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Original article

Efficacy of Calcium Phosphate Bone Allograft with EDTA Root Surface Etching in Treatment of Intrabony Periodontal Defects

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ABSTRACT

Periodontal disease has been considered as a group of inflammatory disorder involving tooth-supporting structure, characterizing by bone destruction in advancing forms. Ultimate goal of periodontal therapy includes arresting disease progression, regeneration of lost structures as well as, attain improved periodontal architecture. In this study efficacy of calcium phosphate bone Allograft with EDTA root surface etching in treatment of intrabony periodontal defects has been carried out. Thirty non-smoking patients (16 males & 14 females) of age between 38 to 52 years with severe chronic periodontitis were included. Patients were assigned to treatment groups blindly into 3 groups: G1: Intra-bony defects were grafted with micro sized (β-TCP) with a particle size that ranged from 63 to 150 μm; G2: Exposed roots were etched for 2 minutes with 24% EDTA gel, followed by intra-bony defect fill of (β-TCP) of same particle size used for G1; G3: Periodontal intra-bony defects were grafted with NHA. Intrabony defect of each group was filled by the graft till the defect margin with moderate condensation. Mucoperiosteal flap was replaced and sutured with interrupted sutures, no periodontal pack were used. It can conclude that, combining micro sized grafts with nano grafts can turn the Osteoconductive bone substitute into an osteo-inductive one, indirectly through stimulation of pro-osteogenic mediators.

Keywords: Calcium phosphate, Bone graft, EDTA, intrabony periodontal defects.

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INTRODUCTION

Periodontal regeneration is a term applied for regeneration of tooth supporting tissue, including alveolar bone, periodontal ligament and cementum over a previously diseased root surface.[1] The goals of periodontal therapy include not only arresting periodontal disease progression, but also regeneration of structures lost due to disease, where appropriate conventional surgical approaches reduce periodontal pockets, and attain improved periodontal architecture.[2]β-Tricalcium phosphate (β-TCP) is a porous form of calcium phosphate, with similar proportions of calcium and phosphate to cancellous bone.[3,4] Studies showed that calcium TCP support the attachment, proliferation and differentiation of osteoblasts and mesenchymal cells and bone growth.[5-8]Tricalcium phosphate ceramic is biocompatible, osteoconductive. Physiochemically, β-TCP is a resorbable material with 99% phase purity, total micro porosity and a homogeneous ceramic structure. [9]

Chemical root surface conditioning has been introduced, using a variety of agents, in order to detoxify, decontaminate and demineralize the root surface, thereby removing the smear layer and exposing the collagenous matrix of dentin and cementum. Various acids have been used for chemical root surface conditioning, including *citric and phosphoric acids, ethylenediaminetetraacetic acid (EDTA)* and *tetracycline hydrochloride.* EDTA preserves the vitality of tissues with direct contact, and removes hydroxyapatite from the collagenous dentin matrix more selectively than low pH etching agents. The effectiveness for smear layer removal of etching of the root surface with EDTA gel providing the most desirable root surface to which maximum periodontal ligament cells can adhere and on which they can grow was reported.[10,11] A number of studies [12, 13] have evaluated the effect of particle size on regenerative procedures. Generally, smaller particles are preferable because of more rapid resorption, greater surface area, and enhanced osteogenesis. A particle size of 300–500 μm is optimal for the treatment of periodontal bone defects. Therefore, it was speculated that small β-TCP particles with a diameter of 250– 1,000 μm could be used as a scaffold. Advantages of a nanostructured material in comparison to bulk material are the close contact with surrounding tissues, quick resorption characteristics and a high number of molecules on the surface. In vivo studies demonstrated rapid healing of critical size defects after application of NHA graft material.[14, 15] Particle size appears to be an important variable in the success of alloplast as a bone-inductive material. Particles in the range of 125–1,000 μm possess a higher osteogenic potential than do particles below 125 μm. Optimal particle size appears to be between 100 and 300 μm. This may be due to a combination of surface area and packing density. Very small particles elicit a macrophage response and are rapidly resorbed with little or no new bone formation.[16]

MATERIALS AND METHODS

Thirty non-smoking patients (16 males and 14 females) were 38 to 52 years of age at the time of baseline examination (mean age 39.8 ± 5.8) with severe chronic periodontitis, [17] were participated in this prospective, blinded clinical trial. Subjects were recruited consecutively from patients seeking care for periodontal problems. They were included according the following criteria: No systemic diseases which could influence the outcome of therapy; Good compliance with plaque control instructions following initial therapy; Teeth involved were all vital with score 0 mobility ; Each subject contributed at least one tooth with a test site of two- or three-walled intra-bony interproximal defects of anterior or premolar upper or lower areas; selected 2- or 3-wall intra-bony defect depth ≥2mm as detected in diagnostic periapical radiographs; Selected pocket depth (PD) ≥ 5 mm and clinical attachment level (CAL) ≥ 4 mm at the site of end osseous defect four weeks following initial cause-related therapy; and end osseous radiographic defect angle ≤55 and ≥15.

Initial periodontal therapy including a thorough full mouth scaling and root planning under local anesthesia; this procedure was performed using a combination of hand and ultrasonic instrumentation. Patients were recalled every 3 days for 3 weeks, received plaque control instructions; supra-gingival plaque removal was performed whenever necessary. Four weeks after initial therapy, a reevaluation was performed to confirm the need for periodontal surgery; applying the following criteria: persistence of sites with PD ≥ 5 mm, CAL ≥ 4 mm, and intrabony defects of ≥ 2 mm. Periodontal disease status of the selected sites were determined by clinical periodontal assessments including Plaque Index (PlI)[18], Gingival Index (GI)[19], Probing Depth (PD) [20], Clinical Attachment Level (CAL) [21].

Patient assignment and Periodontal Surgery

Surgical treatment phase was initiated only if the subject had a full-mouth dental plaque score of less than one and zeros plaque score for the test sites. A mucoperiosteal flap was elevated using intrasulcular incisions under local anesthesia and vertical releasing incisions were used when necessary. Debridement of all inflammatory granulation tissue from the intrabony defect was performed until a sound, healthy bone surface was obtained. The teeth were root planed thoroughly using hand and ultrasonic instruments to obtain a smooth and hard root surface.

Patients were assigned to treatment groups blindly taking 1 of 3 sealed envelopes with group name (10 patients each) Figure (1-3):

1. Group 1 (G1): Intra-bony defects were grafted with micro sized (β-TCP) with a particle size that ranged from 63 to 150 μm.
2. Group 2 (G2) Exposed roots were etched with 24% EDTA gel, followed by intra-bony defect fill of (β-TCP).
3. Group 3(G3): Periodontal intra-bony defects were grafted with NHA.

Intrabony defect of each group was filled by the graft till the defect margin with moderate condensation. Mucoperiosteal flap was replaced and sutured with interrupted sutures, no periodontal pack were used.

Postsurgical Treatment

All patients received oral and written postoperative instructions. Patients were prescribed amoxicillin/clavulanate potassium, three times a day for 1 week. During this initial healing phase, plaque control effort was supplemented with chlorhexidine mouth rinse for 1 minute by 0.12% Chlorhexidine digluconate (2 times/ day/2 weeks). Patients were instructed to refrain from tooth brushing and interdental cleaning was avoided at the surgical areas during this time. Sutures were removed 14 days postoperatively and recall appointments for observation of any adverse tissue reaction and oral hygiene reinforcement were scheduled every second week during the first 2 months after surgery. All patients were instructed to resume their normal mechanical oral hygienic measures, which consisted of brushing using a soft toothbrush with a roll-technique and flossing, 1 month after surgery. Supportive periodontal maintenance of oral hygiene reinforcement and supragingival scaling whenever necessary were performed during each recall appointment.

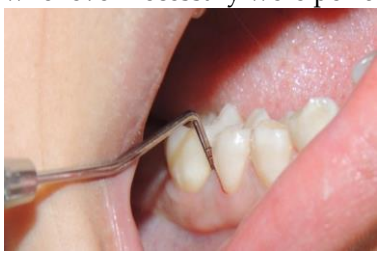

Figure 2: Probing at baseline showing CAL =8mm on distobuccal line angle of lower first molar.

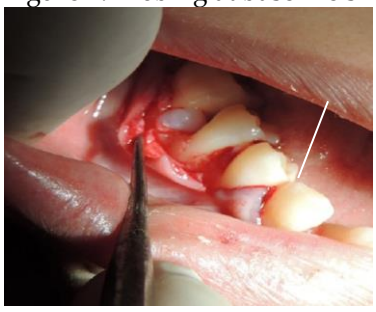

Figure 3: EDTA applied on root surface.

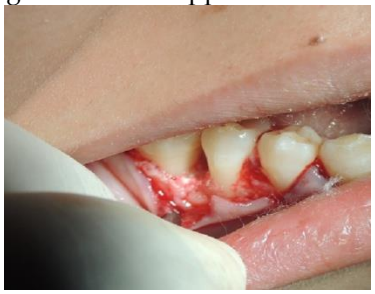

Figure4: β-TCP application on root surface

RESULTS

1. Pocket Depth(PD)

A. **Effect of bone particle size:** ***With EDTA:*** At base line as well as after 6 months, there was no statistically significant difference between the bone particle sizes. After 3 months, Nano bone particle size showed statistically significant highest mean PD. There was no statistically significant difference between mixed and Micro bone particle sizes; both showed statistically significant lowest mean PD, at 6 months no significant difference found between the three groups. ***Without EDTA:*** At base line as well as after 6 months, there was no statistically significant difference between bone particle sizes. After 3 months, Nano bone particle size showed the statistically significantly highest mean PD; no statistically significant difference between mixed and Micro bone particle sizes; both showed statistically significant lowest mean PD.

Table (1): comparison between PD with different bone particle sizes

		Mixed	Nano	Micro	*P*-value
EDTA	Time	Mean ± SD	Mean ± SD	Mean ± SD	
EDTA	Base line	5.6 ± 0.1	5.7 ± 0.3	5.5 ± 0.4	0.068
	3 months	2.2 ± 0.5 [b]	3.7 ± 0.5 [a]	3.4 ± 0.3 [b]	<0.001*
	6 months	2.2 ± 0.3	2.4 ± 0.8	2.7 ± 0.5	0.125
No EDTA	Base line	6.1 ± 0.1	6.2 ± 0.5	6.5 ± 0.1	0.051
	3 months	3.9 ± 0.3 [b]	4.4 ± 0.3 [a]	4.0 ± 0.9 [b]	<0.001*
	6 months	3.3 ± 0.5	3.4 ± 0.2	3.4 ± 0.9	0.703

**: Significant at P ≤ 0.05, Different superscripts in the same row are statistically significantly different*

Using Nano bone particle size: With and without EDTA, there was a statistically significant decrease in mean PD through all periods. ***Using Micro bone particle size:*** With EDTA, there was a statistically significant decrease in mean PD after 3 months. From 3 months to 6 months, there was no statistically significant change in mean PD. Without EDTA, there was a statistically significant decrease in mean PD through all periods.

Table (2): Comparison between PD at different time periods

Bone particle size		Base line	3 months	6 months	*P*-value
	EDTA	Mean ± SD	Mean ± SD	Mean ± SD	
Mixed	EDTA	5.6 ± 0.1 [a]	2.2 ± 0.5 [b]	2.2 ± 0.3 [b]	<0.001*
	No EDTA	6.1 ± 0.1 [a]	3.9 ± 0.3 [b]	3.3 ± 0.5 [c]	<0.001*
Nano	EDTA	5.7 ± 0.3 [a]	3.7 ± 0.5 [b]	2.4 ± 0.8 [c]	<0.001*
	No EDTA	6.2 ± 0.5 [a]	4.4 ± 0.3 [b]	3.4 ± 0.2 [c]	<0.001*
Micro	EDTA	5.5 ± 0.4 [a]	2.4 ± 0.3 [b]	2.7 ± 0.5 [b]	<0.001*
	No EDTA	6.5 ± 0.1 [a]	4.0 ± 0.9 [b]	3.4 ± 0.9 [c]	<0.001*

2. Plaque Index (PI)

A. Effect of bone particle size

With EDTA: Through all periods, there was no statistically significant difference between the three bone particle sizes. **Without EDTA:** Through all periods, there was no statistically significant difference between the bone particle sizes. Through all periods, there was no statistically significant difference between bone particle sizes.

Table (3): Comparison between PI with different bone particle sizes

		Mixed	Nano	Micro	*P*-value
EDTA	Time	Mean ± SD	Mean ± SD	Mean ± SD	
EDTA	Base line	0.5 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.757
	3 months	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.928
	6 months	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.897
No EDTA	Base line	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	1.000
	3 months	0.4 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.928
	6 months	0.5 ± 0.3	0.5 ± 0.2	0.5 ± 0.2	0.928

**: Significant at P ≤ 0.05*

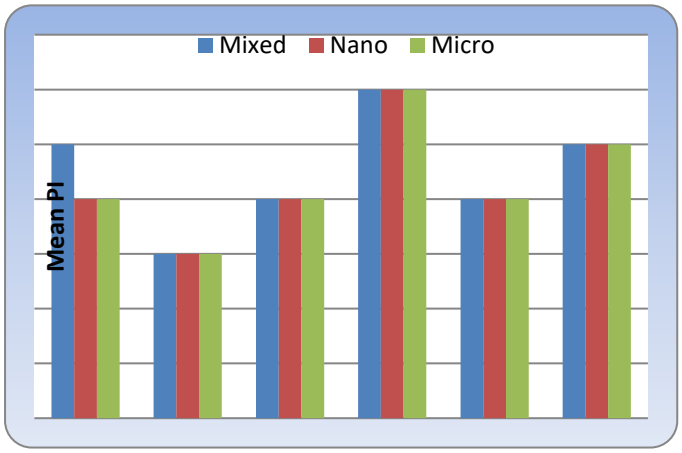

Figure (4): Bar chart representing mean PI with different bone particle sizes

3. Gingival Index (GI)

A. **Effect of bone particle size:**

With EDTA: Through all periods, there was no statistically significant difference between the three bone particle sizes. W**ithout EDTA:** Through all periods, there was no statistically significant difference between the three bone particle sizes. Through all periods, there was no statistically significant difference between the three bone particle sizes.

Table (4): Comparison between GI with different bone particle sizes

EDTA	Time	Mixed	Nano	Micro	*P*-value
		Mean ± SD	Mean ± SD	Mean ± SD	
EDTA	Base line	0.4 ± 0.3	0.5 ± 0.3	0.6 ± 0.3	0.514
	3 months	0.6 ± 0.4	0.6 ± 0.4	0.3 ± 0.2	0.072
	6 months	0.5 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.885
No EDTA	Base line	0.3 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.108
	3 months	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.3	0.955
	6 months	0.3 ± 0.2	0.4 ± 0.3	0.5 ± 0.2	0.615

**: Significant at P ≤ 0.05*

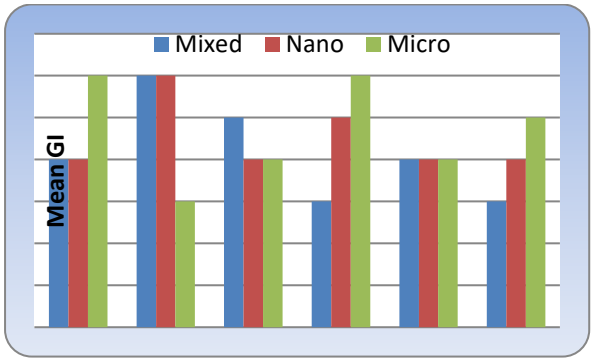

Figure (5): Bar chart representing mean GI with different bone particle sizes

DISCUSSION

One of the most important factors that could optimize the clinical outcomes of periodontal regenerative therapies is the ability to seal the regenerating wound from the contaminated oral environment. Clot adhesion appears vitally dependent on the formation of a resilient union between the fibrin clot and root surface elements. The root surface becomes covered by a smear layer of instrumentation debris following routine root preparation [22-24]which appeared to compromise fibrin clot adhesion to such altered root surfaces.[25]To achieve appreciable periodontal regeneration, a large number of studies have supported the necessity of removing endotoxin and the smear layer of instrumentation on the root surface which is exposed in the periodontal pocket.[26,27] Chemical root conditioning of periodontitis affected root surfaces has been described with a number of agents of varying PH in order to eliminate factors that render the root surface bio-incompatible for PDL associated with periodontal healing. EDTA etching has been found to be as effective as low pH etchants with respect to smear removal and superior in exposing root surface associated collagen[28]. It was hypothesized [11] that EDTA gel smear removal and dentinal tubule exposure effects could enhance bone graft – root surface adhesion, a factor that could ensure complete protection of the underlying defect filled regenerative materials and blood clot. In the present study the same hypothesis with various bone particles sizes was implemented in order to test the effect of EDTA root surface etching on clinical outcomes.

The present study attempted to search for the efficient particle size that could enhance osteo-inductive capacity of the remaining periodontal tissues. There are 2 main types of commonly used β-TCP: one consists of small particles with a diameter of 250–1,000 μm, and the other of large particles with a diameter of 1,000–2,000 μm. The porosity and particle size of a graft material is important feature in the graft remodeling, as they affect its resorption rate and ability to promote regeneration of bone. A minimum space between graft particles is necessary for effective vascularization and bone formation at the same site, wide spaces has been shown to promote bone growth. [29] According to Yukna[30], a particle size of 300–500 μm is optimal for the treatment of periodontal bone defects. Therefore, it was speculated that small β-TCP particles with a diameter of 250– 1,000 μm could be used as a suitable micro particle in the present study. A synthetic nanocrystalline hydroxyapatite (NHA) graft material has been introduced for augmentation procedures in osseous defects. Advantages of such a nanostructured material in comparison to bulk material are the close contact with surrounding tissues, quick resorption characteristics and a high number of molecules on the surface. In vivo studies demonstrated rapid healing of critical size defects after application of NHA graft material.[31, 32] In addition, an undisturbed osseous-integration and complete resorption of the material could be observed. Thus, the resorption process was carried out by osteoclastic cells during the remodelling of the new developed bone tissue. According to many reports, dimensions of biological apatite in the calcified tissues always possess a range of a few to hundreds of nanometers with the smallest building blocks on the nanometer size scale. The nano-structured materials were found to provide a better capability for the specific interactions with proteins and may have many potential advantages in the context of promoting bone cell responses.[33]

Clinical data of the present study revealed more significant pocket reduction and attachment gain following the use of mixed nano micro sized grafts followed by EDTA etching compare to the other groups. A recent study[34] compared the effect of NHA and β-TCP in the treatment of human periodontal defects. They concluded that both NHA and β-TCP have proved to be beneficial in the management of periodontal defects. Treatment of intrabony periodontal defects with NHA leads to significant improvement in early clinical and radiographic outcomes as compared to β-TCP. The effect of silk scaffolds on one-wall periodontalintrabony defects was invistegated[35]. They conjugated nHA onto a silk scaffold and then seeded periodontal ligament cells or dental pulp cells onto the scaffold. They concluded that nHA-coated silk scaffolds can be considered to be potentially useful biomaterials for periodontal regeneration.

A study [36] evaluated outcome of nanocrystalline hydroxyapatite bone graft in combination with collagen membrane compared with OFD only in the treatment of intrabony periodontal defects. They concluded that NcHA bone graft in combination with collagen membrane demonstrated better clinical outcomes compared with OFD alone. Kasaj et al[37] evaluated the clinical efficacy of NHA paste in intrabony defects and reported PPD reduction of 3.9±1.2 mm and CAL gain of 3.6±1.6 mm. This slightly higher PPD reduction may be due to effect of combination technique (BG+GTR). It has been demonstrated that combination of nHA collagen bone graft with ePTFE membrane resulted in higher PD reduction (5.85 mm) and greater CAL gain (3.80 mm) compared with the test groups results of our study. Contrary to these findings of present study demonstrated significantly less pocket reduction and attachment gain following the use of nano grafts compared to micro grafts. This could be also explained by the rapid nano grafts particles washing in the GCF. It has been mentioned that nanoparticles of graft materials may lead to breakthrough applications in periodontal regeneration. However, due to their small particle size, nanoparticles may be eliminated from periodontal defects by phagocytosis. They concluded that the use of a composite graft consisting of nHA and micro sized β-TCP after root surface treatment with 24% EDTA may be a suitable method to improve nHA retention in periodontal defects with subsequent graft bioreactivity. In the present study, the more significant clinical and biological outcomes of mixed nano micro graft following EDTA root surface etching support this hypoesthes and through light on the importance of limiting nano graft dissemination for 2 reasons, the 1st is to enhance its local effect and the 2nd is to reduce its unwanted systemic effects. It can conclude that, combining

micro sized grafts with nano grafts can turn the Osteoconductive bone substitute into an osteoinductive one, indirectly through stimulation of pro-osteogenic mediators.

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