Comparative clinical and histological analysis of demineralized bone matrix putty and bioactive glass for fresh extraction socket preservation

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Abstract:

The goal of the study was to compare and evaluate the influence of bioactive glass against demineralized bone matrix putty in enhancing natural bone regeneration by preserving the extraction socket. Twenty patients with teeth having a hopeless prognosis and indicated for extraction were selected. Socket preservation was done by the placement of demineralized bone matrix putty (group I) and bioactive glass (group II) after the extraction. Following extraction and placement of graft material, collagen plug, a bioabsorbable dressing material was kept covered over the remaining 1-2 mm augmented extraction socket. Various parameters evaluated were plaque index, modified gingival index, early wound healing index and buccolingual ridge width. After 6 months, the bone sample was collected by trephine bur and sent for histological analysis. Histomorphometric analysis was also done. Statistical significance was defined as $p<0.05$. Significant improvement was seen in terms of plaque index, modified gingival index, early wound healing index, and buccolingual ridge width from baseline to 6 months in intragroup comparison. But both groups showed no statistically significant difference in intergroup comparison for the same parameters. Histomorphometric analysis, on the other hand, showed a statistically significant difference in the mean values of vital bone and provisional matrix between both groups. Both the graft materials

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produced adequate bone formation and successfully preserved the ridge. However, on histomorphometric analysis, demineralized bone matrix putty showed enhanced bone regeneration.

Keywords Bone augmentation, Biomaterials, Bioactive-glass, Demineralized bone matrix, Osteoinductivity, Tooth socket
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Introduction: The alveolar bone and surrounding soft tissues are destroyed during the painful process of tooth extraction (Caplanis N et al., 2005). This is linked to a complex chain of biochemical and histologic events that occur during the wound healing process, resulting in physiologic changes to the alveolar bone and soft-tissue architecture (Amler., 1969) (Araujo MG., 2005). An otherwise healthy person may get alveolar ridge resorption after having teeth extracted. The alveolar ridge will frequently lose volume and undergo morphological changes as a result of the disorder, which appears to be gradual and irreversible (Irinakis T., 2006) (Bartee BK., 2001). The literature describes up to a 50% drop in bone volume within a year after dental extractions, with two-thirds of that decline occurring in the first three months (Lam, R.V.., 1960) (Chen ST et al., 2004) (Schropp L et al., 2003). With today's aesthetic-conscious population, many patients find the idea of simply extracting a tooth and replacing it later unacceptable (Fowler EB et al., 2004)

Within the past decade, more emphasis has been given to aesthetics, which is influenced by the resorption of the alveolar ridge after tooth extraction. A ridge form with such flaws will not allow for appropriate prosthetic fabrication or endosseous implant placement. To avoid this clinical situation, various authors have described a variety of surgical procedures, ranging from atraumatic tooth extraction to regenerative techniques for socket preservation. Considering the amount of resorption after extraction, various therapeutic strategies have been proposed to prevent or minimise alveolar ridge collapse over the past three decades (Camargo PM et al., 2000). Ridge preservation is a clinical procedure used during tooth extraction to reduce socket wall bone resorption. As a result, studies in which fresh extraction sockets were filled and covered with various bone substitutes and membranes have been conducted (Iasella JM et al., 2003).

Demineralized bone matrix (DBM) is a cadaver bone-derived biomaterial that has been proposed as an alternative to autografts. It is an inexhaustible source of grafts, and it can be mixed with autologous bone as well as growth factors like bone morphogenic protein 2 and fibroblast growth factor. Furthermore, it reduces surgery time and eliminates the possibility of donor site morbidity. High costs, potential immune adverse reactions, and disease transmission are some of the drawbacks of using a demineralized bone matrix. Urist discovered that implantation of demineralized bone tissue in other tissues causes outbreaks of bone development, a phenomenon known as osteoinduction. Recently, it has been established that endogenous morphogen, in the form of morphogenetic proteins, are responsible for the osteoinductive potential of the demineralized bone matrix (bone morphogenetic proteins) (Madrid JR et al., 2014). There have been various studies where the demineralized bone matrix has been used for periodontal bony defects (Banjar AA et al., 2013). extraction sockets (El-Chaar ES., 2013), and alveolar cleft management (Madrid JR et al., 2014). with satisfactory results in cases with a periodontal bony defect and its use in extraction sockets.

Among these grafting materials, Hench and colleagues developed a limited group of surface reactive glass ceramics (calcium sodium phospho silicates), including the original bioactive glass (BG), in the late 1960s. The most important characteristics of BG are its proven history of biocompatibility and its ability to act quickly as a biomimetic mineraliser, matching the mineralizing traits

of the human skeleton (Profeta AC et al., 2015). Clozza E et al 2012 have successfully demonstrated 77% of alveolar width dimensions with less bone loss in width than vertical bone loss. but in one other study, it was concluded that grafting material in fresh extraction sockets delays the healing process of alveolar bone (Clozza E et al ., 2014). Ioannou et al concluded in a review of the literature that when used for ridge preservation, bio-active glass yields a high percentage of true bone regeneration; however, no reliable controlled studies have reported histological outcomes from the use of bioactive glass in ridge augmentation procedures (Ioannou AL et al .,2015).

Thus, the primary goal of this study is to compare and evaluate the influence of bioactive glass versus demineralized bone matrix putty in enhancing natural bone regeneration, as measured by their ability to preserve the fresh extraction socket and alveolar ridge dimensions after tooth extraction, thereby limiting ridge resorption, in order to maximize the bone available for ideal implant placement.

Materials and Methods: In this study, 20 patients were chosen in the outpatient Department of Periodontology and Implantology of the institute with a tooth that had a bleak prognosis and required tooth extraction followed by implant insertion. Before the procedure, the patient gave their informed consent, and the study was given the go-ahead by the institution's pertinent ethics committee.

These patients were randomly divided into two groups.

Group I: Demineralized bone matrix putty

Group II: Bioactive glass

Patients in the age range of 18-60 years of either sex, systemically healthy patients, extraction socket with intact wall configuration, patients who desired to participate in the study, patients planning for delayed implant placement were all considered for inclusion, while patients with acute infection at the time of extraction, subjects with any major systemic illness, cigarette smokers, and patients with uncontrolled periodontal disease were excluded.

Materials used were DBM putty (Steriigraff™ dental putty, Bone Bank Allograft, San Antonio, TX, USA), bioactive glass (Perioglass™ US Biomaterials Corp., Alachua, FL, USA), and commercially available collagen plug (CollaPlug™, Eucare Pharmaceuticals, Chennai, India).

All selected patients were informed about the surgical procedures, the frequency of visits, the importance of maintaining oral hygiene, and the importance of follow-up visits. The patient's detailed case history was obtained. Scaling and root planing were performed as part of the preparation phase.

Following this phase and achievement of good oral hygiene patients signed a written consent form regarding tooth extraction, socket preservation and biopsy procedure followed by implant placement.

Strict aseptic procedures were followed during a surgical procedure. This comprised an extraoral povidone-iodine skin scrub and 0.2 per cent chlorhexidine mouth rinses for the patient's oral cavity prior to surgery. Operations were carried out under local anaesthesia.

The nerve block was administered with 2 percent lignocaine in 1:80000 adrenaline, depending on the site. Tooth extraction was done atraumatically. After extraction, the socket was thoroughly debrided (Figure 1a, 1b, 2a, 2b). All clinical measurements were taken. After that, the graft material was gradually but firmly placed into the extraction socket. DBM putty was used to graft a socket with complete bone walls in group 1 patients. (Figure 1c) and bioactive glass in group 2 patients in 1-2 mm apical to bone level to enhance preservation of the alveolar bone (Figure 2c). A condenser was used for lightly condensing the graft material. Heavy condensing of the graft was avoided to prevent the compromise of the vascularity within the socket and subsequent bone healing. Collagen plug, a bioabsorbable dressing material, covered the remaining 1-2 mm augmented extraction socket over the graft material to compensate for bone remodelling, soft tissue thickness, and

hemostasis. To close the margins and aid in the retention of the graft material placed in the socket, a cross mattress suture made of non-resorbable silk was placed on top of the collagen plug.

Patients were given systemic antibiotics (Cap Amoxicillin 500 mg three times daily for five days) and anti-inflammatory drugs (Tab Ibuprofen 400mg + Tab Paracetamol 325 mg three times daily for five days) following surgery. A bacterial contamination control protocol of 0.2 per cent chlorhexidine mouth rinse once daily for two weeks was prescribed. Patients were asked to refrain from brushing or chewing in the treated area for two weeks. The healing process was monitored weekly for the first month and then every month for the next six months.

Plaque index, modified gingival index, early wound healing index, and buccolingual ridge width were the clinical criteria used (Ridge calliper was used to measure the thickness of buccolingual ridge width to the nearest 0.5 mm at a distance of 2 mm apical to the crest of the bone at the mesiodistal midpoint of the extraction socket) (Figure 3a).

In this study, after socket preservation, surgical re-entry was done after 6 months. At this time bone samples were collected using trephine bur for histological processing at the time of osteotomy preparation followed by implant placement (Figure 3b, 3c). A bone biopsy was sent to the department of oral pathology for histopathological analysis. Biopsy samples were fixed in 10% neutral buffered formalin for 12 hours, decalcified in ethylenediamine tetraacetic acid for 4 hours, dehydrated in ethanol and embedded in paraffin. The specimen was cut in the apico-coronal plane, 4 um thick, and stained with haematoxylin and eosin stains. New bone was evaluated in all histological sections. Vessels in intratrabecular mesenchyma, containing erythrocytes and surrounded by mild infiltration of plasma cells were looked for, in all sections for histological analysis. Residual graft particles were also evaluated in the specimen for histological analysis. The presence of tightly packed mesenchymal cells in the collagen-rich connective tissue matrix was also examined in the histological sections. (Figure 4a, 4b).

The statistical method used - By using repeated measure ANOVA, the intragroup comparison of the plaque index (PI) and modified gingival index (MGI) in groups I and II at baseline, three months, and six months was examined. Unpaired T-test was used to evaluate the intergroup comparison of PI & MGI at baseline, 3 months, and 6 months. The Friedman test was used to compare the Early Wound Healing Index between groups I and II at 1 week, 2 weeks, and 4 weeks. The Mann-Whitney U test was used to compare the data between groups. A paired T-test was used to compare the buccolingual ridge width between groups I and II at baseline and after six months, but an unpaired T-test was used to compare the same between groups. Groups I and II's histomorphometric analysis was carried out using an unpaired T-test. Software called SPSS version 22 was used to examine the data (IBM Co., Chicago, IL, USA)

Results: Results were analyzed in two ways, firstly intragroup analysis of group I and group II were done for all the indexes and thereafter intergroup analysis of the same data was done between group I and group II.

In intragroup analysis, mean plaque index score was 0.47±0.08 at baseline, 0.57±0.11 at 3 months and 0.66±0.08 at 6 months. mean plaque index score was 0.55±0.16 at baseline, 0.60±0.15 at 3 months and 0.67±0.17 at 6 months. Statistically, a significant difference was present in the change of value at various times in group I and group II. Similarly, in the mean gingival index score, the results were statistically significant. For group I the values were 0.61±0.26 at baseline, 0.76±0.21 at 3 months and 0.84±0.19 at 6 months, while for group II the score was 0.61±0.19 at baseline, 0.70±0.18 at 3 months and 0.81±0.14 at 6 months. Both the groups showed considerable healing which was evident through the data also, the mean early wound healing index scores were 2.10±0.87 at 1 week, 1.60±0.69 at 2 weeks, and 1.10±0.31 at

3 weeks for group I and for group II the scores were 2.30±1.16 at 1 week, 1.70±0.67 at 2 weeks, and 1.10±0.31 at 3 weeks. Lastly, the mean buccolingual ridge width score was 9.60±1.50 at baseline and 8.4±1.17 at 6 months for group I and 9.80 ± 1.54 at baseline and 8.00±1.24 at 6 months for group II. Here too, a statistically significant difference was present in the change of value at various times in both the groups.

Plaque index and modified gingival index intergroup comparisons between the two groups did not reveal any statistically significant differences at baseline, 3 months, or 6 months in either group (Table 1 and 2). Buccolingual width displayed comparable mean values as well, although there was no statistically significant difference between baseline and six months later (Table 3). The early wound healing index did not show a statistically significant difference in either group at 1, 2, or 4 weeks (Table 4), although histomorphometric analysis revealed a difference. The mean values of the vital bone and provisional matrix were statistically significantly different between the two groups. According to statistics, there was no discernible difference between the two groups in the residual graft. (Table 5).

Table 1. Intergroup comparison of plaque index
PI wise distribution between Group I and Group II (Unpaired T-test)

Time	Groups	Number	PI		p Value
			Mean	SD	
Base line	Group I	10	0.47	0.08	>0.05
	Group II	10	0.55	0.16	
3 Months	Group I	10	0.57	0.11	>0.05
	Group II	10	0.60	0.15	
6 Months	Group I	10	0.66	0.08	>0.05
	Group II	10	0.67	0.17	

PI - Plaque Index
SD - Standard Deviation

Table 2. Intergroup comparison of modified gingival index GI wise distribution between Group I and Group II (Unpaired T-test)

Time	Groups	Number	GI		P Value
			Mean	SD	
Base line	Group I	10	0.61	0.26	>0.05
	Group II	10	0.61	0.19	
3 Months	Group I	10	0.76	0.21	>0.05
	Group II	10	0.70	0.18	
6 Months	Group I	10	0.84	0.19	>0.05
	Group II	10	0.81	0.14	

GI - Gingival Index
SD - Standard Deviation

Table 3. Intergroup comparison of Early wound healing index Early wound healing index wise distribution between Group I and Group II (Mann Whitney U test)

Time	Groups	Number	EWHI		p Value
			Mean	SD	
1 Week	Group I	10	2.10	0.87	>0.05
	Group II	10	2.30	1.16	
2 Week	Group I	10	1.60	0.69	>0.05

	Group II	10	1.70	0.67	
4 Week	Group I	10	1.10	0.31	>0.05
	Group II	10	1.10	0.31	

EWHI - Early wound healing Index
SD - Standard Deviation

Table 4. Intergroup comparison of bucco lingual ridge width
Bucco lingual ridge widthwise distribution between Group I and Group II (Unpaired T-test)

Time	**Groups**	**Number**	**BLRW**		**p Value**
			Mean	**SD**	
Base line	Group I	10	9.60	1.50	>0.05
	Group II	10	9.80	1.87	
6 Months	Group I	10	8.40	1.17	>0.05
	Group II	10	8.00	1.50	

BLRW - Bucco lingual ridge width
SD - Standard Deviation

Table 5: Histomorphometric analysis of Group I and Group II
(Unpaired T-test)

Histomorphometric type	**Group I**	**Group II**	**p Value**
	Mean ± SD	**Mean ± SD**	

Vital Bone	54.61 ± 3.16	47.74 ± 3.78	≤ 0.005*
Provisional matrix	37.03 ± 2.88	43.75 ± 4.33	≤ 0.005*
Residual Graft	8.36 ± 3.16	8.51 ± 4.25	> 0.005

SD - Standard Deviation
***** Statistically significant difference

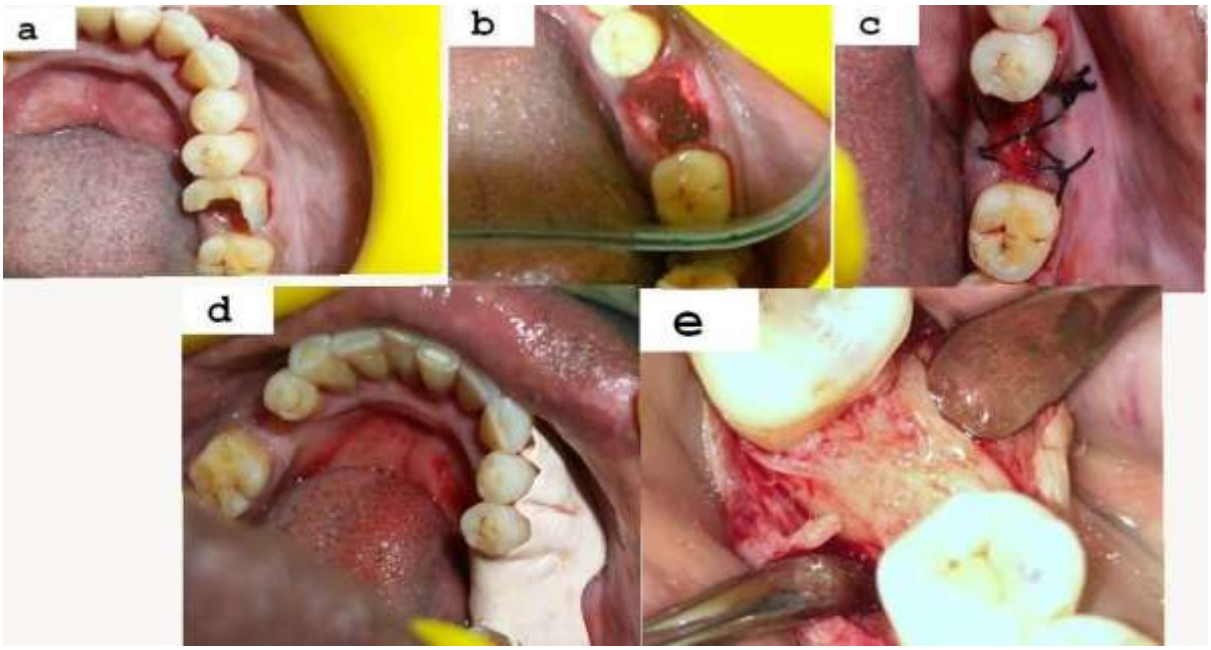

Figure 1a Preoperative
Figure 1b Extraction socket after atraumatic extraction
Figure 1c Demineralized bone matrix putty and collagen plug placed in the extraction socket
Figure 1d Periodontal dressing placed
Figure 1 e Bone formation after 6 months

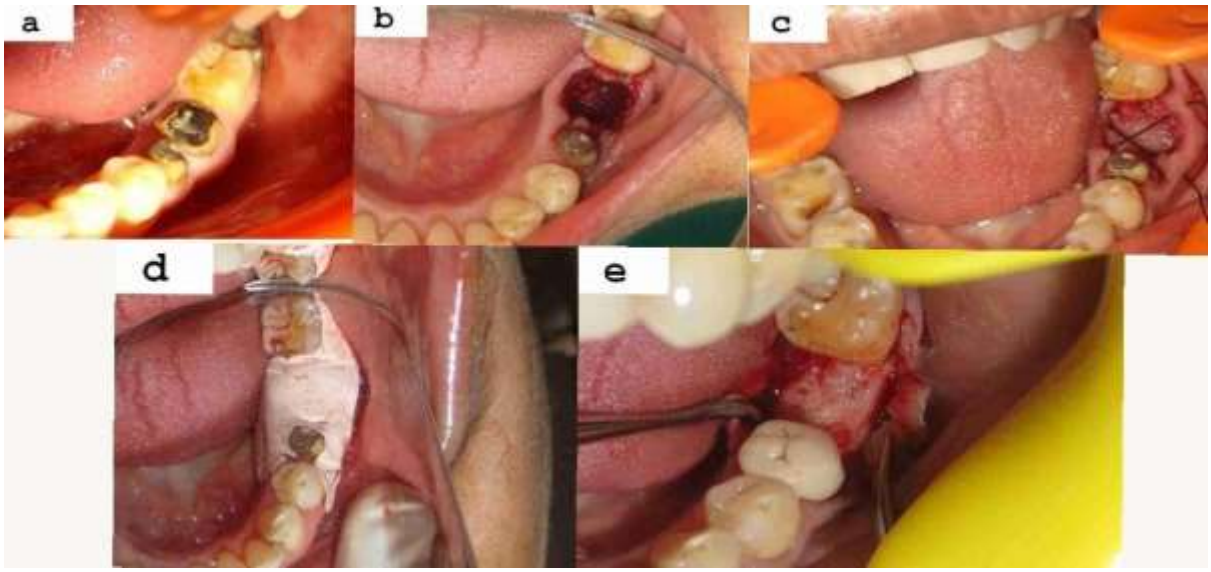

Figure 2a Preoperative
Figure 2b Extraction socket after atraumatic extraction
Figure 2c Bioactive glass and collagen plug placed in the extraction socket
Figure 2d Periodontal dressing placed
Figure 2e Bone formation after 6 months

Figure 3a Buccolingual ridge measurement done after atraumatic extraction
Figure 3b Bone sample for histological assessment taken with the help trephine bur
Figure 3c Bone sample collected in trephine bur

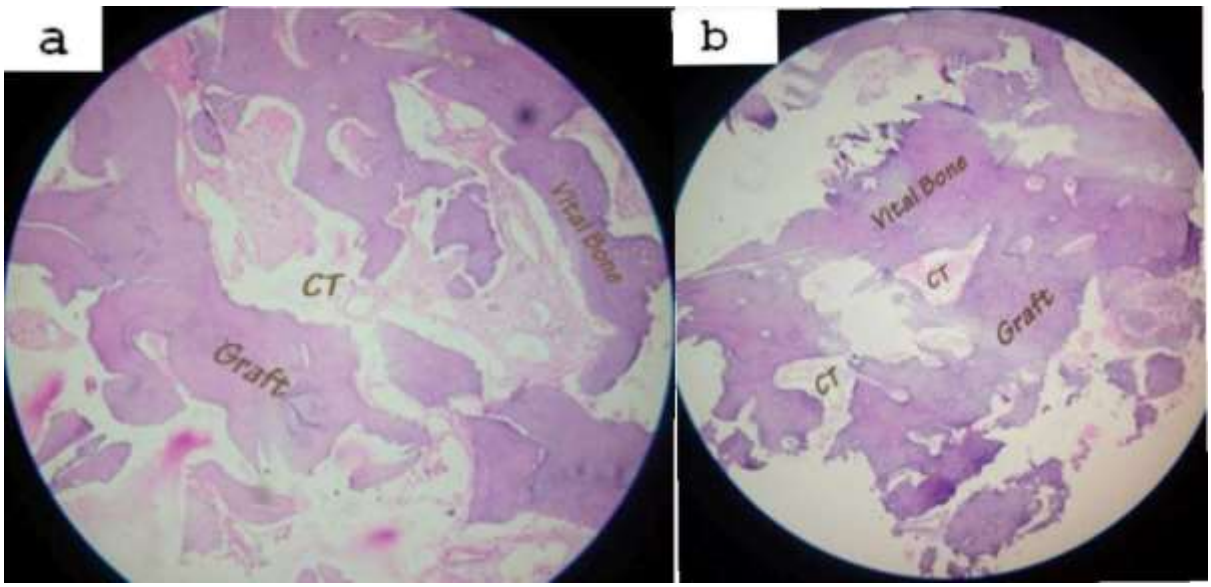

Figure 4 Histologic slide stained with hematoxylin and eosin depicting the composition of specimens

a. Vital bone can be seen in close approximation to a segment of residual graft particles. Connective tissue (CT) consists of large marrow spaces with osteoblasts lining surface of vital bone.
b. Residual graft particles intimately surrounded by vital new bone, most of which is woven bone. Connective tissue interspersed among bony compartments

Discussion: In a study on the effects of removing a single premolar or molar tooth on bone healing and soft tissue changes, Schropp et al. used clinical and radiographic measurements to demonstrate that significant changes occur 12 months after an extraction, with an average 50% reduction in the width of the alveolar ridge. Within the first three months, this reduction happened in two-thirds. The ridge's average width was 12 mm (8.6-16.5 mm) immediately following tooth removal, and at 12 months it was 5.9 mm (2.6-12.2mm) (Schropp L et al ., 2003). So the present study was planned to preserve ridge so as to attain adequate height and width of the ridge after extraction of the tooth for providing a proper emergence profile for future implant placement and to measure the clinical and histological success rate of both grafts demineralized bone matrix putty and Bioactive glass.

The duration of the present study for ridge preservation was 6 months and 40-60% loss of socket width was estimated post extraction. Cardaropoli et al., 2003, investigated bone modelling and remodelling that occurred within the extraction socket after the distal root of the premolar was removed. They concluded that the formation of a coagulum, which was replaced by a provisional connective tissue matrix, woven bone, lamellar bone, and bone marrow, was involved in the healing of an extraction socket. A hard tissue bridge - cortical bone - formed during the healing process, "closing" the socket.

When (Loe et al., 1986) studied Sri Lankan tea workers, they discovered that severe periodontal disease was already common between the ages of 20 and 45 and had caused a significant amount of tooth loss. (Baelum et al., 1986) and (Okamoto et al., 1986) both reported similar findings after looking at subject populations in Tanzania and Japan, respectively. In his study, Wenstrom J. L. showed that mean bone level values > 6mm, which indicate a noticeable bone loss, were first noticed in only 11% of the individuals investigated, were nonexistent in ages below 35 years and were most frequently found in the age groups of 70 years and above (27 per cent) (Wennstrom JL., 1988). Hence, the age group between 28 to 60 were selected for the present study.

Patients with good systemic health and compliance were included in the study since patients suffering from systemic diseases like uncontrolled diabetes mellitus or those patients on immunosuppressive therapy almost always show a compromised wound healing response, such patients were not part of the study. Diabetes is known to impede a microbial defence mechanism, and hyperglycemia and the degree of glucose control appear to be associated with infection susceptibility. Diabetes patients are particularly vulnerable to infections brought on by wound healing or surgery. (McMahon MM et al., 1995). A woman's hormonal changes may influence her periodontal health status and the effectiveness of her treatment. Pregnancy and lactation are when periodontal changes are most noticeable. Delaying periodontal surgery till after delivery is a possible course of treatment for people with periodontal disease who are pregnant (J periodontol., 2000). Therefore, participants in the trial who had diabetes, as well as pregnant and lactating women were excluded. Smoking can negatively affect the normal healing process as well as osseointegration of implant survival (Bain CA et al., 1993). Therefore, smokers too were excluded from the study too.

Plaque index is significant because it might have an impact on the health of the soft tissue surrounding the implant. It represents the level of oral hygiene practised by the patient. Plaque management and recall compliance have been directly linked to long-term success with regeneration operations

(Bragdon CR et al., 1996). Plaque scores were assessed prior to the second surgery, after three months, and at the baseline stage. In intergroup comparison at any clinical time point, there was no statistically significant difference. All patients maintained their dental hygiene throughout the duration of the trial, and only a small amount of plaque did not impede the soft tissue healing.

The modified gingival index is thought to be an accurate indicator of gingival health and illness (Lobene RR et al., 1986). Statistically, no significant difference was present in values at baseline, 3 months, and 6 months between both the groups in intergroup comparison. This is in congruence with the plaque scores and suggests that all the patients were maintaining their oral hygiene.

To ensure soft tissue healing; early wound healing index was taken at an interval of 1 week, 2 week and 4 weeks. No statistically significant difference was present in the change of value at various times in Group I as well as Group II. It showed that in all patients the soft tissue healing was good and uneventful.

Thin buccal bone, primarily in the most coronal part, is vulnerable to vascular supply interruption as a result of flap elevation. The loss of the buccal plate could result in esthetic issues due to significant resorption (Capelli M et al., 2013) (Chappuis V et al., 2017). The extent of buccal bone resorption is determined by the initial thickness of the buccal crestal bone (Merheb J et al., 2014). As a result, the thickness of the buccal bone is a critical factor to consider when placing implants. In Group I and Group II, there was no statistically significant difference in the change of value over time. As a result, it should be noted that early remodelling can begin immediately after tooth extraction and continue in a haphazard manner even after delayed implant placement.

In histological analysis, the percentage of new bone, provisional matrix and the residual graft was measured using IMAGE J^{TM} software in both groups. In intergroup comparison, the mean value of vital bone (54.61±3.16) was higher in Group I than in Group II (47.74 ± 3.78). Statistically, a significant difference was present between the two groups. Mean value of the provisional matrix (37.03±2.88) was less in Group I than in Group II (43.75±4.33). Statistically, a significant difference was present between the two groups. Mean value of residual graft (8.36±3.16) was less in Group I than Group II (8.51±4.25). Statistically, no significant difference was present between the two groups. So the range of vital bone formation found after ridge preservation varies widely depending on the material used for grafting. Similarly, a study was done by (Clozza E et al., 2014) to investigate the ability of bioactive glass for socket preservation. In all 22 biopsies, they found newly formed immature bone around residual particles of bioactive glass in a histological study. 6 months after the ridge preservation procedure, histomorphometry of the amount of bone, provisional matrix, and residual graft yielded a mean standard deviation (SD) value of 5431 percent, 37.925.6 percent, and 8.17.8, respectively (RPP). In addition, they did an immunohistochemical study and found levels of CD31, CD68, BMP-7 and cathepsin-k 6 months after ridge preservation procedure (RPP).

A central excavation was visible in the residual particles. Schepers and colleagues (Schepers E et al.,1991) previously reported this phenomenon in an animal study, observing that most glass particles are internally eroded via small cracks. The excavated area was hypothesised to provide a protected environment with minimal fluid flow, allowing mesenchymal stem cells to adhere to the internally formed calcium phosphate layer in the aforementioned study. When primitive cells were immobilised on a bone-like surface at this time, mesenchymal stem cells would have differentiated into osteoblasts.

This study attempted to clinically and histologically evaluate various materials utilized for treatment of extraction sockets. This study describes the efficacy of demineralized bone matrix putty and bioactive glass for socket preservation. By

taking into account ridge preservation and retaining enough bone for ideal implant placement and subsequently improving the look, implant treatment can be facilitated at the time of extraction (Darby I et al.,2008). It should be noted that the use of osteoconductive-mineralized grafts does not hasten bone healing; rather, it may enable improved ridge volume preservation, which is highly desirable for future purposes both aesthetically and functionally.

Conclusion: The current study found that implanting demineralized bone matrix putty and bioactive glass as graft materials in extraction sockets for ridge preservation resulted in varying degrees of bone formation as well as acceptable biocompatibility. Both demineralized bone matrix putty and bioactive glass showed similar bone healing as well as bone formation. However, on histomorphometric analysis, demineralized bone matrix was found superior in terms of enhancement of bone regeneration and graft material resorption than bioactive glass.

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