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HORIZONTAL ALVEOLAR BONE AUGMENTATION USING GUIDED BONE REGENERATION FOLLOWING ALVEOLAR BONE DECORTICATION OR DEMINERALIZATION (CLINICAL AND HISTOLOGICAL STUDY)

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ABSTRACT

Background: Bone volume affects the long term success of an implant. Augmentation of the existing edentulous alveolar bone is often necessary to obtain excellent functional and esthetic restorations of the implants. The reconstruction of alveolar ridges for implant placement is still a challenging procedure, especially in the case of extensive vertical and horizontal atrophy. Here, we aimed to evaluate the effect of EDTA bone demineralization on bone graft consolidation to the native bone in comparison to alveolar bone decortication.

Methods: A total of 14 subjects were divided into two groups (n = 14) . In the test group I (n = 7), alveolar cortical bone in the area of regeneration was demineralized by 24% EDTA. While, decortication was performed in group II (n = 7). Subsequently, defects in both groups were augmented by guided bone regeneration using resorbable membrane and bovine bone. After a healing period of 6 months, trephine cores were harvested for histological and histomorphometric analysis of the grafted areas and the buccolingual width dimension was evaluated radiographically

Results: Histomorphometrical analysis demonstrated that the amount of newly formed bone in the test group (3.63±1.35 %) was greater than that in group II (2.52±0.78 %), and the difference was statistically significant (P = 0.029)

Conclusions: Bone demineralization results in more width gain than mechanically decorticating the alveolar bone.

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INTRODUCTION

After tooth extraction, a process of remodeling of alveolar bone occurs which result in horizontal and vertical bone resorption ***(Van der Weijden et al., 2009; Liu and Kerns, 2014)***. *Carlsson* and his collogues studied changes of the mandible after tooth extraction. The percentage of bone resorption was estimated as 21 % after 3 months, 36% after double this period, and 44% after 1 year ***(Carlsson and Persson, 1967)***. The most dimensional tissue changes (about 50%) happened at the extraction site during the first year after tooth extraction ***(Schropp et al., 2003)*** Studies concluded that the amount of horizontal bone resorption is higher than the amount of vertical bone resorption clinically as well as radiographically ***(Van der Weijden et al., 2009)***.

Endo-osseous dental implants have been a good solution to restore missing teeth. The bone volume affects the long term success of an implant. In case of bone volume inadequacy, additional techniques may be implemented to reach acceptable outcomes. Accordingly, local bone augmentation, accompanied by guided bone regeneration, has been brought into consideration ***(Lekholm et al. 1999)***.

Both the morphology of the bone defect and the ridge contour dictate the best treatment protocol and the selection of materials ***(Benic and Hämmerle, 2014)***. It had been determined that the least dimensions for inserting cylindrical implants are 5-8 mm in height and 6 mm in width. Alveolar bone 1 mm thick should encircle the implant after insertion. Therefore, widening of the alveolar ridge before implant insertion is mandatory in cases of ridge width of 4 mm or less. Alveolar ridge augmentation is highly recommended, if implant stability or appropriate positioning cannot be achieved. ***(Bahat 1994; Miyamoto et al., 2012)***.

Procedures that use barrier membranes to direct the growth of new bone toward sites of bone defects is called Guided bone regeneration (GBR).This is done to restore function and esthetics. ***(Dahlin et al., 1988; Miloro et al., 2004)***. To ensure successful GBR, four principles need to be met ,these are, wound closure, blood supply maintenance of space, and stability of the initially formed blood clot **(PASS principle)** ***(Wang and Boyapati, 2006)***.

Different materials have been used in studies in terms of GBR. Barrier membranes can be classified into three generations. First generation membranes are non-absorbable membranes, that were used for periodontal regeneration. These include cellulose acetate (Millipore), expanded polytetrafluoroethylene (e-PTFE), titanium re-enforced ePTFE, high density- PTFE. Second generation membranes are absorbable membranes, which could be natural such as collagen or synthetic which are made from polyester such as poly-glycolic acid (PGA), poly-lactic acid (PLA) and their copolymers. Third generation membranes act as barriers and as delivery vehicles for local agents such as antibiotics and growth factors ***(Scantlebuty, 1993; Hardwick et al., 1995; Saad et al., 2012; Sam and Pillai, 2014)***

Non-resorbable membranes are not biodegradable, that is, they require another surgical intervention to be removed. Moreover, their exposure may lead to total failure of the regeneration process ***(Rocchietta et al., 2008).***

Resorbable membranes do not require a second procedure to remove the membrane. It requires less surgical time with less potential postsurgical morbidity ***(Tolstunov et al., 2019).*** However, their limitations include, uncontrolled duration of barrier function and the need of a membrane supporting material to minimize its failure. ***(Schwarz et al. 2006; Becker et al. 2009)***.

Pericardium membrane has been utilized as a part of cardiac repair. The xenogenic pericardium is derived from bovine sources. It includes collagen strands, and has elastic properties enabling adjustment to complex anatomy ***(Nair 2018)***.

Bone graft materials function as scaffolds that maintain space for osteogenic cells, and the host response to these scaffolds is accordingly one of the success factors in GBR ***(Hockers e al., 1999; Esposito et al. 2009).***

Bone fillers could be autogenous bone chips, allograft (same species), xenograft (another species), or alloplast (synthetic). They are commonly used in the GBR process. They promote bone ingrowth and healing through osteoconduction, by offering mechanical support of the membrane and stabilizing the blood clot ***(Jensen et al., 2006)***.

The gold standard material is the autogenous bone as it is the only type of graft that has osteoconductive, osteoinductive and osteogenic potential ***(Brunsvold and Mellonig, 1993; Pandit and Pandit 2016)***. The main drawbacks of autogenous bone grafts are the limited bone volume availability that can be obtained and the morbidity of the harvesting site ***(Dragoo and Sullivan, 1973; Jensen et al., 2016)***

Allografts are fresh, frozen-fresh, freeze- dried grafts that are harvested from two dissimilar members of the same species. The main problem with fresh and frozen allografts was the immunologic potential that could occur when used. This could lead to complications such as, graft infections, a nonunion or, delayed union at the graft host interface ***(Lord et al. 1988; Aro and Aho 1993; Gazdag et al., 1995; Kumar et al., 2013)***

Xenografts are grafts that are obtained from another species which could be bovine, porcine, equine or coralline. Chemical and physical properties of xenografts were found to be similar to that of human bone, when used they provided osteoconductive properties ***(Traini et al., 2007; Wong and Griffiths, 2014)***. Alloplasts are synthetic inorganic graft substitutes which have osteoconductive properties. They can be made from ceramic based materials such as calcium phosphates {hydroxyappetite (HA), tricalcium phosphate} and bioactive glass or they can be made from polymer based material which can be either natural or synthetic ***(Kumar et al., 2013).***

For attaining GBR, angiogenesis and adequate blood supply are of prime importance as they follow a sequence of events. In 1994, ***Schmid et al***., concluded that new bone regeneration is mainly dependent on the development of new blood vessels that stimulate and nurture the surgical site. 24 hours after a GBR procedure, a blood clot is formed then it is resorbed by neutrophils and macrophages and hence is replaced by granulation tissue containing numerous blood vessels that transport cells and nutrients involved in bone matrix formation. Osteoid is unmineralized bone matrix and is referred to as woven bone upon mineralization. Woven bone acts as a scaffold. ***(Schmid et al., 1994; Hämmerle et al., 1995 ; Schmid et al., 1997)***. Regeneration of new bone is established after 4 weeks from initiating GBR ***(Hämmerle et al., 1995; Hämmerle et al., 1996; Schmid et al., 1997; Glowacki, 1998; Lu et al., 2007)***.

Bone decortication is done by drilling holes through the cortical bone into the spongy bone or by complete removal of the cortical bone. Bone decortication has been used as a part of GBR and stated in several cases to enhance the ridge thickness and height prior to implant placement ***(Buser et al., 1990; Buser et al., 1993; Buser et al., 1995; Greenstein et al., 2009)***.

Many trials stated that bone decortication resulted in increased apposition of lamellar bone in the grafted site. The new bone formation was related to regional acceleratory phenomena after traumatizing the alveolar bone mechanically by making bone perforations. Bone decortication in GBR allows the release of bone and blood forming cells from the bone marrow space, resulting in the synthesis of the new bone matrix ***(Frost, 1983; Buser et al., 1990; Greenstein et al., 2009; Saghiri et al., 2016)***.

Localized osteoporosis is a part of the healing process, that can accelerate hard and soft tissue healing two to ten times. The mechanical interlocking of a bone graft and a recipient site can be increased by perforating the bony cortex and may also improve its stability by firm bonding to the newly formed bone. Bone decortication improved mineralized bone and newly regenerated augmented tissue during guided bone regeneration ***(Alberius et al., 1996; Amit et al., 2012; Acar and Yolcu, 2018)***.

On the other hand, decortication has some dis advantages such as long operating time, extra blood loss, increased postoperative pain, and some bone loss in case the procedure fails. ***Maestre-ferrin et al***., observed that the union of onlay bone graft to the recipient bed does not have adequate strength to maintain adequate integrity when preparing the surgical sites and or implant placement due to shear forces produced during those procedures and may lead to detachment of the bone graft ***(Greenstein et al., 2009; Maestre Ferrín et al., 2009)***.

Therefore, authors used other ways not to mechanically treat bone by decortication but chemically by means of acid demineralization of alveolar bone cortex surface to enhance bone formation and integration ***(Greenstein et al., 2009)***

The extracellular matrix of bone contains a reservoir of growth factors. Following trauma, these proteins, such as platelet derived proteins, bone morphogenetic proteins, insulin like growth factors and transforming growth factor-β (TGF-β), target cells in the injury site. ***(Hauschka et al., 1986; Frolik, Ellis, and Williams 1988; Taipale And Keski-Oja, 1997; Schonherr and Hausser, 2000; Ramirez and Rifkin, 2003)***.

Citric acid, (EDTA), and calcium hydroxide have the ability to dissolute TGF-β1 from dentine. They act by mineral demineralization or calcium chelation. TGF-β1 released from dentine after calcium hydroxide treatment differs in level than that released after the use of EDTA ***(Zhao et al., 2000; Graham et al., 2006; De-Deus et al., 2008)***.

In 1965, ***Urist et al.*** was the pilot to introduce the principle of intentional bone demineralization, when Hcl was used to decalcify bone grafts implanted in animals subcutaneously. Bone demineralization could have osteoinductive potential where demineralization is done using diluted acid which removed inorganic component of bone ***(Urist and McLean, 1965)***.

Urist et al in 1970, tested the induction of new bone formation after a demineralized bone or dentin matrix was implanted in rabbit muscles. Results had showed ectopic new bone formation intramuscular after 4 to 6 weeks from implantation. In a sequence of studies, this induction was found to be a result of the released bone morphogenic proteins to the surrounding environment as a result of the bone demineralization process ***(Urist et al., 1970; Urist, 1973)***.

The physiologic mechanism of bone remodeling involves demineralization by acid production from osteoclasts. These acids attack minerals and release enzymes on the bone surface, that hydrolyze the organic matrix ***(Roodman, 1999; Hadjidakis and Androulakis, 2006).*** Preosteoblasts differentiate on a rough surface and deposit new bone. It was found that demineralization with tetracycline resulted in surface roughness comparable to that produced by osteoclasts acting on dentin. ***(Schwartz et al., 2000)***.

In 2015, ***Rezende et al.***, stated that demineralization by citric acid resulted in surface roughness equivalent to that produced by osteoclasts during bone resorption.

Osteoinductive property was stated to be due to the exposure of certain proteins found in the bone matrix which stimulated the surrounding mesenchymal cells to differentiate to osteoblasts and produce new bone ***(Bauer and Muschler, 2000).*** Superficial bone demineralization has been revealed

to be a promising adjunctive during regenerative procedures. In addition to the favorable biological effects presented above, some relevant advantages of bone demineralization can be cited as: the low cost of the acid solutions, the ease of its clinical use, as there are no need for perforations or decortication of the bone bed, the resorption of the bone grafts is minimized as the demineralization mimics osteoclasts function, there is an anticipation of the remodeling events and consequently, a reduced healing time ***(Salmeron and Rezende, 2017)***

EDTA (Ethylene di-amine tetra-acetic acid) gel preparation was studied as a root surface conditioning material because of its neutral PH that would not damage the organic component of the root, accompanying surgery following conventional flap in PDL intraosseous defects, the results showed bone gain of about 1mm to 1.5mm. Scanning electron microscope study of root dentine revealed dentinal tubule opening free from smear layer and collagen fiber exposure in the intertubular dentin ***(Blomlöf et al., 1997; Mayfield et al., 1998).***

Increased surface exposure after treatment of bone surfaces with either EDTA or calcium hydroxide resulted in the release of active growth factors, having more osteogenic effect on bone marrow stromal cells. EDTA and citric acid have shown similar patterns of TGF-β1 surface dissolution . ***(Smith et al., 2011)***.

Owing to the lack of necrotizing effect of EDTA etching, it is more beneficial to periodontal and bone healing because of its ability to selectively expose collagen fibers in dentin and bone, which in turn produces a matrix to retain implants of biologically active substances and provides a biocompatible surface for periodontal membrane cell colonization ***(Blomlöf, 1996; de Vasconcellos et al., 2006).***

So, the current study aimed to evaluate the effect of demineralization of alveolar bone in comparison to bone decortications prior to bone graft application in cases that needs horizontal ridge augmentation.

MATERIALS AND METHODS

The study consisted of 14 subjects (age range: 25-55 years) attending the outpatient clinic of Oral Medicine and Periodontology department, faculty of Dentistry, Ain Shams University, Cairo, Egypt. Those who agreed to participate voluntarily have written informed consent and ethical clearance, FDASU-Rec1M011613, was obtained from the Institution's Ethical Committee. Inclusion criteria include subjects: 1- free from any systemic disease as evidenced by health questionnaire using modified Cornell Medical Index ***(Abramson, 1966)***. 2-Single lower posterior edentulous area, for more than 6 months since the time of extraction with remaining 5 mm or less in horizontal dimension. 3- Sufficient zone of attached gingiva ***(Bouri et al., 2008).*** Patients with previous medical history and patients who had any systemic or local factors that would inhibit a normal wound healing process were excluded.

They were randomly assigned into two groups: test patients (n = 7) received EDTA etching of the recipient bed; Group II patients (n = 7) had perforation of the recipient bed prior to GBR. using computer generated random tables (IBM SPSS statistics for windows, version 22.0. Armonk, NY: IBM comp).

Each subject underwent a full mouth scaling and debridement. Presurgical baseline tomography CBCT was taken to determine the severity of ridge resorption at the osteotomy site. Measurements were taken 1mm subcrestal, at the middle of the mesiodistal, buccolingual edentulous area.

After administration of local anesthesia, intrasulcular, crestal and 2 vertical releasing incisions were done to elevate a full thickness periosteal flap and expose the recipient bone. The intrasulcular incision was extended two teeth mesial or distal to the defect. The buccal flap was extended beyond the mucogingival junction and at least 5 mm

beyond the bone defect ***(Urban et al., 2013)***. For group I, after isolation of the field, the bone surface was etched by application of EDTA*, 24% (PH 6.7) for 1 minute to the bone surface ***(*Blomlöf et al., 1997*)***. **(Fig.1)**

For group II, decortication of bone surface was done by size 2 round bur** to induce bleeding under copious water irrigation. **(Fig.2)**

Subsequently, Bovine pericardium membrane (Tutopatch, tutogen medical GmbH, Germany) was inserted and fixed with fixation pins (Titan, Botiss, Germany) at the apical part of the defect. Particles (particle size 0.5–1 mm) of deproteinized bovine bone (Cerabone, Botiss, Germany) were placed in the defect area and covered by the membrane. A tension-free primary closure was achieved and the flap was sutured with non-resorbable suture material (Polypropylene blue, Assut medical, Switzerland). **(Fig.1: I&II)**

The patients were instructed to have Augmentin 1gm twice for 7 days, (ibuprofen 600 mg) for 3 days and to rinse with 0.12% chlorhexidine digluconate oral rinse 3 times daily for 2 weeks. Postoperative examination and suture removal were performed after 14 days.

6 months later, (mean months), patients were clinically assessed for any local problem that might interfere the surgical intervention. Before implant placement, a CBCT was taken for both groups to assess the width of alveolar ridge and for implant planning.

Bone core samples (2 mm in diameter and 5 mm in length) were obtained from within the boundaries of the augmented site using a trephine drill, under copious irrigation, without compromising implant placement. **(Fig.1: III&IV)**

* Prefgel, Straumann, switzerland

** Hager Meisinger GmbH

Dental implants (Neobiotech), were placed according to standard protocols in a prosthetically ideal position and the flap repositioned and sutured. All biopsy specimens were placed in 10% neutral

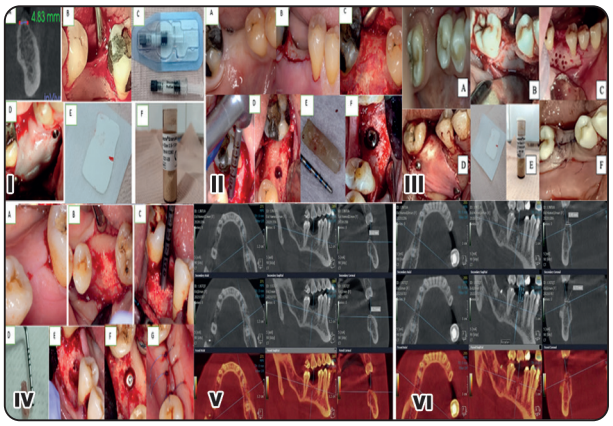

Fig (1: I): EDTA group. **a** Coronal slice of the site of implant that needs GBR. **b** Mucoperiosteal flap reflection. **c** Showing the EDTA 24% gel (Prefgel). **d** Showing Pericardium membrane fixed with tacks in place supported by the underlying bone graft material. **e** The Tutopatch membrane & the demineralized bovine bone mineral (DBBM) (Cerabone, Botiss, Germany).

Fig (1: II): Decortication group. **a** Site of implant that needs GBR. **b** Mucoperiosteal flap reflection. **c** decortication of the buccal surface of cortical bone. **d** Pericardium membrane fixed with tacks in place supported by the underlying bone graft material. **e** The Tutopatch membrane & the demineralized bovine bone mineral (DBBM) (Cerabone, Botiss, Germany). **f** Immediate postoperative suturing of the flap using 4-0 propylene blue suture

Fig (1: III): EDTA group Re-entry. a **t**he augmented site of missing lower left first molar. **b** Mid- crestal incision of the augmented site. **c** Showing the alveolar ridge width gain 6 months after GBR procedure. **d** Showing the site of core biopsy intake using trephine bur drill. **e** Showing 7 mm length core biopsy **f** Showing the augmented site received a dental implant.

Fig (1: IV): Decortication group Re-entry. **a** Showing the augmented site of missing lower left first molar. **b** Showing the alveolar ridge width gain 6 months after GBR procedure. **c** Showing the site of core biopsy intake using trephine bur drill. **d** Showing core biopsy. **e** Showing the augmented site received a dental implant. **f** Postoperative suturing.

Fig (1: V&IV): V : a showing the superimposition method between T1&T2 for group I. **IV** showing the superimposition method between T1&T2 for group II

buffered formalin for 5 days to fix the dissected block sections.

CBCT taken at reentry were assessed by fusion method for buccolingual width dimensions after mean of 6 months.

For histological studies, the specimens were cleared with xylene and embedded in paraffin wax. 4 microns thick Sections were cut longitudinally using a Jung K microtome (Leica microtome type sm2500s; Leica, Wetzlar, Germany). The prepared slices were stained with haematoxylin and eosin (H&E) and observed by a light (Carl Zeiss, Oberkochen, Germany. (This was done in the Oral Histology laboratory, Oral Biology Department, Faculty of Dentistry Ain Shams University.

All clinical and biochemical data were tabulated and statistically analyzed.

Statistical analysis:

The averages and ranges for the percentage of newly formed bone, residual graft particles were calculated. Numerical data were presented as mean and standard deviation (SD) values. They were explored for normality by checking the data distribution, and using Shapiro-Wilk test. Data showed parametric distribution so they were analyzed using paired t-test. R statistical analysis software version 4.1.1 for Windows was used to obtain the statistical results.

RESULTS

All surgical sites healed without complications. No flap dehiscence and no exposure of membranes were noted. Good primary stability of all implants and after 4 months of healing were restored. No residual parts of the collagen membrane could be detected at re-entry surgery and the regenerated tissue appeared as mineralized bone tissue and enough to place implants without grafting.

Histological Assessment:

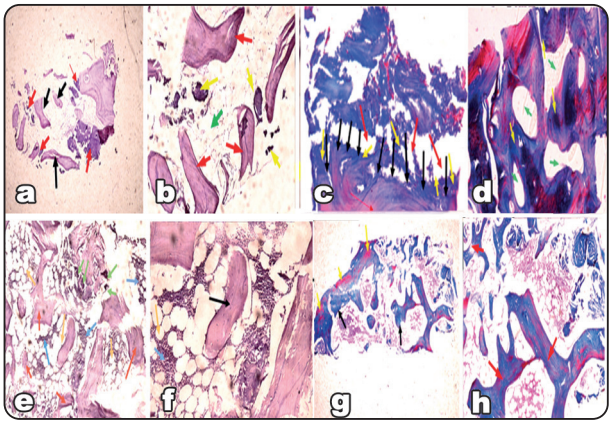

Figure (2)

Group I (EDTA): as shown in Figure (2: A, B,C,D)

Fig. (2: A&B) Photomicrograph of bone core biopsy taken after 6 months of horizontal augmentation. The newly formed bone trabeculae shown by red arrow surrounded by fat cells shown by green arrow and residual bone graft particles shown by the yellow arrows after 6 month **A**: (H&E stain org.mag.X100). **B:** (H&E stain org.mag.X400).

Fig. (2: C&D): Photomicrograph showing integration of the new collagen shown by yellow arrow on the surface of the native cortical lamellated bone as shown by the red arrow, the blue arrow represents the spaces of DBBM particles after decalcification. Black arrows represent the interface between native bone and bone graft. **C:**(Masson's Trichrome stain org.mag.X200). **D:** (X400). **Group II (Decortication)**:as shown in (E, F,G,H).

Fig. (2: E&F): A photomicrograph showing islands of new bone shown by red arrow surrounded by inflammatory cells and fat cells.The blue arrow shows areas of inflammatory cells and the orange arrow shows areas of fat cells surrounding islands of new bone. Residual graft paticles shown by green arrow. The black arrow showing an island of new bone surrounded by inflammatory cells as shown by the orange arrow and fat cells as shown by the blue arrow. **E:** (H&E stain org. mag.X200). **F:** (H&E stain org.mag.X400)

Fig. (2: G&H): Photomicrograph showing a biopsy stained by Masson's Trichrome stain. Black arrow shows areas of new collagen formation. Yellow arrow represents the native bone. Areas of new collagen formation shown by red arrow. **G:** (Masson's Trichrome stain org.mag. X100). **H:** (X400).

Histomorphometric analysis

The histomorphometric analysis was done by processing the images from the microscope with a camera (Olympus BX50; Olympus Optical Co., Tokyo, Japan) and a frame grabber. The images from each area of the biopsy core were obtained and analyzed using image analysis software (Image j) to calculate the percentages of residual graft particles (RG), area percentage of newly formed bone (NB), in each specimen.

- Group (I) (3.63±1.35) had a significantly higher value of area percentage of new bone formation than group (II) (2.52±0.78) (p=0.029).

TABLE (1): Mean, Standard deviation (SD) values of area percentage of new bone formation (%) for different groups

Area percentage of new bone formation (%) (mean±SD)		p-value
Group (I)	**Group (II)**	
3.63±1.35	2.52±0.78	**0.029***

; significant (p ≤ 0.05) ns; non-significant (p>0.05)

- Group (II) (0.63±0.39) had a higher value of residual bone graft particles than group (I) (0.25±0.03) yet the difference was not statistically significant (p=0.186).

TABLE (2): Mean, Standard deviation (SD) values of residual bone graft particles (%) for different groups

Residual bone graft particles (%) (mean±SD)		p-value
Group (I)	**Group (II)**	
0.25±0.03	0.63±0.39	**0.186ns**

; significant (p ≤ 0.05) ns; non-significant (p>0.05)

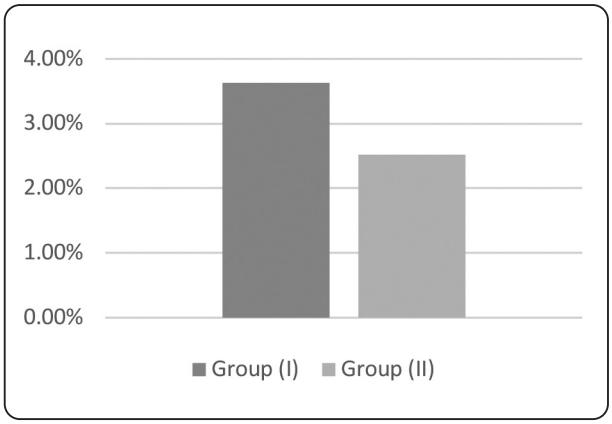

Fig. (3): Bar chart showing average area percentage of new bone formation (%) for different groups

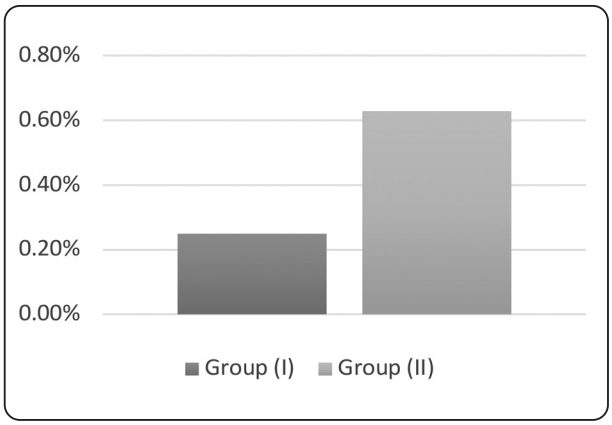

Fig (4): Bar chart showing average residual bone graft particles (%) for different groups

Radiographic Assessment

1- Intergroup comparisons:

Baseline:

Group (I) (4.81±0.68) had a higher value than group (II) (4.40±0.59) yet the difference was not statistically significant (p=0.178).

6 months:

Group (I) (8.17±0.99) had a significantly higher value than group (II) (6.37±0.77) (p=0.001).

Difference:

Group (I) (3.37±0.61) had a significantly higher value than group (II) (1.97±0.27) (p<0.001).

2- Intragroup comparisons

Group (I)

Value measured at 6 months (8.17±0.99) was significantly higher than value measured at baseline (4.81±0.68) ($p<0.001$).

Group (II)

Value measured at 6 months (6.37±0.77) was significantly higher than value measured at baseline (4.40±0.59) ($p<0.001$).

TABLE (3) Mean, Standard deviation (SD) values of alveolar bone width (mm) for different groups

Interval	Alveolar bone width (mm) (mean±SD)		p-value
	Group (I)	Group (II)	
Baseline	4.81±0.68	4.40±0.59	**0.178ns**
6 months	8.17±0.99	6.37±0.77	**0.001***
p-value	**<0.001***	**<0.001***	
Difference	3.37±0.61	1.97±0.27	**<0.001***

; Significant (p ≤ 0.05) ns; non-significant (p>0.05)

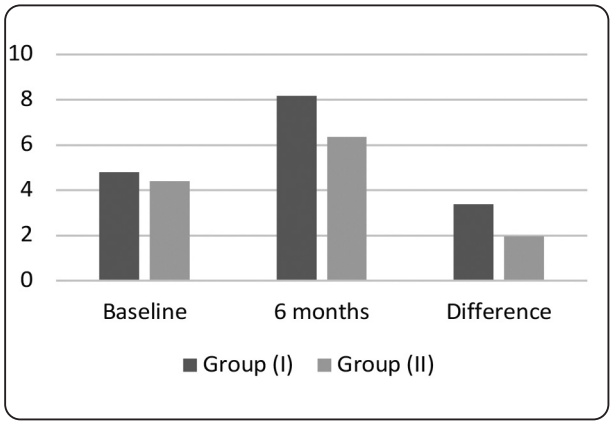

Fig (5): Bar chart showing average alveolar bone width in different groups

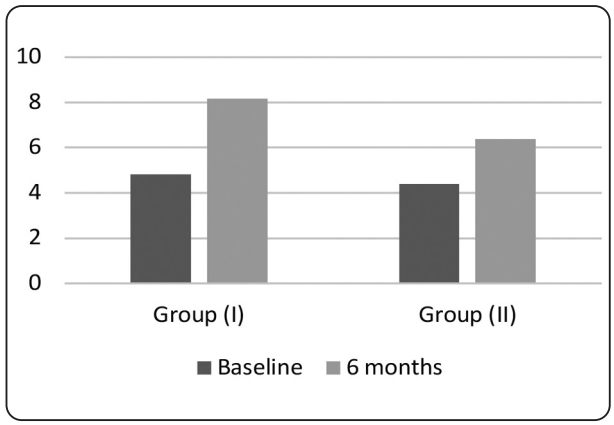

Fig (6): Bar chart showing average bone width change

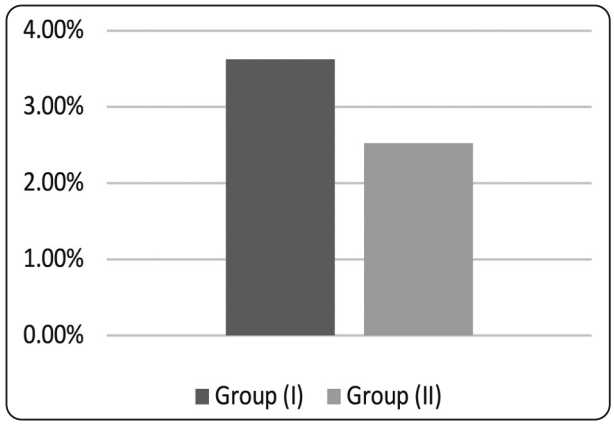

Fig (7): Bar chart showing average area percentage of new bone formation (%) for different groups

DISCUSSION

This clinical comparative study aimed to compare the histological and radiographical outcome of horizontal ridge augmentation after application of EDTA on the surface of cortical bone versus bone decortication. Despite the numerous studies describing the benefits of acids demineralization and bone decortications, there has been no studies describing the effect of EDTA gel on bone surface.

Adequate width and height of alveolar bone are mandatory for a successful placement of a dental implant at the recipient site. Absence of bone at the implant site, can be caused by either periodontitis, tooth extraction, or trauma due to long-term use of a removable prosthesis. In such case , a surgical

procedure, known as guided bone regeneration (GBR), can be used to restore bone height and width ***(Lekovic et al., 1998; McAllister and Haghighat, 2007; Ong et al. 2008).***

In our study,-mid-crestal incision was the incision of choice as it support blood perfusion to the edges of the flap with no risk of necrosis; making the incision in the area of the avascular zone which is located in the mid crest of the ridge prevents the risk of cutting through anastomoses ***(Kleinheinz et al., 2005).*** Two vertical releasing incisions were utilized to accommodate the increased dimension of the grafted ridge, allow for visibility of the edges of the membrane apically, ease of membrane manipulation during the bone tac fixation process and ease of periosteal incision to allow readaptation of soft tissue before tension free closure ***(Urban, 2017).***

To improve bone grafts, decortication of the receptor bone bed have been recommended as proved by an experimental study on thirty sex white rabbit. It was found that the bone density increased on sites of bone perforation and there was increased levels of VEGF, Type 1 collagen and Osteopontin ***(Faria et al. 2008).*** Other studies stated that the addition of bone inducers at the interface between grafts and receptor bone enhances bonding. The latter was first used by **Rezende et al.,2014** who stated the advantages of demineralization over the other methods.

In this study, comparison has been done between two different method of graft bed interface treatments. Graft integration into native bone will be enhanced by making bone penetrations into bone marrow to allow for blood to fill the graft space and increase cellular recruitment and this will lead to increasing the physical bond between the graft and the native bone ***(Carvalho et al., 2000; Lundgren et al., 2000).***

Bone decortication was an integral step of GBR procedures. This was attributed to the increased source of undifferentiated mesenchymal cells that will increase blood supply and new bone formation ***(Buser et al., 1993)***. ***Alberius et al.*** found that penetration of the bony cortex also increase the mechanical interlocking of the bone graft to recipient bed, which improves its stability ***(Alberius et al., 1996).***

On the other hand,, decortication , perforation have multiple limitations as increased bleeding and difficult visibility of the operative field ***(Carvalho et al. 2000;Lundgren et al., 2000).***

Rezende et al.,2014, showed that using bone surface demineralization is better than mechanical bone decortication. Bone remodeling begins with dissolution of minerals by acids from osteoclasts that enzymatically digest the organic bone matrix ***(Melcher, 1976)***

Demineralizing agents have been widely used to isolate collagen ***(Pang et al., 2021).*** This is why a method for bone demineralization using EDTA was chosen, bone tissue is formed by osteoinductive factors contained within the organic matrix. Demineralized fragments of bone induce bone formation subcutaneously and intramuscularly in rats. It was demonstrated that the demineralization process releases BMPs, which induce stromal cells to differentiate into osteoblasts ***(Urist and McLean 1965; Urist et al., 1970); Urist, 1973).***

Although bone demineralization is successful in increasing the osteoinductive potential of bone grafts, in situ demineralization of the contacting surfaces between the bone graft and bone bed has never been used to improve the graft consolidation till Rezende et al. in 2014, studied the citric acid demineralizing effect on the graft bed interface ***(Rezende et al., 2014).***

In previous studies the reaction rate, demineralization efficiency, and the effect on residual collagen were addressed. A comparison of 0.1 M EDTA and 0.6 M HCl for evaluating the efficiency of mineral removal and collagen integrity from bone revealed that EDTA resulted in an almost intact collagen structure, while the HCl destroyed the collagen structure. Thus, EDTA aids in removing min-

eral and preserving collagen integrity, but it takes longer treating time. ***(Pang et al., 2021)***.

EDTA Gel was proved to be a calcium chelating agent that results in removal of calcium ions from their hydroxy appetite crystals leading to exposure of the collagen fibers and other stimulatory proteins that promote osteoblatic differentiation and proliferation such as bone morphogenetic protein ***(Gandolfi et al. 2019)***

The supersaturated concentration of EDTA gel was chosen in this study as it is the best concentration for chelation of calcium and the most efficient rate of demineralization with time ***(Blomlöf et al. 1997).***

Certain inclusion criteria are required to select appropriate barrier membranes these include biocompatibility, and clinical controllability. Although collagen membranes show effective regeneration, yet their major limitation is their fast resorption, which results in early loss of barrier function. The pericardium membrane, the choice for this study, in comparison to collagen membranes have shown an effective crosslinking, suggesting its prolonged resorption time, it also has exceptional handling properties ***(Muto et al. 2009; Gupta and Gupta 2014).***

Using deproteinized bovine bone mineral (DBBM) in this study was due to its slow rate of resorption, act as an osteocoductive scaffold to enhance bone regeneration outside the skeletal envelope and also it has no osteoinductive effect that can interfere with the osteoinductive effect of demineralization of the bone bed ***(Hämmerle et al., 2008; Aludden et al., 2020; Lee, 2021)***.

In this study, CBCT was chosen for the analysis of osseous changes. CBCT uses a two-dimensional detector to scan the head, rather than stacking multiple slices together, as the conventional CT scanner does. It also, does not expend high radiation doses in addition to providing a 3D information ***(Sukovic, 2003)***.

The timing for core biopsy was decided based on studies showed that the area % of new bone significantly increased with the use of DBBM and resorbable barrier membrane after 6 months post-operatively***(Naenni et al., 2017; Lee, 2021)***

Every effort was taken in biopsy procedure to make sure that we evaluate the quality of newly formed bone. Trephine drill was used to obtain the biopsies which were immediately placed in a container filled with 10 % formalin for 5 days. Once fixed, then decalcified by immersing in 12% EDTA for 10 days then using ascending concentrations of alcohol (from 96 to absolute alcohol), each specimen was dehydrated, and then transferred to xylol to free it from alcohol then embedded into paraffin wax to be sliced to semi-thin sections of about 4 microns thick. Sections were transferred in descending concentrations of alcohol solutions (96%, 70%, and then distilled water)***.(Bancroft, 2008)***

Masson trichrome stain was used for the histomorphometric analysis of bone quality and quantity as secondary outcome because it can differentiate between mineralized and osteoid tissue. ***(Suvik, 2012)***

Histological evaluation of the core biopsies conducted 6 months after augmentation. In group I, histological analysis showed increased width of bone trabeculae and more osteoid tissue than Group II as well as basophilic immature collagen areas of woven bone or osteoid tissue which were indicative of the active new bone formation, The edges of the graft showed osteoclastic activity followed by bone matrix formation consistent with remodeling. Group I showed more obvious crosslinking between graft and bed, with shared marrow spaces and trabeculae of newly formed bone, impairing the identification of boundaries of graft, new bone, and recipient bed.

It was found that preosteoblast differentiate upon recognition of a rough surface and begin to deposit new bone. This explains the better results found in demineralized specimens in this study. Osteoblastic differentiation is stimulated by the release of BMPs by the demineralization process they in turn diffuse through the tissue ***(Urist, 1973; Schwartz et al.***

2000). From a histomorphometric standpoint, the highest mean value of new bone was recorded in group I (EDTA group), with a highly statistically significant difference between the two groups.

Regarding CBCT results, both groups showed significant gain in width after six months. In comparison between the two groups, a non-significant difference in baseline measurements were recorded. However, after six months group I (EDTA group) showed higher mean increase in bone width (8.17±0.99) in comparison to (6.37±0.77) in group II (decortication group), with statistically significant difference between the two groups.

Based on the results of the present study we found that, demineralization by EDTA resulted in significantly more area % of newly formed bone and more bonding of this new bone to the graft and recipient bed compared to the decortication Group II. The bone width of EDTA Group I was significantly higher than those of the decortication group after 6 months according to the statistical analysis.

This study raises the possibility that demineralization of the contacting bone surfaces in the bone grafts and native bone can accelerate and intensify the mechanical interlocking of these grafts to the host bed.

CONCLUSION

Demineralization of alveolar bone is an effective way to increase the integration of bone graft and promote the new bone formation whenever ridge augmentation is needed as it has an osteoinductive effect on the osteoblatic cell differentiation and proliferaton with no trauma to the alveolar ridge specially in very thin ridge. Decortication of alveolar bone is effective method to enhance blood supply and nourish the area with the undifferentiated mesenchymal cells which will aid in success of the augmentation procedures but in case of thin ridge the volume will be affected. Bone demineralization results in more width gain than mechanically decorticating the alveolar bone..

REFERENCES

1. Abramson, J. H. 1966. “The Cornell Medical Index , a Health Questionnaire, Has Possibilities as an Epidemiologic Tool. The Author Discusses the Uses of the Index , as Well as Its Limitations, and Suggests Modifications to Enhance Its Usefulness. THE CORNELL MEDICAL INDEX AS.” American Journal of Public Health 56(2):287–98.
2. Acar, Ahmet and Umit Yolcu. 2018. “Bone Decortication Rate and Guided Bone Regeneration under an Occlusive Titanium Dome: Micro- CT Analysis.” Annals of Medical Research 26(3):329–34.
3. Alberius, Per, Monica Gordh, Lisbeth Lindberg, and Olof Johnell. 1996. “Onlay Bone Graft Behaviour after Marrow Exposure of the Recipient Rat Skull Bone.” Scandinavian Journal of Plastic and Reconstructive Surgery and Hand Surgery 30(4):257–66.
4. Aludden, Hanna, Arne Mordenfeld, Christer Dahlin, Mats Hallman, and Thomas Starch-Jensen. 2020. “Histological and Histomorphometrical Outcome after Lateral Guided Bone Regeneration Augmentation of the Mandible with Different Ratios of Deproteinized Bovine Bone Mineral and Autogenous Bone. A Preclinical in Vivo Study.” Clinical Oral Implants Research 31(10):1025–36.
5. Amit, Goyal, J. P. S. Kalra, Bhatiya Pankaj, Singla Suchinder, and Bansal Parul. 2012. “Periodontally Accelerated Osteogenic Orthodontics (PAOO) - A Review.” Journal of Clinical and Experimental Dentistry 4(5): 292–96.
6. Aro, Hannu T. and Allan J. Aho. 1993. “Clinical Use of Bone Allografts.” Annals of Medicine 25(4):403–12.
7. Bahat, O. 1994. “Treatment Planning and Placement of Implants in the Posterior Maxillae.” Implant Dentistry 3(1):151–61.
8. Bancroft, John. 2008. Bancroft’s Theory and Practice of Hisological Techniques. 8th ed. edited by J. Bancroft, C. Layton, and SuvarnaS. Kim Suvarna. Elsevier.
9. Bauer, Thomas W. and George F. Muschler. 2000. “Bone Graft Materials.” Clinical Orthopaedics and Related Research (371):10–27.
10. Becker, Ju¨rgen, Bilal Al-Nawas, Marcus Klein, Hennig Schliephake, Hendrik Terheyden, and Frank Schwarz. 2009. “Use of a New Cross-Linked Collagen Membrane for the Treatment of Dehiscence-Type Defects at Titanium Implants : A Prospective , Randomized- Controlled Double-Blinded Clinical Multicenter Study.” Clinical Oral Implant Research 20:742–49.

11. Benic, Goran I. and Christoph H. F. Hämmerle. 2014. "Horizontal Bone Augmentation by Means of Guided Bone Regeneration." Periodontology 2000 66(1):13–40.

12. Blomlöf, Johan. 1996. "Root Surface Etching at Neutral PH Promotes Periodontal Healing." Journal of Clinical Periodontology 23(1):50–55.

13. Blomlöf, Johan, Leif Blomlof, and Sven Lindskog. 1997. "Effect of Different Concentrations of EDTA on Smear Removal and Collagen Exposure in Surfaces." Journal of Clinical Periodontology 24:534–37.

14. Bouri, Anil, Nabil Bissada, Mohammad S. Al-Zahrani, Fady Faddoul, and Imad Nouneh. 2008. "Width of Keratinized Gingiva and the Health Status of the Supporting Tissues around Dental Implants." The International Journal of Oral & Maxillofacial Implants 23(2):323–26.

15. Brunsvold, Michael and James Mellonig. 1993. "Bone Grafts in Periodontal Regeneration." Periodontology 2000 9:80–91.

16. Buser D, Bragger U, Lang NP, Nyman S. 1990. "Buser 1990 Regeneration and Enlargement of Jaw Bone.Pdf." Clinical Oral Implant Research 1:22–32.

17. Buser, D., K. Dula, U. Belser, H. P. Hirt, and H. Berthold. 1995. "Localized Ridge Augmentation Using Guided Bone Regeneration. II. Surgical Procedure in the Mandible." The International Journal of Periodontics & Restorative Dentistry 15(1):11–29.

18. Buser, Daniel, Karl Dula, Urs Bels, Hans-Peter Hirt, and Hermonn Berthold. 1993. "Localized Ridge Augmentation Using Guided Bone Regeneration . I . Surgical Procedure in the Maxilla Simultaneous Approach of GBR Staged Approach of GBR." The Internatinal Journal of Periodontics & Restoraive Dentistry 13(1):29–47.

19. Carlsson, G. E. and G. Persson. 1967. "Morphologic Changes of the Mandible after Extraction and Wearing of Dentures. A Longitudinal, Clinical, and x-Ray Cephalometric Study Covering 5 Years." Odontologisk Revy 18(1):27–54.

20. Carvalho, P. S., L. Vasconcellos, and J. Pi. 2000. "Influence of Bed Preparation on the Incorporation of Autogenous Bone Grafts: A Study in Dogs." The International Journal of Oral & Maxillofacial Implants 15:565–70.

21. Dahlin, Christer, Anders Linde, Jan Gottlow, and Sture Nyman. 1988. "Healing of Bone Defects by Guided Tissue Regeneration." Plastic and Reconstructive Surgery 81(5):672–76.

22. De-Deus, G., C. Reis, S. Fidel, R. Fidel, and S. Paciornik. 2008. "Dentine Demineralization When Subjected to EDTA with or without Various Wetting Agents: A Co-Site Digital Optical Microscopy Study." International Endodontic Journal 41(4):279–87.

23. Dragoo, Mick R. and Harley C. Sullivan. 1973. "A Clinical and Histological Evaluation of Autogenous Iliac Bone Grafts in Humans Part II. External Root Resorption." Journal of Periodontology 44(10):614–25.

24. Esposito, M., M. G. Grusovin, S. Kwan, H. V. Worthington, and P. Coulthard. 2009. "Interventions for Replacing Missing Teeth: Bone Augmentation Techniques for Dental Implant Treatment." Australian Dental Journal 54(1):70–71.

25. Faria, Paulo E. P., Roberta Okamoto, Ricardo M. Bonilha-Neto, Samuel P. Xavier, Antonio C. Santos, and Luiz A. Salata. 2008. "Immunohistochemical, Tomographic and Histological Study on Onlay Iliac Grafts Remodeling." Clinical Oral Implants Research 19(4):393–401.

26. Frolik, Charles A., Lee F. Ellis, and Daniel C. Williams. 1988. "Isolation and Characterization of Insulin-like Growth Factor-II from Human Bone." Biochemical and Biophysical Research Communications 151(3):1011–18.

27. Frost, Harold M. 1983. "The Regional Acceleratory Phenomenon: A Review." Henry Ford Hospital Medical Journal 31(1):3–9.

28. Gandolfi, Maria Giovanna, Paola Taddei, Anna Pondrelli, Fausto Zamparini, Carlo Prati, and Gianrico Spagnuolo. 2019. "Demineralization, Collagen Modification and Remineralization Degree of Human Dentin after EDTA and Citric Acid Treatments." Materials 12(25):1–20.

29. Gazdag, André R., Joseph M. Lane, David Glaser, and Robert A. Forster. 1995. "Alternatives to Autogenous Bone Graft: Efficacy and Indications." Journal of the American Academy of Orthopaedic Surgeons 3(1):1–8.

30. Glowacki, Julie. 1998. "Angiogenesis in Fracture Repair." Clinical Orthopaedics and Related Research (355 SUPPL.):82–89.

31. Graham, Lee, Paul R. Cooper, Nicola Cassidy, Jacques E. Nor, Alastair J. Sloan, and Anthony J. Smith. 2006. "The Effect of Calcium Hydroxide on Solubilisation of Bio-Active Dentine Matrix Components." Biomaterials 27(14):2865–73.

32. Greenstein, G., B. Greenstein, J. Cavallaro, and D. Tarnow. 2009. "The Role of Bone Decortication in Enhancing the Results of Guided Bone Regeneration: A Literature Review." Journal of Periodontology 80(2):175–89.

33. Gupta, Dr. Swati and Dr. Richa Gupta. 2014. "Guided Bone Regeneration with Pericardium Membranes." IOSR Journal of Dental and Medical Sciences 13(11):61–65.
34. Hadjidakis, Dimitrios J. and Ioannis I. Androulakis. 2006. "Bone Remodeling." Annals of the New York Academy of Sciences 1092:385–96.
35. Hämmerle, C. H., J. Schmid, N. P. Lang, and A. J. Olah. 1995. "Temporal Dynamics of Healing in Rabbit Cranial Defects Using Guided Bone Regeneration." Journal of Oral and Maxillofacial Surgery : Official Journal of the American Association of Oral and Maxillofacial Surgeons 53(2):167–74.
36. Hämmerle, C. H., J. Schmid, A. J. Olah, and N. P. Lang. 1996. "A Novel Model System for the Study of Experimental Guided Bone Formation in Humans." Clinical Oral Implants Research 7(1):38–47.
37. Hämmerle, Christoph H. F., Ronald E. Jung, Duygu Yaman, and Niklaus P. Lang. 2008. "Ridge Augmentation by Applying Bioresorbable Membranes and Deproteinized Bovine Bone Mineral: A Report of Twelve Consecutive Cases." Clinical Oral Implants Research 19(1):19–25.
38. Hardwick, Ross, Byron K. Hayes, and Charles Flynn. 1995. "Devices for Dentoalveolar Regeneration: An Up-To-Date Literature Review." Journal of Periodontology 66(6):495–505.
39. Hauschka, P. V., A. E. Mavrakos, M. D. Iafrati, S. E. Doleman, and M. Klagsbrun. 1986. "Growth Factors in Bone Matrix. Isolation of Multiple Types by Affinity Chromatography on Heparin-Sepharose." Journal of Biological Chemistry 261(27):12665–74.
40. Hockers, Thierry; Abensur, David; Valentini, Pascal; Legrand, Roman; Hammerle, Christoph H. F. 1999. "The Combined Use of Bioresorbable Membranes and Xenografts or Autografts in the Treatment of Bone Defects around Implants." Clinical Oral Implant Research 10:487–98.
41. Jensen, Anders Torp, Simon Storgård Jensen, and Nils Worsaae. 2016. "Complications Related to Bone Augmentation Procedures of Localized Defects in the Alveolar Ridge . A Retrospective Clinical Study." Oral and Maxillofacial Surgery 20:115–22.
42. Jensen, Ole T., Lee Kuhlke, Jean-Francois Bedard, and Dawn White. 2006. "For Anterior Maxillary Vertical Augmentation Prior to Implant Placement." Journal of Oral Maxillofacial Surgery 64:290–96.
43. Kleinheinz, Johannes, Birgit Kruse-lo, and Dieter Weingart. 2005. "Incision Design in Implant Dentistry Based on Vascularization of the Mucosa." Clinical Oral Implants Research 16:518–23.
44. Kumar, Prasanna, Belliappa Vinitha, and Ghousia Fathima. 2013. "Bone Grafts in Dentistry." Journal of Pharmacy and Bioallied Sciences 5(SUPPL.1):125–28.
45. Lee, Sung-Jo. 2021. "The Sausage Technique Using Collagen Membrane without Autogenous Bone Graft: A Case Report." Journal of Implantology and Applied Sciences 25(2):74–83.
46. Lekholm, Ulf, Karin Wannfors, Sten Isaksson, and Björn Adielsson. 1999. "Oral Implants in Combination with Bone Grafts: A 3-Year Retrospective Multicenter Study Using the Brånemark Implant System." International Journal of Oral and Maxillofacial Surgery 28(3):181–87.
47. Lekovic, Vojislav, Paulo M. Camargo, Perry R. Klokkevold, Michael Weinlaender, E. Barrie Kenney, Bozidar Dimitrijevic, and Melica Nedic. 1998. "Preservation of Alveolar Bone in Extraction Sockets Using Bioabsorbable Membranes." Journal of Periodontology 69(9):1044–49.
48. Liu, Jie and David G. Kerns. 2014. "Mechanisms of Guided Bone Regeneration: A Review." The Open Dentistry Journal 8(1):56–65.
49. Lord, C. F., M. C. Gebhardt, W. W. Tomford, and H. J. Mankin. 1988. "Infection in Bone Allografts. Incidence, Nature, and Treatment." The Journal of Bone and Joint Surgery. American Volume 70(3):369—376.
50. Lu, Chuanyong, Theodore Miclau, Diane Hu, and Ralph S. Marcucio. 2007. "Ischemia Leads to Delayed Union during Fracture Healing: A Mouse Model." Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society 25(1):51–61.
51. Lundgren, Anna Karin, Dan Lundgren, Christoph H. F. Ha, Sture Nyman, and Lars Sennerby. 2000. "Influence of Decortication of the Donor Bone on Guided Bone Augmentation." Clinical Oral Implants Research 11:99–106.
52. Maestre Ferrín, Laura, Miguel Penarrocha, and Maria Penarrocha-Diago. 2009. "Augmentation Procedures for Deficient Edentulous Ridges, Using Onlay Autologous Grafts : An Update Augmentation Procedures for Deficient Edentulous Ridges, Using Onlay Autologous Grafts : An Update." Medicina Oral, Patología Oral y Cirugía Bucal · 8(June):402–7.
53. Mayfield, L., G. Söderholm, O. Norderyd, and R. Attström. 1998. "Root Conditioning Using EDTA Gel as an Adjunct to Surgical Therapy for the Treatment of Intraosseous Periodontal Defects." Journal of Clinical Periodontology 25(9):707–14.

54. McAllister, Bradley S. and Kamran Haghighat. 2007. "Bone Augmentation Techniques." Journal of Periodontology 78(3):377–96.

55. Melcher, A. H. 1976. "On the Repair Potential of Periodontal Tissues." Journal of Periodontology 47(5):256–60.

56. Miloro, M., Ghali, G.E., Larsen, P.E. and Waite, P.D. (2004) Peterson's. Principles of Oral and Maxillofacial Surgery. 2nd Edition, BC Decker Inc., Hamilton, London, Vol- 1: 583.

57. Miyamoto, Ikuya, Katsuyuki Funaki, Kensuke Yamauchi, Takashi Kodama, and Tetsu Takahashi. 2012. "Alveolar Ridge Reconstruction with Titanium Mesh and Autogenous Particulate Bone Graft: Computed Tomography-Based Evaluations of Augmented Bone Quality and Quantity." Clinical Implant Dentistry and Related Research 14(2):304–11.

58. Muto, Akihito, Toshiya Nishibe, Herbert Dardik, and Alan Dardik. 2009. "Patches for Carotid Artery Endarterectomy: Current Materials and Prospects." Journal of Vascular Surgery 50(1):206–13.

59. Naenni, Nadja, David Schneider, Ronald E. Jung, Jürg Hüsler, Christoph H. F. Hämmerle, and Daniel S. Thoma. 2017. "Randomized Clinical Study Assessing Two Membranes for Guided Bone Regeneration of Peri-Implant Bone Defects: Clinical and Histological Outcomes at 6 Months." Clinical Oral Implants Research 28(10):1309–17.

60. Nair, Vinod. 2018. "Review Article Pericardium Membranes - A Critical Appraisal." Manipal Journal of Dental Sciences 3(1):27–30.

61. Ong, Constantine T. T., Saso Ivanovski, Ian G. Needleman, Maria Retzepi, David R. Moles, Maurizio S. Tonetti, and Nikolaos Donos. 2008. "Systematic Review of Implant Outcomes in Treated Periodontitis Subjects." Journal of Clinical Periodontology 35(5):438–62.

62. Pandit, Nymphea and InderKumar Pandit. 2016. "Autogenous Bone Grafts in Periodontal Practice: A Literature Review." Journal of the International Clinical Dental Research Organization 8(1):27.

63. Pang, Siyuan, Frances Y. Su, Amesha Green, Justin Salim, Joanna McKittrick, and Iwona Jasiuk. 2021. "Comparison of Different Protocols for Demineralization of Cortical Bone." Scientific Reports 11(1):1–10.

64. Ramirez, Francesco and Daniel B. Rifkin. 2003. "Cell Signaling Events: A View from the Matrix." Matrix Biology 22(2):101–7.

65. Rezende, Maria L., Alberto Consolaro, Adriana C. Sant'Ana, Carla A. Damante, Sebastião L. Greghi, and Euloir Passanezi. 2014. "Demineralization of the Contacting Surfaces in Autologous Onlay Bone Grafts Improves Bone Formation and Bone Consolidation." Journal of Periodontology 85(5):e121–29.

66. Rocchietta, Isabella, Filippo Fontana, and Massimo Simion. 2008. "Clinical Outcomes of Vertical Bone Augmentation to Enable Dental Implant Placement: A Systematic Review." Journal of Clinical Periodontology 35(SUPPL. 8):203–15.

67. Roodman, G. David. 1999. "Cell Biology of the Osteoclast." Experimental Hematology 27(8):1229–41.

68. Saad, Moustapha, André Assaf, and Hassan Maghaireh. 2012. "Guided Bone Regeneration: Evidence & Limits." Smile Dental Journal 7(1):8–16.

69. Saghiri, Mohammad Ali, Armen Asatourian, Franklin Garcia-Godoy, and Nader Sheibani. 2016. "The Role of Angiogenesis in Implant Dentistry Part II: The Effect of Bone-Grafting and Barrier Membrane Materials on Angiogenesis." Medicina Oral, Patologia Oral y Cirugia Bucal 21(4):e526–37.

70. Salmeron, Samira and Maria O. O. Rezende. 2017. "Trends in Clinical Periodontology and Implant Dentistry." Elsevir (4):31–39.

71. Sam, George and Madhavan Pillai. 2014. "Evolution of Barrier Membranes in Periodontal Regeneration- " Are the Third Generation Membranes Really Here ?"." Smile Dental Journal 8(12):12–15.

72. Scantlebuty, Todd V. 1993. "1982-1992 : A Decade of Technology Fof Guided Tissue Regeneration." Journal of Periodontology 64:1129–37.

73. Schmid, J; Hämmerle, CHF; Olah, AJ; Lang, NP. 1994. "Membrane Permeability Is Unnecessary for Guided Bone Regeneration of Bone." Clinical Oral Implants Research 5:125–30.

74. Schmid, J., Beat Wallkamm, Christoph H. F. Hammerle, Sylwester Gogolewski, and Niklau P. Lang. 1997. "The Significance of Angiogenesis in Guided Bone Regeneration." Clinical Oral Implants Research 8:244–48.

75. Schonherr, E. and H. J. Hausser. 2000. "Extracellular Matrix and Cytokines: A Functional Unit." Developmental Immunology 7(2–4):89–101.

76. Schropp, Lars, Ann Wenzel, Lambros Kostopoulos, and Thorkild Karring. 2003. "Bone Healing and Soft Tissue Contour Changes Following Single-Tooth Extraction: A

Clinical and Radiographic 12-Month Prospective Study." International Journal of Periodontics Restorative Dentistry 23(4):313–23.

77. Schwartz, Z., C. H. Lohmann, M. Wieland, D. L. Cochran, D. D. Dean, M. Textor, L. F. Bonewald, and B. D. Boyan. 2000. "Osteoblast Proliferation and Differentiation on Dentin Slices Are Modulated by Pretreatment of the Surface With Tetracycline or Osteoclasts." Journal of Periodontology 71(4):586–97.
78. Schwarz, Frank, Daniel Rothamel, Monika Herten, and Jurgen Becker. 2006. "Angiogenesis Pattern of Native and Cross-Linked Collagen Membranes : An Immunohistochemical Study in the Rat." Clinical Oral Implants Research 17:403–9.
79. Smith, E. L., J. S. Colombo, A. J. Sloan, and Rachel Waddington. 2011. "TGF- β 1 Exposure From Bone Surfaces by Chemical Treatment." European Cells and Materials 21(0):193–201.
80. Sukovic, P. 2003. "Cone Beam Computed Tomography in Craniofacial Imaging." Orthodontics \& Craniofacial Research 6(s1):31–36.
81. Suvik, Assaw. 2012. "The Use of Modified Massion's Trichrome Staining in Collagen Evaluation in Wound Healing Study." Malaysian Journal of Veterinary Research 3(1):39–47.
82. Taipale, Jussi and Jorma Keski-Oja. 1997. "Growth Factors in the Extracellular Matrix." The FASEB Journal 11(1):51–59.
83. Tolstunov, Len, John F. Eri. Hamrick, Vishtasb Broumand, Dekel Shilo, and Adi Rachmiel. 2019. "Bone Augmentation Techniques for Horizontal and Vertical Alveolar Ridge Deficiency in Oral Implantology." Oral and Maxillofacial Surgery Clinics of North America 31(2):163–91.
84. Traini, Tonino, Pascal Valentini, Giovanna Iezzi, and Adriano Piattelli. 2007. "A Histologic and Histomorphometric Evaluation of Anorganic Bovine Bone Retrieved 9 Years After a Sinus Augmentation Procedure." Journal of Periodontology 78(5):955–61.
85. Urban, Istvan A. 2017. "Principles of Vertical and Horizontal Ridge Augmentation in the Posterior Mandible." P. 400 in Vertical and Horizontal Ridge Augmentation, edited by I. Urban. London ,UK: Quintessence Pub. Co.
86. Urban, Istvan A., Heiner Nagursky, Jaime L. Lozada, and Katalin Nagy. 2013. "Horizontal Ridge Augmentation with a Collagen Membrane and a Combination of Particulated Autogenous Bone and Anorganic Bovine Bone–Derived Mineral: A Prospective Case Series in 25 Patients." The International Journal of Periodontics and Restorative Dentistry 33(3):299–307.
87. Urist, Marshall R. and Franklin C. McLean. 1965. "Bone Formation by Autoinduction." Science 150:893–99.
88. Urist MR, Jurist Jm Jr, Dubuc FL, Strates BS. 1970. "Quantitation_of_New_Bone_Formation_in Intramucular Implants of Bone Matrix in Rabbits." Clinical Orthopaedics and Related Research (68):280–93.
89. Urist MR, Iwata H. 1973. "Preservation and Biodegradation of the Morphogenetic Property of Bone Matrix ?" Journal of Theoretical Biology (38):155–67.
90. de Vasconcellos, Luana Marotta Reis, Lucilene Hernandes Ricardo, Ivan Balducci, Luis Gustavo Oliveira de Vasconcellos, and Yasmin Rodarte Carvalho. 2006. "Histological Analysis of Effects of 24% EDTA Gel for Nonsurgical Treatment of Periodontal Tissues." Journal of Oral Science 48(4):207–14.
91. Wang, Hom Lay and Lakshmi Boyapati. 2006. "'PASS' Principles for Predictable Bone Regeneration." Implant Dentistry 15(1):8–17.
92. Van der Weijden, Fridus, Federico Dell'Acqua, and Dagmar Else Slot. 2009. "Alveolar Bone Dimensional Changes of Post-Extraction Sockets in Humans : A Systematic Review." Journal of Clinical Periodontology 36:1048–58.
93. Wong, Maelene L. and Leigh G. Griffiths. 2014. "Immunogenicity in Xenogeneic Scaffold Generation: Antigen Removal vs. Decellularization." Acta Biomaterialia 10(5):1806–16.
94. Zhao, S., A. J. Sloan, P. E. Murray, P. J. Lumley, and A. J. Smith. 2000. "Ultrastructural Localisation of TGF-β Exposure in Dentine by Chemical Treatment." Histochemical Journal 32(8):489–94.