

## EFFECT OF HYALURONIC ACID MIXED WITH BIPHASIC CALCIUM PHOSPHATE ON BONE HEALING AROUND IMMEDIATELY PLACED IMPLANTS IN THE POSTERIOR AREA OF THE MANDIBLE

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### ABSTRACT

**Objective:** To evaluate the effect of hyaluronic acid mixed with biphasic calcium phosphate bone graft on bone healing around immediately placed implant in the posterior region of the mandible.

**Patients and Methods:** This clinical study included 16 implants placed for 14 patients who were seeking for extraction of non-restorable mandibular premolar or molar teeth and replacement with immediate implant. The patients were randomly and equally divided into two groups; extraction of the intended non restorable tooth with immediate implant placement and the gap between the implant and socket walls was filled with BCP bone graft only in group I (control group) and with BCP bone graft mixed with HA in group II (study group) and The evaluation was done immediately (Tx), postoperatively at loading (T0), at 3&6 months after loading (T3&T6) respectively to assess implant stability, modified sulcus bleeding index, Peri-implant pocket depth, bone loss around the dental implant.

**Results:** The study included 7 females (50 %) and 7 males (50%) with average age of 35 years. No statistically significant differences were recorded between both groups regarding age, sex (P=0.642, 0.451) and implant characters (length, width) (P=0.751, 0.851) respectively. The implants used were 10 and 12mm in length with 4.1 mm and 4.8 mm diameter with no significant difference between studied groups. No statistically significant differences were found between both groups in implant stability (P=0.735, 0.722, 0.410), mSBI (P=0.662, 0.642, 0.448) PPD (P=0.278, 0.06, 0.120) and bone loss (P= 0.562, 0.492) at different time intervals of assessment.

**Conclusion:** The effect of HA with BCP bone graft was not superior on bone healing around immediately placed implant than BCP bone graft alone in posterior region of the mandible.

**KEYWORDS:** Immediate Implant, Hyaluronic acid, Biphasic calcium phosphate Bone Graft

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## INTRODUCTION

Placement of dental implants in deficient alveolar bone continues to be the main challenge in implant dentistry. Often bone augmentation is required to achieve the ideal implant position for prosthesis restoration due to physiologic resorption of bone after tooth extraction. Reducing the loss of bone during the physiological remodeling stages of socket healing is another advantage of immediate implant placement.<sup>1,2</sup> Survival rates of immediate implants are close to that of delayed implant placement.<sup>3</sup>

Hyaluronic acid (HyA) is a naturally occurring extracellular biodegradable polymer that is distributed widely throughout neural, epithelial, and connective tissues. HyA is a non-sulfated glycosaminoglycan that is composed of repeating glucuronic acid and N-Acetyl-D-glucosamine. It plays a vital role in the control of tissue hydration through its negative charge and high hydration ratio.<sup>4</sup> HyA has both elastic and viscous properties owing to polymer chain entanglements.<sup>5</sup>

The viscous properties provide lubrication, while the elastic properties have a shock absorption effect.<sup>6</sup> HyA plays a vital role in wound healing. The formation of a non-adhesive and hydrated environment improves cell migration, whereas, the interactions of pericellular HyA with proteoglycans impede migratory movements.<sup>7</sup> Previous experiments showed that low molecular-weight HyA augments angiogenesis and increases collagen creation by endothelial cells.<sup>8</sup> When HyA is added to a particle type of bone graft, viscosities are increased, and, thus, graft handling properties improved and stability of the grafted site can be optimized.<sup>9</sup>

On the other hand, biphasic calcium phosphate (BCP) has presented significant advantages over other alloplastic bone grafting material due to its controlled bioactivity and balance between resorption, which guarantees the stability of the biomaterial while promoting bone ingrowth.<sup>10,11</sup> BCP is also able to form a direct bond with the host bone through formation of a carbonate hydroxyapatite

layer to form a strong interface compared to inert biomaterials, which form a fibrous interface.<sup>12</sup>

Authors revealed that by changing the HA/TCP ratio, it has been possible to alter the substitution rate and the bioactivity of these materials,<sup>13</sup> which has led to their clinical use in oral and orthopedic surgery.<sup>14, 15</sup> The advantages of biphasic calcium phosphate over other allografts and xenografts is that it does not cause an immunogenic host response as this synthetic material is chemically similar to natural bone. In addition, the material has unlimited availability and does not cause disease transmission.

From the above forementioned data, it was found to be interesting to evaluate the effect of Hyaluronic Acid mixed with biphasic calcium phosphate on bone healing around immediately placed implants in posterior mandible.

## PATIENTS AND METHODS

Sixteen immediate implants were placed in fourteen patients seeking for extraction of non-restorable teeth in the posterior mandible and replacement by immediate implants were chosen from the Out-patient Clinic of the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Mansoura University. This study was approved by the Ethical Committee of Faculty of Dentistry, Mansoura university (No. A03140420). Expected benefits and risks were explained, and written consents were signed from all individuals included in this study.

All included patients were with age ranged from 18 to 50 years and medically free from any systemic or local diseases that contraindicate surgery or implant placement, Intact buccal and lingual cortical plates of at least 1 mm thickness and sufficient bone height between the apices of the tooth to be extracted and the inferior alveolar canal  $\geq 5$  mm were considered as a pivotal inclusion criteria.

On the other hand, all patient with psychiatric problems, Parafunctional habits, heavy smokers were excluded from the study.

### **Radiographic examination**

Preoperative CBCT was taken for each patient to measure buccolingual and mesiodistal dimensions, bone quantity and quality and to evaluation of presence of infection or bone resorption.

### **Preoperative measures and patient grouping:**

Patients were under antimicrobial prophylaxis with antibiotic of clavulanic acid and amoxicillin (Augmentin, Medical Union Pharmaceutical, Egypt) 1gm one hour before surgery and mouth rinse of Chlorhexidine (Listermix Plus mouthwash, SIGMA, Egypt) prior to surgery.

### **Patients' preparation & grouping**

After extraction of the non-restorable posterior premolar or molar tooth and placement of an immediate implant, the fourteen patients were randomly classified into two equal groups as follows:

**Group I (Control group):** consisted of 7 patients with the gap between the implants and the socket walls was filled with biphasic calcium phosphate bone graft (Genoss co.,ltd, Korea).

**Group II (Study group):** consisted of 7 patients with the gap between the implants and the socket walls was filled with biphasic calcium phosphate bone graft mixed with hyaluronic acid (Curavisc vials; Manufacturer Biological GMBh (curasan,Benelux B.V.). With concentration (2mg/2ml). Sole agent: PHARMA CON, made in Germany).

### **Surgical procedure:**

#### **A. First stage surgery**

After induction of inferior alveolar, lingual nerve block and long buccal local anesthesia by 4% articaine hydrochloride (Articaine, Artinibsa 4% Inibsa Dental manufactured in Spain) of 1:100000 epinephrine, full thickness mucoperiosteal flap was reflected buccally to expose the intended tooth to be extracted and the socket at implant site.

The non-restorable mandibular premolar/molar tooth was atraumatically extracted. For molars, separation was made between the two roots of the molar then luxation using straight elevator. Bone curettage was done as well as socket debridement by irrigation with normal saline solution.

Drilling was done in the inter-radicular bone at speed of 600 to 800 rpm in a precise direction. Sequential drilling depending on implant size was performed following the instructions of implant manufacturer (Nucleoss implant made in Turkey) under copious saline solution irrigation. Implant was placed 1 to 2 mm under the buccal bone level. Implant stability quotient (ISQ) was recorded for all fixtures (Osstell, Integration Diagnostics, Savadale, Sweden).

For both groups, resorbable membrane (Bio-guard membrane connect-biofarm LTD, Moscow) of a suitable size was tucked under buccal and lingual flaps to cover the graft material and the implant. Releasing incision was done at the base of the mucoperiosteal flap to obtain primary closure without tension. The flap was closed using vicryl suture 3/0 using simple interrupted sutures.

### **Postoperative phase:**

Patients were instructed to have a soft or semi-solid diet for the first 3 days after surgery and then gradually return to normal diet. Patients were instructed to apply cold packs on the cheek in the first 24 hours. Patients were recalled after 7 days for follow up and suture removal. Postoperative medication included continue of the amoxicillin and clavulanic acid 1 gm antibiotic twice daily for 5 days. Analgesic (Ibuprofen 400 mg) (Manufacturer's PIL, Brufen® tablets, Abbott laboratories limited, electronic medicines compendium) was prescribed when needed as non-steroidal anti-inflammatory (NSAID). Patients were asked to use 0.2% chlorhexidine mouthwash for 1 minute twice daily for one week

The initial bone height was measured immediately after implant placement (Tx) using CBCT at the four aspects (mesial, distal, buccal and lingual).

### **B. Second stage surgery**

Exposure of the implant cover screw was done through crestal incision to record stability for all fixtures and then replaced with a healing abutment for 2 weeks. (Fig.1D) (Fig.2D)

### **Prosthetic phase**

The gingival former was replaced by the abutment and impression was taken by closed tray technique using abutment cap and implant analogue before pouring the working cast. The final porcelain

fused to metal crown was temporary cemented for 6 months. (Fig.1E) (Fig.2E).

### **Evaluation:**

All patients were followed up at different time intervals for evaluating the following criteria:

#### **A. Clinical evaluation:**

##### **1. Implant stability:**

Implant stability was assessed immediately after implant insertion Tx, at loading T0 and 6 months after loading T6.

The ISQ scale ranges from 0 to 100 that measures implant stability. The values less than 60 indicate

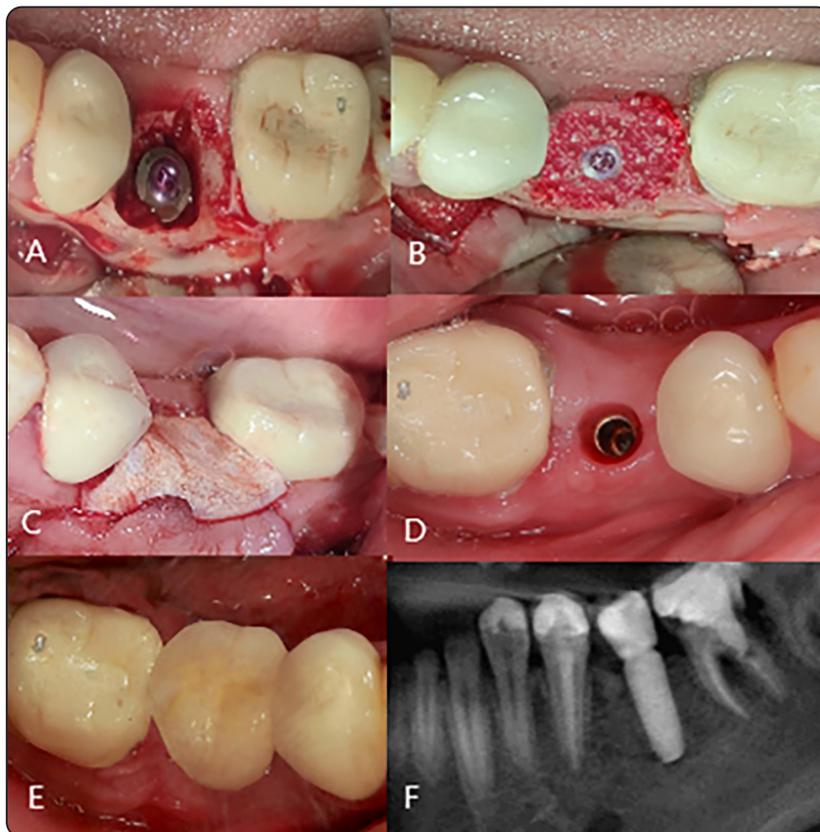


Fig. (1) Control group case of an immediate replacement of anon-restorable mandibular left first molar by immediate implant, photographs showing: A) Immediate implant placement in the planned osteotomy site of fresh extraction socket. B) Packing of biphasic calcium phosphate around immediately inserted implant into fresh socket C) Showing collagen membrane covering the grafted site and tucking underneath buccal and lingual mucosa D) Emergence profile after healing abutment removal E) Final PFM crown in place F) CBCT after 6 months from loading

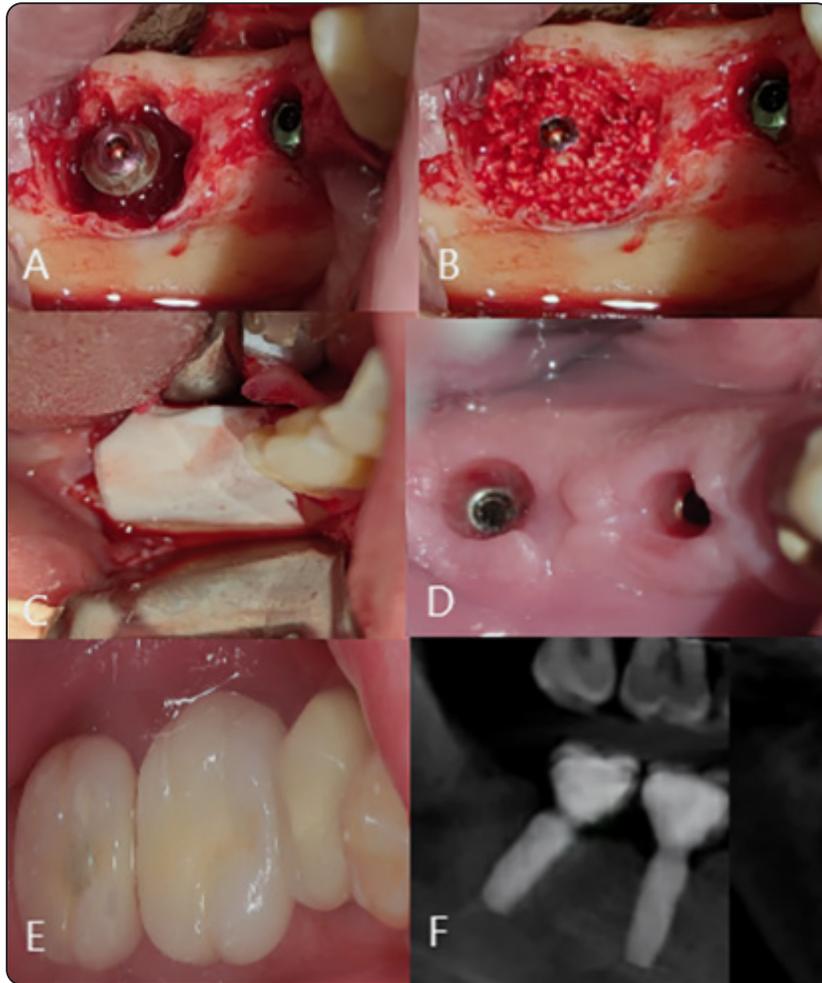


Fig. (2) Study group case of an immediate replacement of anon-restorable mandibular left second molar by immediate implant, photographs showing. A) Immediate implant placement in the planned osteotomy site of fresh extraction socket. B) Packing of biphasic calcium phosphate that was mixed with HA around immediately inserted implant. C) Showing collagen membrane covering the grafted site and tucking underneath buccal and lingual mucosa D) Emergence profile after healing abutment removal after 6 months. E) Final PFM crown in place after 6 months. F) CBCT after 6 months from loading

low implant stability, values from 60 to 69 indicate medium stability where values between 70 and 79 indicate high stability.<sup>16</sup>

## 2. Modified sulcus bleeding Index (MSBI)

Clinical signs and symptoms of inflammation of peri-implant mucosa were assessed at loading time T0, 3 months and 6 months after loading T0, T3 and T6. peri implant inflammation was evaluated at four aspects around the implants, (mesial, distal, buccal and lingual) according to the following (MSBI)

criteria documented by Mombelli *et al*<sup>17</sup>:

- Score 0; no bleeding when a periodontal probe was passed along the gingival margin adjacent to the implant.
- Score 1: isolated visible spot of bleeding.
- Score 2: blood form a confluent red line on the gingival margin.
- Score 3: profuse or heavy bleeding is present.

The average mean of the 4 readings was recorded for each implant at the different follow up intervals.

### 3. Peri-implant pocket depth:

The distance between the gingival margin and the base of the pocket was measured immediately after loading T0, 3 months after loading T3 and 6 months after loading T6 using a graduated periodontal probe. The probe was inserted buccally, lingually, mesially and distally around each implant in a line parallel to the vertical axis of the implant until the probe's blunt end reached the pocket base. The average mean of the 4 readings was recorded and approximated to the nearest 0.5mm.<sup>18,19</sup>

### Radiographic evaluation:

Using CBCT, the marginal bone height was measured at loading (T0) and 6-months after loading (T6) at the four aspects mesial, distal, buccal and lingual then the average of the 4 readings was recorded.

Bone loss was measured at (T0) and (T6) by subtraction average bone height recorded at T0 and T6 from average bone height at Tx.

### Statistical analysis

Data was analyzed statistically using Statistical Package for Social Science Program (SPSS 21 for PC, IBM Inc., Armonk, NY). Comparisons between the study groups were done using the independent sample t test for age, stability and marginal bone loss. The Mann-Whitney test was used for probing depth and bleeding index and the Chi-Square tests for gender. All p values less than 0.05 were considered significant.

## RESULTS

This study was conducted on 14 patients; 7 females (50 %) and 7 males (50%) with average age of 35 years, seeking replacement of unrestorable single or multiple mandibular posterior teeth. No statistically significant differences were recorded between both groups regarding age and sex

(p=0.642, 0.451). The implants used were 10 and 12mm in length with 4.1 mm and 4.8 mm diameter with no significant difference between studied groups (P=0.751,0.851) respectively

### A. Clinical evaluation:

#### Implant stability assessment by Osstell

The mean ISQ values were recorded for both groups immediately at (Tx), at loading time (T0), and 6 months after loading (T6) (Table 1). Comparing between the two groups regarding implant stability assessment, lack of statistically significant differences was established at Tx, T0 and T6. (P=0.735, 0.722, 0.410 respectively) (Table 1).

TABLE (1) Showing mean, SD and intra/inter group level of significance at the successive periods of assessment regarding implant stability

Stability	Group I (Control group) N=8	Group II (Study group) N=8	Test of significance
	Mean ±SD	Mean ±SD	
Tx	70.0±2.0 <sup>ab</sup>	69.63±2.0 <sup>ab</sup>	t=0.346 p=0.735
T0	72.0±2.07 <sup>ac</sup>	72.38±2.07 <sup>ac</sup>	t=0.363 p=0.722
T6	74.13±1.46 <sup>bc</sup>	74.75±1.49 <sup>bc</sup>	t=0.849 p=0.410
Repeated Measures ANOVA test	F=55.52 P<0.001*	F=121.10 P<0.001*	

t: Student t test, \*statistically significant, similar superscripted letters denote significant difference between different periods by post Hoc Tukey test within same column. P<0.05 is considered as significant difference

#### Modified sulcus bleeding index (MBI):

The mean modified sulcus bleeding index values were recorded for both groups at loading time (T0), and 3&6 months after loading (T3 &T6) (Table 2). Comparing both groups regarding MBI, lack of statistically significant difference was established at the successive periods of assessment T0, T3 and T6 (P=0.662, 0.642, 0.448 respectively)(Table 2).

**Peri-implant pocket depth (PPD):**

The mean PPD values were recorded for both groups at loading time (T0), and 3&6 months after loading (T3 &T6). The results are shown in (Table 2).

Comparing both groups, there were no statistically significant differences between PPD values recorded at T0, T3 and T6 (P=0.278, 0.06, and 0.12 respectively) (Table 2).

**B. Radiographic evaluation**

CBCCT radiograph were made for evaluation of marginal bone height immediately after implant placement Tx, at loading T0 and 6 months post loading T6. Bone loss was evaluated at loading T0

and 6-months after loading T6.

Comparing both groups, there were no statistically significant differences between marginal bone height values recorded at Tx, T0 and T6 (P=0.634, 0.874 and 0.204) respectively.

The median bone loss values recorded at T0 in both groups were 0.388mm (0, 2.0) and 0.275mm (0.48, 1.4) for control and study group respectively, while the median bone loss values recorded at T6 were 0.6mm (0.55, 2.5) and 0.8mm (0, 1.9) for control and study group respectively Table (3). Comparing both groups, there were no statistically significant differences between them at T0 and T6 (P=0.562,0.492). (Table 3).

TABLE (2) Showing mean, SD and inter/intra group level of significance between the two studied groups regarding the modified sulcus bleeding index and the peri-implant pocket depth values.

Intervals of follow up	Modified sulcus bleeding index			Peri-implant pocket depth		
	Group I (Control group) N=8	Group II (Study group) N=8	Test of significance	Group I (Control group) N=8	Group II (Study group) N=8	Test of significance
	Mean ±SD	Mean ±SD		Mean ±SD	Mean ±SD	
T0	1.75±0.70	1.88±0.35	t=0.447 p=0.662	1.87±0.35 <sup>ab</sup>	1.63±0.52	t=1.12 p=0.278
T3	1.38±0.52 <sup>a</sup>	1.50±0.53	t=0.475 p=0.642	2.37±0.51 <sup>a</sup>	2.0±0.046	t=2.05 p=0.06
T6	2.13±0.64 <sup>a</sup>	1.87±0.64	t=0.780 p=0.448	2.50±0.53 <sup>b</sup>	2.13±0.35	t=1.66 p=0.120
Repeated Measures ANOVA test	F=1.34 P=0.285	F=0.0 P=1.0		F=5.4 P=0.018*	F=3.37 P=0.01*	

*t: Student t test, \*statistically significant, similar superscripted letters denote significant difference between different periods by post Hoc Tukey test within same column. P<0.05 is considered as significant difference*

TABLE (3) Showing median and inter & intra group level of significance between the two studied groups regarding radiographic evaluation of bone loss at T0 & T6

	Group I (Control group) N=8	Group II (Study group) N=8	Test of significance
	Median (min-max)	Median (min-max)	
Bone loss at T0	0.388(0, 2.0)	0.275(0.48, 1.4)	Z=0.579 P=0.562
Bone loss at T6	0.6(0.55, 2.5)	0.8(0, 1.9)	Z=0.687 P=0.492
Wilcoxon signed rank test P	1.40 0.161	2.55 0.06	

*Z: Mann Whitney U test P<0.05 is considered as significant difference*

## DISCUSSION

The immediate implant is a treatment option for replacement of non-restorable teeth that has recently gained popularity as it decreases overall treatment time while preserving the alveolar ridge and preventing migration of teeth into the edentulous space. In this study, the thick gel of cross-linked HA enabled it to be easily bonded to the particles of the bone graft to improve soft as well as hard tissue healing and bone regeneration.<sup>20-24</sup> When reticulated HA is combined with TCP granules, it has a greater regeneration activity than linear HA, according to Zhao N. et al.<sup>25</sup>

BCP is a synthetic material in nature, osteoconductive and biocompatible.<sup>26</sup> An apatite layer is essential for the BCP ceramics bioactivity, and it is created over time by the controlled release of calcium ions. It is possible that this bioactivity explains the ceramic's osteoconductivity.<sup>26</sup>

Moreover, resorbable collagen membrane was used in the current study to cover the bone graft placed around the immediate implant because barrier function longevity is critical for regenerative function. The use of collagen membranes has improved soft tissue healing, and rapid resorption when exposed, thus avoiding bacterial contamination and self-limiting infection.<sup>27</sup>

All implants in the current study proved effective osseointegration. In terms of implant stability, significant statistical differences were detected in the mean ISQ values of the different follow-up intervals when compared against each other respectively within the two groups ( $P < 0.01$ ,  $P < 0.01$ ). The implant stability has improved with time, which is supported by new bone creation on the implant surface.<sup>28</sup> After six months, both groups showed increased stability as a result of increased new bone growth correlated with osseointegration on the bone implant contact.<sup>29</sup> These findings were in accordance with Monje et al.,<sup>30</sup> who determined that the resonance frequency values were high immediately after implant placement

as a result of initial stability but had a tendency to decrease as soon as the healing process and bone remodeling begin then tends to raise once again over time. Additionally, the studies by Sjöstrom et al.<sup>31</sup> and Guler et al.<sup>32</sup> have documented that implant stability peaked at one month and continued to rise until it completed osseointegration at 6 months after functional loading.

According to the results of this study, no statistically significant difference was recorded regarding ISQ values at Tx, T0 and T6 when comparing both groups ( $P = 0.735$ ,  $0.722$ ,  $0.410$ ) respectively. In spite of its osteoinductive activity and beneficial effects on morphogenesis and tissue healing, HA had no significant influence on primary or secondary stability over time.<sup>33</sup> This might be because of low sample size.

The modified sulcus bleeding index was used in this study to assess clinical inflammatory indicators of peri-implant mucosa at loading, 3- and 6-months post loading at four different locations surrounding the implants. There were no statistically significant differences in the average bleeding index scores from all surfaces for both groups ( $P = 0.662$ ,  $0.642$ ,  $0.448$ ), and there was no detected pus discharge during the follow-up period.

In line with the current study, Sánchez et al.<sup>34</sup> documented a study on the effect of HA on peri-implantitis. At 45 and 90 days, their study group had a higher decrease of bleeding on probing than control group which was near to statistical significance after 90 days. In contrary to current study, De Araujo et al.<sup>35</sup> conducted a study on peri-implant maintenance of immediate function implants and concluded that HA has a statistically significant effect on MSBI in comparison with chlorohexidine. This can be attributed to the different method of application of HA between the two studies

In the current study, regarding PPD readings although the recorded measurements were less in the study group than the control group, no significant differences were detected between both

groups at different time intervals of follow up at T0, T3&T6 (P=0.278, 0.06, 0.12) respectively. These findings were comparable to those documented by De Araujo M. et al.<sup>35</sup> who compared the effect of HA and chlorhexidine after implant placement and reported absence of statistically significant difference between the two groups.

In the current study, **CBCT** was used as a radiographic modality for implant assessment as it provides sufficient diagnostic quality to allow evaluation of bony structures with high contrast in the oral and maxillofacial region with low exposure time and dose.<sup>36</sup>

The recorded results of this study showed an increase in bone loss throughout the follow-up periods in both groups which comes in accordance with Bungthong W. et al.<sup>37</sup> who recorded significant bone loss after six months following immediate implant placement even with bone grafting, as the immediate implant placement cannot completely preserve the hard tissue dimension of the ridge at the extraction socket.<sup>38-39</sup>

Regarding the median bone loss at T0 and T6, there were no significant statistical differences between the two groups (P=0.562, 0.492). Coinciding with our findings, Eric Aguado et al.<sup>40</sup> who used HA as a binder to bone graft, have reported that the amount of formed bone was not significantly higher than with BCP granules alone. Also, in accordance with our study, Kaya A. et al.<sup>41</sup> who used a combination of HA and xenografts in filling peri-implant bone defects, concluded that HA did not have a significant positive result on the repair of defects around dental implants.

In contrary, Kim et al.<sup>42</sup> showed that HA increased bone formation and improved bone healing in new extraction sockets. Additionally, Mendes et al.<sup>43</sup> discovered that HA might improve tooth socket repair by increasing the expression of bone morphogenetic protein-2 and osteopontin. Moreover, Shamma et al.<sup>44</sup> used HA in conjunction with BCP around implants in their experimental study,

and found that the combination of BCP and HA was more effective than BCP alone.

Aguado et al.<sup>45</sup> examined the usage of HA as an aqueous binder of BCP bone granules. They drilled holes in rabbit femoral condyles and filled half of them with BCP alone and the other half with BCP mixed with HA. Rabbits were given a month to recover before being sacrificed and then their femurs were retrieved and examined histologically. They discovered that the amount of produced bone was not much higher than when only BCP granules were utilised.<sup>45</sup>

Based on the limitations of the current study and regarding to the best of our knowledge through reviewing the literature, HA with BCP has no superior effect on bone healing around immediately placed implant than BCP alone when placed in the gap around immediate implant after extraction in the mandibular posterior region.

## CONCLUSION

Despite the success of both techniques of using bone graft around immediate implant, the clinical outcomes and bone healing effect of using HA combined with BCP bone graft around immediately placed dental implant in the posterior mandibular region was not significantly superior to BCP bone graft only.

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