

A Clinical and Histomorphometric Evaluation of Socket Preservation Using Resorbable Collagen Membrane and an Alloplast: A Comparative Interventional Study

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Abstract: There are various grafting materials used for socket preservation of the alveolar ridge following tooth extraction. The purpose of this study was to evaluate clinically and histomorphometrically healing after tooth extraction with or without placement of a synthetic bone graft and determine the effect on alveolar ridge preservation following extraction. 30 subjects in need of extraction of non-molar teeth were recruited for this study. Recruited subjects were randomly assigned to the group A (with graft material) or control (without graft material) group B. Data were recorded at 6 months after socket preservation procedures. At 6 months, a surgical re-entry was performed; Clinical Measurements and bone core biopsies were obtained for histomorphometric analysis. The control group B had a mean reduction in ridge height of 1.26mm, whereas alveolar ridge height in the test group A was 0.73mm. The test group A was 7.33 ± 1.01 mm compared to 7.23 ± 1.13 mm in group B which were not statistically significant ($p = 0.684$). Histomorphometric analysis revealed total new bone volume in group A represented $65.6 \pm 11\%$ connective tissue $25.6 \pm 10.18\%$ and 8.8% was occupied by residual graft material. The mean new bone volume in group B was $31.23 \pm 7.24\%$, whereas connective tissue formation was approximately $68.78 \pm 7.24\%$. Both these values were statistically significant when compared with group A. There was no relation with residual graft material as there was no graft material used in group B. A positive response was observed when synthetic graft was applied to extraction sockets, suggesting that it may be useful for preservation of ridge height prior to dental implant placement.

Keywords: Tooth extraction, bone graft, socket preservation, Implant

INTRODUCTION

Dental implants continue to have improved success. As patient access and acceptance grows, the standards for implant treatment and success are raised. Clinicians strive to fulfil both the aesthetic and functional needs of the restoration, to include a gingival profile harmonious with the adjacent healthy dentition. In general, the alveolar bone remodelling that occurs after tooth loss yields diminished alveolar ridge dimensions in both the vertical and horizontal planes. Hence, preservation of the original alveolar ridge should aid the surgeon in achieving the optimal placement of the implant with the desired implant diameter while maintaining the aesthetics of the overlying soft tissues. Hydroxyapatite and porous β -TCP have been found to be biocompatible and bioactive materials, which might be effective in socket preservation technique. Very few studies are available in literature to prove the potential of β -TCP and Hydroxyapatite combination with a barrier membrane technique to show the ridge height preservation along with good quality bone formation. The projected study

has been undertaken to evaluate the effectiveness of an alloplastic graft material along with bio-resorbable membrane clinically and histologically. The main objectives of this study was to establish a protocol for ridge preservation in post extraction socket using an Alloplast (Biograft[®]-HT) containing porous biphasic synthetic hydroxyapatite & β tri calcium phosphate granules with a resorbable collagen membrane (Proguide[™]).

MATERIAL AND METHODOLOGY

This study was conducted in the division of Periodontology of our institute. Thirty patients who visited the outpatient department (OPD) of our hospital were recruited into this study based on the inclusion and exclusion criteria. An ethical clearance was obtained before conducting the study from the ethical committee of our institution. The criteria based on which the patients were selected are as follows:

Inclusion Criteria

- Patients should be between 18-60 years of age
- Patient should be in good general health
- Patients with hopeless or non-restorable non-molar tooth bilaterally with adjacent teeth present. [Fig1]
- The extraction socket maintaining a four-walled configuration with intact buccal plate
- Patients should have the desire to have an implant restoration done.
- Patient should be available for follow-up examinations and be compliant.



Fig-1: Hopeless non-restorable tooth

Exclusion Criteria

- Patients who will be unable to undergo oral surgery procedures because of active systemic conditions
- Patients who have the habit of smoking
- Female patients who are pregnant or lactating
- Any condition that would be contraindicated for dental implant placement.
- Patients with compromised health that would affect the ability of the patient's tissues to heal will not be included in this clinical trial.

The ongoing study was a case control study. Patients were explained about the elected procedure in detail and were included for the study after obtaining their signature on the consent form. The study group comprised 16 males and 14 females aged 18 to 60 years with a mean age of 36.5 years. Demographic information and medical history of all the subjects were obtained by patient interviews. The study group included six maxillary incisors, four maxillary canines, five maxillary premolars, four mandibular canines and eleven mandibular premolars.

A split mouth case selection was done in the present study. The cases included bilateral single rooted teeth indicated for extraction in either of the arches. The aim of the case selection was to avoid the bias being originated because of the difference in healing in

different individuals rather the study was concentrated on comparing the effectiveness of two different procedures, one with use of osseous substitute along with a membrane and the other without using any substitute and allowing for natural healing in the same individual. The differences could easily be elicited by using the above-mentioned procedures in the same individual to find out the dimensional changes and quality of bone formation [1].

In the ongoing study, only single-rooted teeth were included, all teeth were extracted with minimal trauma. [Fig 2] All roots had to have angulations similar to the angulations of the implant to be placed at the site. This ensured adequate depth of socket and angulations of the core trephine to allow biopsies to contain only newly formed bone, as opposed to mature alveolar bone present at the root apex or along the socket wall.



Fig-2: Atraumatic forceps extraction

The methods of socket preservation are using various graft materials including autogenous graft [2], xenograft [3], alloplast [4] has been used by various workers alone or in combination with or without a GTR membrane.

The side to be grafted and the one not to be grafted was decided by the flip of a coin method of randomization to avoid any bias. Similar method of randomization was done in studies carried out by Iasella JM and Greenwell H [5]. The methodology included the use of an alloplast (Biograft HT[®]) [Fig3]. There are very limited studies that have been carried out with this material [6].



Fig3: Use of an alloplast (Biograft HT)

This graft material was used along with a collagen membrane (Proguide™) for socket preservation on one side of split mouth and compared it with a socket without any graft or membrane by just leaving it to be healed physiologically in the same patient on the opposite side of the jaw.

The clinical parameters included in our study were measurement of ridge width and vertical ridge height from a standardized reference point immediately after extraction. Post extraction measurement of bone width and height were measured six months later from the same standardized reference points just before the placement of implants. This method was similar to the method adopted by Zubillaga G [7].

The horizontal width dimensions as measured from the midpoint of the alveolar crest with the reference point being 2mm below the line joining the cement enamel junction of the adjacent teeth showed significant reduction at the sixth month evaluation. This method was similar to the studies carried out by Schropp L *et al.* who used an bioactive alloplastic graft and reported the mean width of the test sites to be 5.7 (\pm 1.2) mm compared to 3.9 (\pm 0.8) mm at the control sites [8].

The vertical height was measured using standardized acrylic stents that were fabricated with the help of study models before the extraction. The vertical height was measured from these acrylic stents and a probe (Williams /UNC 15) to the mid-buccal and mid lingual crest after the extraction were done. [Fig 4] A clean operating field was maintained during the height measurement.



Fig-4: Measurement of vertical height from a fixed reference point

The average ridge height, at 6 months were tabulated and sent for statistical analysis. Pietrokovski J and Massler M showed that the placement of bone replacement grafts into extraction socket maximized bone formation and helped to maintain them for future endosseous implant placement [9].

The extraction sockets were assessed for the number of walls present and the extent of resorption of the individual walls by a number of studies and showed that defects of the original buccal plate do not heal completely without use of grafting techniques [10]. Other studies have shown that extraction sockets with completely intact bony walls are capable of complete bone regeneration by physiologic healing alone [11].

Our findings were in concurrence with studies carried out by Nemcovsky CE, who reported that ridge width slightly decreased during follow up [12]. As per the author, most of the shrinkage was recorded during the first six months. No change in ridge dimensions was observed subsequently.

Unlike the present study, there are studies carried out by Lekovic V *et al.*, who filled the test sockets with bioactive glass and then calcium sulphate. The authors reported that the unfilled sockets showed slightly better results than the sockets filled with graft material [13, 14].

One of the reasons for placing a graft is to provide a scaffold for new bone formation. This objective was met in the current study. Histological evaluation post 6 months of the preserved socket revealed larger amounts of new bone formation, confirming to the osteoconductive property of Biograft® HT.

Second Surgical procedure

Patients were recalled approximately 6 months after baseline appointment when healing of the bone grafting would have been achieved. A cone beam Computed Tomography Scan was taken as a requirement for implant placement. [Fig 5]

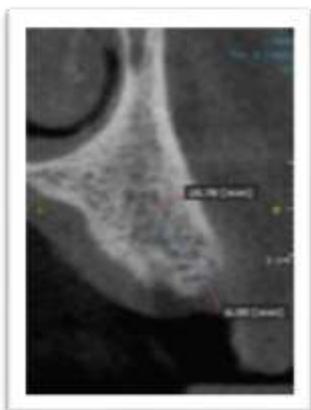


Fig-5: cbct

After local anesthesia administration, papilla sparing crestal incision was made, and full-thickness flap was reflected. Osteotomy for implant insertion was prepared using a trephine with irrigation. Cylindrical core samples of newly regenerated bone will be retrieved from the centre areas of both the grafted [Fig 6] and the non-grafted sockets using a bone trephine. [Fig 7] After the sample collection they were placed immediately in 10% buffered formalin. [Fig 8] The samples were taken to the laboratory for further histological analysis. A dental implant was placed in the trephined site subsequent to performing a complete osteotomy according to the recommendations of the manufacturer. Healing abutments or cover screws were placed based on primary stability of the implant and flaps were secured with sutures.



Fig-6: Core sample of grafted site



Fig-7: Core sample of non grafted site

Histomorphometric analysis of the samples exhibited only residual particles of the bone graft present. The formation of vital bone was appreciated. These results were in accordance with the histomorphometric analysis by Araujo M *et al* on extraction sockets augmented with Bio-Oss [15].



Fig-8: Samples collected in 10% formalin

RESULTS AND DISCUSSION

The graft particles were surrounded by newly formed bone which suggested that during continued healing, these biomaterial particles may become integrated with and further enhance hard tissue formation. The material used in our study was Biograft[®] HT which contains 40% β -Tri Calcium Phosphate with 60% porous biphasic synthetic Hydroxyapatite. This material is an alloplast with granule size of 350-500 μ m with osteoconductive properties. Biograft[®] HT used in our study has been reported to act as a scaffold for de novo bone formation.

As per the studies carried out by Froum *et al.*, osteoconductive process leads to the formation of bridges of woven bone between the bone graft substitutes particles, connecting them into a mass of mineralized tissue. Bone formation is followed by the remodelling and replacement of woven by mature lamellar bone. In the present study, bone formation was still ongoing after six months, as indicated by the rather large content of non-mineralized bone tissue in all samples and the relatively low number of osteon-like structures. The bone graft substitute particles seemed to act as local bone growth centres throughout the grafted area, according to the osteoblasts that were lying on the surface of the grafted material. In addition, because of osteoblasts activity, bone formation started around grafted particles with the production of non-mineralized bone matrix proceeding to its mineralization. Generally, the amount of bone formed at the former defect site after ridge preservation varies between studies, presumably due to differences in study populations, defect anatomies, observation time points and material characteristics [16].

Cardaropoli G *et al.* reported that in the grafted site with Bio-Oss[®] Collagen, total relative volume of

newly formed mineralized bone amounted to 46.7% [17], where in the present study showed that the sites grafted with Biograft® HT(β -TCP +HA) had a relative volume of new bone to be 65.6%.

In most respects, the current histological findings are in accordance with observations made in clinical and experimental studies showing that an intimate contact frequently is established between pure graft particles and newly formed mineralized bone.

Most of the grafted biomaterial particles were surrounded by newly formed bone and rare gaps or connective, fibrous tissues were found at the biomaterial- bone interface. The connective tissue volume was found to be $25 \pm 10.1\%$ in the grafted site while compared to $68 \pm 7.2\%$ in the non-grafted site. This was because the normal physiologic bone formation as we know starts from the apical portion of the extraction socket towards the crest. Other similar clinical studies by Brkovic *et al.* indicated that bone formation starts from existing bone surfaces in the most apical part of the extraction defect, propagating along the surface of the bone graft substitute particles [18].

Most of the newly formed bone in the current study was woven bone, and the relative lack of newly formed lamellar bone was an interesting finding. We expected minimal lamellar bone in the early healing group, but not in the late healing group where we anticipated a higher degree of lamellar bone formation. In other studies by Tsao V-Po showed a combination of woven and lamellar bone was often found at 5 to 6 months post grafting [19].

Although β -TCP is expected to degrade three times faster than HA, the predictability of β -TCP degradation in humans remains poor. It has been recently documented that non-resorbable particles of beta-TCP well incorporated inside a new bone formation were detected nine months after ridge preservation [18]. The same was observed in our study also where a core sample was removed using a trephine bur before placement of an implant six months after socket preservation. The core sample removed showed non-resorbed particulate along with new bone attached to it. Furthermore, *in vivo* experiments using rabbit, critical-sized defects showed less bone formation in β -TCP treated animal compared with autogenous bone grafts.

After four months of healing, Kesmas S *et al.* reported that the mean percentage of new bone formation, connective tissue, and residual graft particles in six biopsies after β -TCP treatment of post-extraction sockets were $28.00 \pm 36.75\%$, $65.50 \pm 25.85\%$, and

$15.85 \pm 8.70\%$, respectively [20]. While our study showed contrasting results at 6 months of healing which reported mean percentage of new bone formation, connective tissue, and residual graft particles were $65.63 \pm 11.8\%$, $25.66 \pm 10.1\%$ and $8.7 \pm 4.7\%$ respectively [20].

The main complication encountered in using the barrier membranes for ridge preservation technique is that it cannot be left exposed to the oral environment and the primary closure of the extraction site is mandatory. Out of the thirty cases, four patients reported after two to three days with dislodgement of sutures. These patients were treated by simple suture placements and an extended course of antibiotics. No other severe complication was accounted that would hamper the outcome of the study. There were no cases lost during follow up of six months. All patients were present till the end of the study.

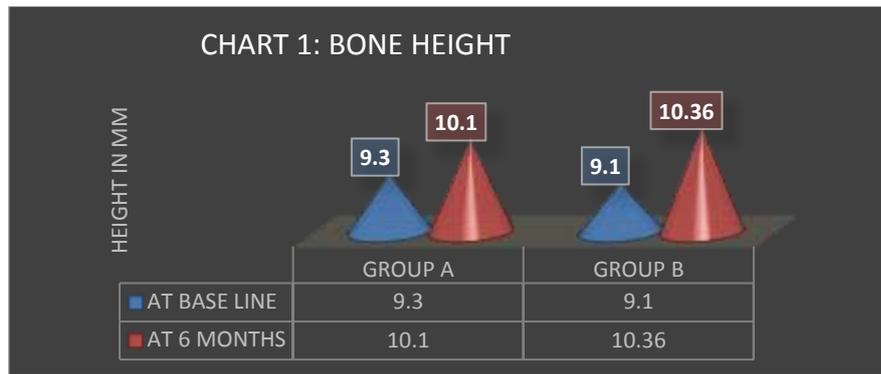
However, the limitations of the present study are small sample size and shorter follow up period. Although case control studies provide a high strength of evidence as compared to cross sectional studies, it is still laden with biases. Our results cannot be generalized to the entire population. Further prospective randomized control trials with a larger sample size, longer follow up period, different population and different bone graft materials are needed in the future to authenticate our hypothesis.

Ridge width

The average ridge width immediately after extraction in group A was 7.33 ± 1.01 mm compared to 7.23 ± 1.13 mm in group B which were not statistically significant ($p = 0.684$). 6 months later, the mean bone widths were 5.56 mm and 5.53 mm for group A and group B respectively. These values were not statistically significant.

Ridge height

The average ridge height, at baseline was 9.366 mm and 9.100 mm in group A and group B respectively. 6 months after socket preservation was done in group A, the height was again measured using the same stent and probe. The results showed a ridge height of 10.100 and 10.366 mm in group A and B respectively which is pictorially depicted in **Chart 1**. This increase in height was attributed to the amount of bone loss or loss of vertical height. In Group A (side with bone graft) there was minimal loss of height of 0.73mm whereas, Group B (side without graft) showed a loss in vertical height of 1.26 mm. These results were statistically significant.



The intra group analysis also showed a significant change in both height and width at baseline when compared to the values at the end of six months. In both groups, average reduction in ridge height was approximately 1mm and loss of ridge width was approximately 2 mm. The dimensional stability of the alveolar ridge after ridge preservation was clinically indistinguishable when grafted with B-TCP and HA versus the side without graft.

Histological parameters

On histological analysis, all biopsies showed newly formed mineralized tissue. No evidence of inflammatory infiltrates, necrosis, foreign body reaction and no other signs of adverse reactions were detected. In all analysed samples, bone tissue formed predominantly in the apical part of the extraction socket. The remaining bone graft particles, which are identified by their colour and round shape, were mainly detected in the coronal portion of the samples.

On image analysis using Biowizard software, graft particles were partially surrounded by trabecular woven bone. Haversian canals surrounded by mature, lamellar bone were noted occasionally, suggesting that the formation of osteon-like structures had already started. Active osteoblasts lined the osteoid surface and produced an osteoid layer (Fig.9).

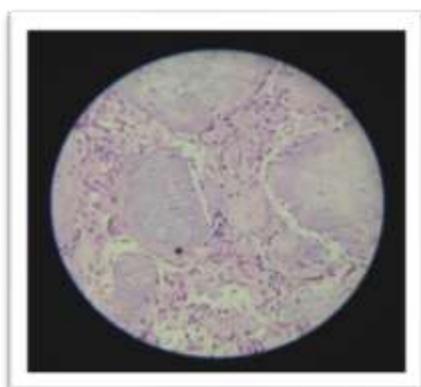


Fig-9: Histological section of grafted site

Many trabeculae demonstrated very active bone remodelling, with a thick layer of osteoid surface at one side, which is indicative of new bone formation. In many areas, wide osteocyte lacunae with osteocytes were present. In all specimens, graft material particles surrounded by the connective tissue with blood vessel, fibroblasts and collagen fibers, intermixed with newly formed bone tissue could be recognized. The presence of collagen fibres and cells inside the grafted material is an indication that resorption of the grafted material had started. Together with bone formation around the grafted particles, this could represent a sign of material integration.

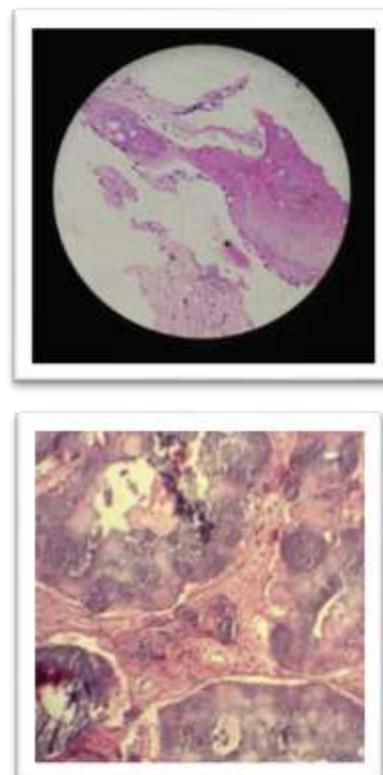


Fig-10: Histological section of non grafted site

Table-1: Histomorphometric Parameters

	Group	N	Mean	Std. Deviation	P-value
Percentage of New Bone (%)	A	30	65.6333	11.85686	.000
	B	30	31.2333	7.24775	
Connective Tissue Volume (%)	A	30	25.6667	10.18563	.000
	B	30	68.7667	7.24775	

The histologic parameters included percentage of new bone formation, percentage of connective tissue as well as residual bone graft volume. Total new bone tissue in group A represented $65.6 \pm 11\%$ connective tissue $25.6 \pm 10.18\%$ (Table 1) and 8.8% was occupied by residual graft material. The mean new bone volume in group B was $31.23 \pm 7.24\%$, whereas connective tissue formation was approximately $68.78 \pm 7.24\%$ (Chart 2). Both these values were statistically significant when compared with group A. There was no relation with residual graft material as there was no graft material used in group B. [Fig 10]

graft volume and the newly formed connective tissue histologically.

Numerous technical and surgical solutions are showed in the literature for augmentation of dental sockets. Various types of bone grafting materials have been suggested for this purpose and some have shown promising results. The implantation of graft material, whether natural or synthetic, results in a host response. This response is dependent on the morphology, chemical composition, porosity and particle size of the biomaterial.

Many biomaterials are proposed as alternative to bone autografts like allografts and xenografts materials are available, however, with limitations. Synthetic bone grafts such as hydroxyapatite HA, beta-tricalcium phosphate β -TCP and the BCP (HA/ β -TCP) have been used successfully because their chemical composition is closely related to that of bone mineral. This non-immunogenic and resorbable material provides the basis for complete, predictable, and reproducible bone regeneration. It is easy to handle and its change in radio opacity allows healing in the area to be monitored over time.

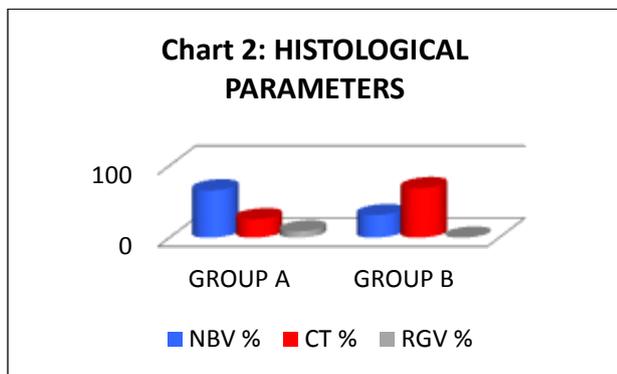


Chart 2: Histological Parameters

CONCLUSION

Ridge preservation after tooth extraction prior to implant placement is essential for successful placement of implants. The success of Osseointegrated dental implants depends on whether there is a sufficient volume of healthy bone at the recipient site at the time of implant placement. The placement of an implant at a site with a thin crestal ridge (eg. Post extraction ridge) could result in a significant buccal dehiscence. The bone complex is a dynamic tissue due to its property of constantly changing (remodelling) and is also capable of self-repair. In order to control the modelling effect in fresh sockets, passive stimulation is required in preventing the bone resorption.

Thus, it seems prudent to prevent alveolar ridge destruction and make efforts to preserve it during extraction procedures. This study aimed at preserving the socket height and width by using an alloplastic graft material (Biograft® HT) along with a membrane (Proguide™) and compared it to a socket that healed physiologically without a graft and membrane. The parameters compared were ridge width and its height clinically; the amount of new bone volume, remaining

This study demonstrates that most biomaterials can prevent alveolar crest resorption after teeth extraction. Moreover, the alveolar bone preservation leads to better aesthetic results in oral implantology. It is the method used in socket preservation that is more important than the material used for the same. In this context, our data open new therapeutic windows for preserving or reconstructing the extraction socket of a failed tooth according to the principles of guided bone regeneration enhances our ability to provide aesthetically pleasing restorations to our patients without violating the predictability and function of those prostheses.

It can be concluded and recommended for routine use of socket preservation technique applying the principle of guided bone regeneration for better hard tissue preservation. The innovative research involving both anterior and posterior teeth with larger samples and varieties of regenerative osseous substitutes and membranes will bring out a condition where the bone loss can be avoided after extraction. The long-term analysis following socket preservation procedure will establish new protocol in the field of implantology.

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