and indications. J Acad Orthop Surg 3: 1, 1995.
2. Ashman A, Froum S and Rosenlicht J. Replacement therapy. Int J Prothodont 15:12, 1994.
3. Babbush CA. The use of a new allograft material for osseous reconstruction associated with dental implants. Implant Dentistry 7:205, 1998.
4. Babush CA, Kerry SV, Jacobson MS. An in vitro and in vivo evaluation of autologous platelet concentrates in oral reconstruction. Implant Dentistry. 24–34, 2003.
5. Babush CA. Histologic Evaluation of Human Biopsies after Dental Augmentation with Demineralized Bone Matrix Putty. Implant Dent 12:325, 2003.
6. Bae, H., Zhao, L., Linda E., Wong, P., BS, Rick B. Delamarter, MD, and Dawson, E. Intervariability and Intravariability of Bone Morphogenetic Proteins in Commercially Available Demineralized Bone Matrix Products. Spine. 31:1299, 2006.
7. Becerra J, Andrades JA, Ertl DC, Sorgente N, Nimni ME. Demineralized bone matrix mediates differentiation of bone marrow stromal cells in vitro: Effect of age of cell donor. J Bone Miner Res 11:1703, 1996.
8. Bendall SW. Effect of particulate bioactive glass on human synoviocyte cultures. J Biomed Mater Res 29: 359, 1995.
9. Becker W, Urist M, Becker B, et al. Clinical and histologic observations of sites implanted with intraoral autologous bone grafts and allografts. 15 human case reports. J Periodontol 67:1025, 1996
10. Becker W, Becker BE, Caffesse R. A comparison of demineralized freeze-dried bone and autologous bone to induce bone formation in human extraction sockets. J Periodontol 65:1128, 1994
11. Boyan B.D., Ranly D.M., Schwartz Z.: Use of growth factors to modify osteoinductivity of demineralized bone allografts: lessons for tissue engineering of bone. Dent Clin North Am 50, 217, 2006
12. Byrne DJ, Hardy J, Wood RAB. Effect of fibrin glues on the mechanical properties of healing wounds. Br J Surg 78:841, 1991.
13. Camargo PM, Lekovic V, Weinlaender M. Influence of bioactive glass changes in alveolar process dimensions after exodontia. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 90: 581, 2000.
14. Carmagnola D, Adriaens P, Berglundh T. Healing of human extraction sockets filled with Bio-Oss. Clin Oral Implants Res 14:137, 2003.
15. Callon DP, Salkaled SL. Histological analysis of implant sites after grafting with DBM putty and sheets, Part II. Implant Dent 9:59, 2000.
16. Cancian DC, Hochuli-Vieira E, Marcantonio RA, Marcantonio E Jr. Use of BioGran and Calcitite in bone defects: Histologic study in monkeys (Cebus apella). Int J Oral Maxiliofac Implants 14:859, 1999.
17. Christensen GJ. Ridge preservation: why not? J Am Dent Assoc 127:669, 1996.
18. Christopher JD, Russel JP, Annamarie BP, Huismans F, Ralph E H. Effect of demineralized bone matrix on bone growth within a porous HA material: A histologic and histometric study. J App Biomat 9:275, 1995.
19. Coulson R, Clokie CML, Peel S. Collagen and a Thermally Reversible Poloxamer Deliver Demineralized Bone Matrix (DBM) and Biologically Active Proteins to Sites of Bone Regeneration. Bone Symposium; Portland, OR; 1999.
20. Douglass G, Alveolar ridge preservation at tooth extraction. CDA J 33:223, 2005
21. Darby I., Stephen T., Buser D., Dent M. Ridge Preservation Techniques for Implant Therapy. J Oral Maxillofac Implants 24: 260, 2009.
22. Fiorellini J, Nevins M. Localized ridge augmentation/preservation. A systematic review. Ann Periodontol 8:321, 2003.
23. Froum S, Cho SC, Rosenberg E, Rohrer M, Tarnow D. Histological comparison of healing extraction sockets implanted with bioactive glass or demineralized freeze-dried bone allograft: A pilot study. J Periodontol 73:94, 2002.
24. Furusawa T, Mizunuma K. Osteoconductive properties and resorbable bioactive glass as a bone-grafting material. Implant Dent 62:93, 1997.
25. Glowacki J, Altobelli D, Mulliken JB. Fate of mineralized and demineralized osseous implants in cranial defects. Calcif Tissue Int 33:71, 1981.
26. Guarnieri R, Pecora G, Fini M. Medical grade calcium sulfate hemihydrate in healing of human extraction sockets: Clinical and histological observations at 3 months. J Periodontol 75:902, 2004
27. Guo MZ, Xia ZS, Lin LB. The mechanical and biological properties of demineralised cortical bone allografts in animals. J Bone Joint Surg Br 73:791, 1991.
28. Harakas NK. Demineralized bone-matrix-induced Osteogenesis. Clin Orthop 188:239, 1984.
29. Hass HJ, Krause H: Bone formation using DBM Grafton for the treatment of bone cysts in children. Eur j Pediatr Surg; 17 (1):45, 2007.
30. Han J, Meng H, Xu L. Clinical evaluation of bioactive glass in the treatment of periodontal intrabony defects. Zhonghua Kou Qiang Yi Xue Za Zhi 37:225, 2002
31. Hench LL, LaTorre GP. The kinetics of bioactive ceramics part III: Surface reactions for bioactive glasses compared with an inactive glass. Bioceramics 4: 156, 1991.
32. Hollinger J.O., Schmitz J.P., Mizgala J.W., Hassler C.: An evaluation of two configurations of tricalcium phosphate for treating craniotomies. J Biomed Mater Res 23, 17, 1989.
33. Hoexter D.L. Osseous regeneration in compromised extraction sites: a ten-year case study. J Oral Implantol 28, 19, 2002
34. Iasella JM, Greenwell H, Miller RL. Ridge preservation with freeze-dried bone allograft and a collagen membrane compared to extraction alone for implant site development: A clinical and histologic study. J Periodontol 74:990, 2003
35. Johnson MW, Sullivan SM, Rohrer M and Collier M. Regeneration of peri-implant infra-bony defects using Perioglass: A pilot study in rabbits. Int J Oral Maxillofac Impl 12:825, 1997.
36. Johnson MW, Sullivan SM, Rohrer M and Collier M. Regeneration of peri-implant infra-bony defects using

Perioglass: A pilot study in rabbits. Int J Oral Maxillofac Impl 12:825, 1997.

37. Kusumoto K, Bessho K, Fujimura K, Ogawa Y, and Lizuka T. Intramuscular osteoinduction and bone marrow formation by the implantation of rhBMP-2 with type I collagen. Br J Oral Maxillofac Surg 35: 433, 2000.

38. Krauser JT, Garg AK. Membrane barriers for guided tissue regeneration. In: Dental Implants: The Art and Science. Philadelphia: WB Saunders 133, 2001.

39. Landesberg B, Roy M, Glickman RS. Quantification of growth factor levels using a simplified method of platelet-rich plasma gel preparation. J Oral Maxillofac Surg 58:297, 2000.

40. Lindsey RW, Wood GW: Grafting long bone fractures with demineralized bone matrix putty enriched with bone marrow: Orthopedics 29:939,2006.

41. Low SB, King CJ, and Krieger T. An evaluation of bioactive ceramic in the treatment of periodontal osseous defects. Int J Peridont Rest Dent 17: 359, 1997.

42. Martin JY, Schwartz Z, Hummert TW, Schraub DM, Simpson J, Lankford J Jr, et al. Effect of titanium surface roughness on proliferation, differentiation, and protein synthesis of human osteoblast-like cells (MG63). J Biomed Mater Res 29(3): 389, 1995.

43. Mellonig JT: Histological and clinical evaluation of allogenic bone matrix for treatment of periodontal osseous defects. Int J Periodontics Restorative Dent 26:561, 2006.Wozney JM. Growth factors influencing bone development. J Cell Sci 13(suppl):149, 1990.

44. Minichetti J.C., D'Amore J.C., Hong A.Y.J., Cleveland D.B.: Human histologic analysis of mineralized bone allograft (PUROS) placement before implant surgery. J Oral Implantol 30, 74, 2004.

45. Molly L, Vandromme H, Quirynen M, Schepers E, Adams JL, van Steenberghe D. Bone formation following implantation of bone biomaterials into extraction sites. J Periodontol 79: 1108, 2008.

46. Mulliken JB, Glowacki J. Induced Osteogenesis for repair and construction in the craniofacial region. Plast Reconstr Surg 65:553, 1980.

47. Nevins M, Camelo M, De Paoli S. A study of the fate of the buccal wall of extraction sockets of teeth with prominent roots. Int J Periodontics Restorative Dent 26:19–29, 2006.

48. Norton M, Wilson J. Dental implant placed in extraction sites implanted with bioactive glass: Human histology and clinical outcome. Int J Oral Maxillofac Implants17:249, 2002.

49. Oonishi H, Hench LL and Wilson J: Comparative bone formation in several kinds of bioceramic granules. Bioceramics 8:137, 1995.

50. Pals SD, Wilkins RM. Giant cell tumor of bone treated by curettage, cementation, and bone grafting. Orthopedics 15:703, 1992

51. Pinho MN, Roriz VL, Novaes AB. Titanium membranes in prevention of alveolar collapse after tooth extraction. Implant Dent 15:53–61, 2006.

52. Rummelhart JM, Mellonig JT, and Gray JL. A comparison of freeze-dried bone allograft and demineralized freeze-dried bone allograft in human periodontal osseous defects. J Periodontol 60: 655, 1989.

53. Ripamonti U, Magan A, Ma S, van den Heever B, Moehl T, Reddi AH. Xenogeneic osteogenin, a bone morphogenetic protein, and demineralized bone matrices, including human, induce bone differentiation in athymic rats and baboons. Matrix 11:404, 1991.

54. Schwartz Z, Mellonig JT, Carnes DL Jr., de la Fontaine J, Cochran DL, Dean DD, et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. J Periodontol 67(9): 918, 1996

55. Schepers E, De Clercq M, Ducheyne P, Kempeneers R. Bioactive glass material as filler for bone lesions. J Oral Rehabil 18:439, 1991.

56. Schwartz Z.; Somers A.; Mellonig J.T.; Carnes D.L. Jr., Wozney J.M., Dean D.D. et al.: Addition of human recombinant bone morphogenetic protein-2 to inactive commercial human demineralized freeze-dried bone allograft makes an effective composite bone inductive implant material. J Periodontol 69, 1337, 1998.

57. Shapoff CA, Alexander DC, Clark AE. Clinical use of a bioactive glass particulate in the treatment of human osseous defects. Compend Educ Dent 18:352, 1997.

58. Stanley HR, Ha UB, Clark AE, Colaizzi F C. Residual alveolar ridge maintenance with a new endosseous implant material. J Proth Dent 58:607, 1987.

59. Sonis ST, Kaban LB, Glowacki J. Clinical trial of demineralized bone powder in the treatment of periodontal defects. J Oral Med 38:117, 1983.

60. Syftestad G, Urist MR. Degradation of bone matrix morphogenetic activity by pulverization. Clin Orthop 141:281, 1979

61. Tamimi FM, Torres J, Tresguerres I, Clemente C, Cabarcos EL. Bone augmentation in rabbit calvariae: comparative study between Bio-Oss® and a novel β-TCP/DCPD granulate. J. Clin. Periodontol 33, 922, 2006

62. Takikawa S, Bauer T, Kambic H, Togawa D. Comparative evaluation of the osteoinductivity of two formulations of human demineralized bone matrix. J Biomed Mater Res 65A: 37, 2003.

63. Tiedeman JJ, Huurman WW, Connolly JF, Strates BS. Healing of a large non ossifying fibroma after grafting with bone matrix and marrow. A case report. Clin Orthop 265:302, 1991.

64. Torricelli P, Fini M, Giavaresi G, Giardino R. In vitro osteoinduction of demineralized bone. Artif Cells, Blood Subs, Immob Biotechnol 26:309–315, 1998.

65. Urist M.R., Lietz A., Dawson E.: Beta-tricalcium phosphate delivery system for bone morphogenetic protein. Clin Orthop 187, 277, 1984.

66. Vallet R M. study in vitro growth of an appetite-like layer on sol-gel glasses. J Biomed Mater Res 44: 416, 2004.

67. Vance GS, Greenwell H, Miller RL, Hill M, Johnston H, Scheetz JP. Comparison of an allograft in an experimental putty carrier and a bovine-derived xenograft used in ridge preservation: A clinical and histologic study in humans. Int J Oral Maxillofac Implants 19:491, 2004.

68. Vasilic N, Henderson R, Jorgenson T, Sutherland E, Carson R. The use of bovine porous bone mineral in combination with collagen membrane or autologous fibrinogen/fibronectin system for ridge preservation following tooth extraction. J Okla Dent Assoc 93:33, 2003.

69. Wang HL, Tsao YP. Histologic evaluation of socket augmentation with mineralized human allograft. Int J Periodontic Restorative Dent 28:231,2008

70. Weitraub J and Burt B. Oral health status in the United States: Tooth loss and edentulism. J Dent Educ 49: 368, 1985

71. Wilson J, Low SB. Bioactive ceramics for periodontal treatment: Comparative studies in the monkey. J App Biomat 3: 1129, 1992.

72. Wilson J, Clark AE, and Hall M: Tissue response to bioglass endosseous ridge maintenance implants. J Oral Impl; 19: 295, 1993.

73. Yilmaz S, Efeog˘ lu E, Kiliç AR. Alveolar ridge reconstruction and/or preservation using root form bioglass cones. J Clin Periodontol 25:832, 1998.

74. Zambonin G, Grano M. Biomaterials in orthopaedic surgery: effects of different hydroxyapatites and demineralized bone matrix on proliferation rate and bone matrix synthesis by human osteoblasts. Biomaterials 16:397, 1995.

8/11/2011