ORIGINAL ARTICLE

CONTENTS

Radiographic evaluation of the impact of hyaluronic acid and bone graft on peri-implant tissue: A randomized clinical trial

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ABSTRACT

Aim: The objective of this randomized clinical trial was to enhance the effect of HY alone or in combination with a bone graft in changing peri-implant tissue dimensions radiographically.

Materials and Methods: Thirty-six patients who required posterior dental implants were randomly assigned to three groups (n=12 each): a control group (CONT) with conventional implant placement was performed, one group treated with HA (HY), and another test group treated with HA and bone graft together (HYB). The same surgical procedure was performed for all implants in the posterior regions of jawbones. Measurements were taken before surgery, after surgery, and at eight and sixteen weeks after surgery.

Results: Hybrid group (HYB) had a significantly greater increase radiographic soft tissue thickness at all-time points ($P < 0.001$). The HYB group had higher radiographic bone height at week 16 and stability of bone width ($P = 0.088$) than the other groups ($P < 0.001$)

Conclusion: The combined application of a bone graft and hyaluronic acid (HYB) significantly improves the radiographic soft tissue thickness and bone height.

KEY WORDS: hyaluronic acid, bone substitutes, dental implant, peri-implant tissues

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INTRODUCTION

High long-term survival rates, functional rehabilitation, and favorable aesthetic outcomes have made the dental implant therapy the gold standard for replacing missing teeth. Continuous advancements in implant surface technology, surgical protocols, and biomaterials have significantly improved treatment predictability, making implant-supported restorations an integral part of modern evidence-based oral rehabilitation [1-6]. However, successful implant therapy is not limited to osseointegration but requires, in addition, the long-term maintenance of both peri-implant hard and soft tissue to ensure biological stability, esthetic harmony and patient satisfaction [7, 8]. The peri-implant tissue phenotype has recently been known as one of the greatest important variables to implant success. It contains several anatomical parameters, including keratinized mucosal width (KMW), mucosal thickness (MT), and supracrestal tissue height (STH), which are involved in the maintenance of the peri-implant mucosal seal and protection of the underlying supporting bone [9-10]. Clinical evidence suggests that insufficient keratinized mucosa, thin peri-implant mucosa, or reduced supracrestal tissue height are associated with increased plaque formation, peri-implant inflammation, mucosal recession, esthetic complications, and progressive marginal bone remodeling [9-11]. Thus, contemporary implant therapy not only aims at successful osseointegration, but also on the optimization of the peri-implant tissue phenotype to enhance long-term clinical results. Hyaluronic acid (HA) is an agent that has been extensively studied to improve the peri-implant wound healing as a biological agent because of its superior biocompatibility and various biological properties. Hyaluronic acid is a naturally occurring glycosaminoglycan that plays an essential role in the organization of the extracellular matrix, modulating inflammation, angiogenesis, fibroblast proliferation, and collagen synthesis and tissue regeneration. Besides promoting soft tissue healing, HA has also shown antimicrobial activity and the ability to stimulate osteogenic differentiation, making it a valuable adjunct in oral regenerative procedures [12-13]. These biological properties have led to its successful use in periodontal surgery, management of oral mucosal diseases, temporomandibular joint disorders and reduction of postoperative complications after oral surgical procedures. In implant dentistry, bone graft substitutes are widely used to preserve alveolar ridge dimensions, reconstruct osseous defects and provide a stable scaffold for new bone formation. Graft materials are classified as autogenous, allogeneic, and xenogeneic and

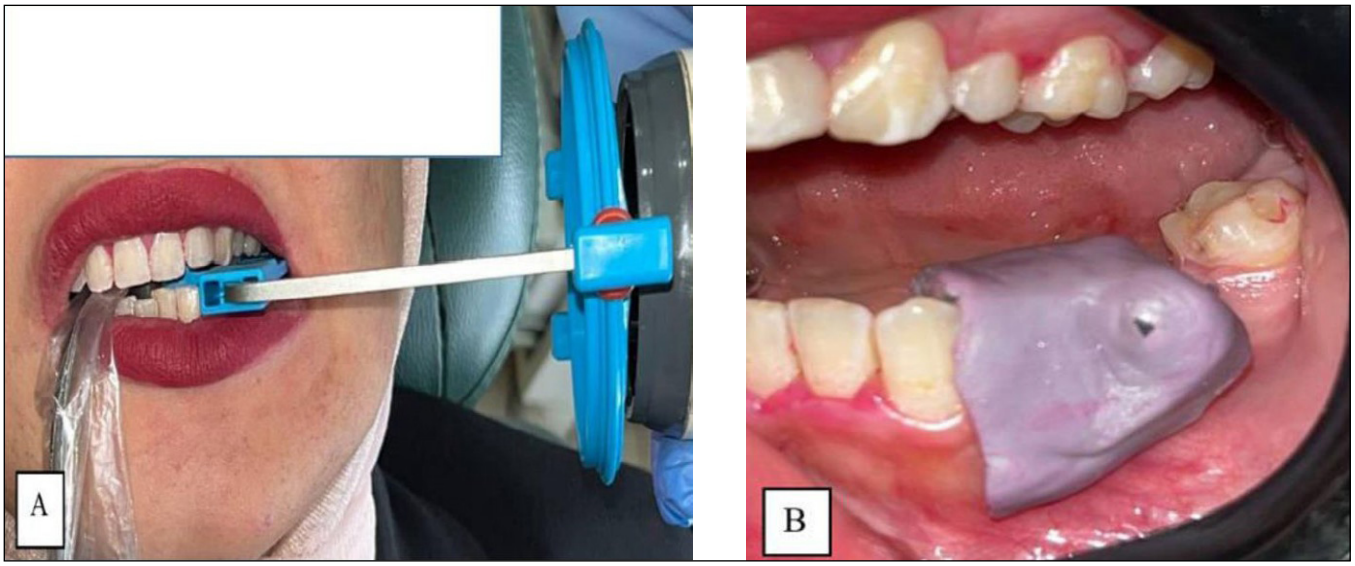


Fig. 1: Radiographic Setup and Calibration (A) Radiograph acquisition process, (B) Placement of the calibration ball
Source: Own materials

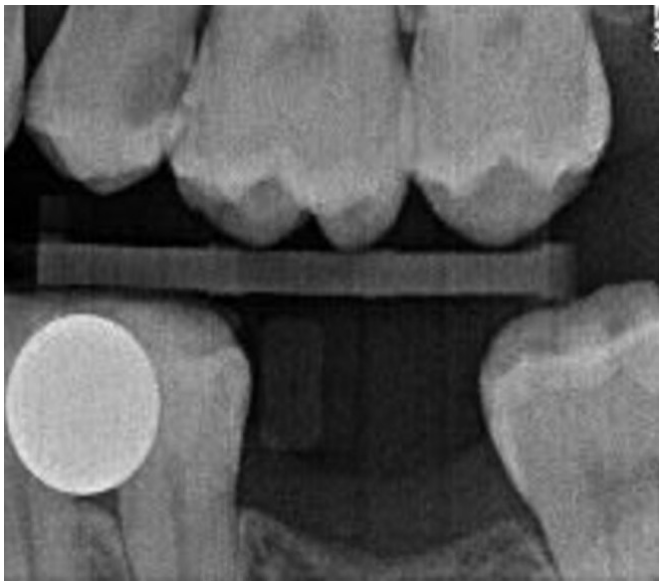


Fig. 2: Assessment of radiographic soft tissue thickness using a bite-wing projection
Source: Own materials

alloplastic based on their source, with different biological and mechanical properties [14]. Although hyaluronic acid and bone grafting materials have been shown to have good biological and regenerative properties separately, evidence of their combination to improve peri-implant tissue phenotype is scarce. Most previous studies have focused on bone regeneration, implant stability or post-operative discomfort, but the comprehensive evaluation of the peri-implant hard and soft tissue phenotype after the combined application of HA and bone graft is still missing.

AIM

The aim of this study was to investigate whether the combination application of hyaluronic acid and bone graft material has a substantial impact on peri-implant tissue parameters.

MATERIALS AND METHODS

The study was performed at two clinical sites, The Implantology Unit, Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Babylon (Babylon, Iraq) and a private specialized dental center (Babylon, Iraq), between December 2025 and July 2026.

The null hypothesis was that hyaluronic acid with bone graft does not have a significant effect on mucosal thickness and marginal bone height around the implants. The alternative hypothesis is that this treatment combination leads to significant changes both in crestal bone levels and peri-implant soft tissue thickness.

Ethical guidelines of the Declaration of Helsinki, as outlined by the World Medical Association, were strictly followed, obtaining informed consent from all participants. All patients gave consent that was written especially for the study prior to enrollment. In order to guarantee voluntary participation, each participant received an information sheet and a consent form that clearly explained the study and its significance. The patients were explicitly informed of their right to withdraw from the investigation at any stage without penalty while it was being conducted. The obtained results were the assessment of comprised the evaluation of radiographic soft tissue thickness (RSTT) alongside crestal bone height (BH). These quantitative assessments were made preoperative (just for RSTT), immediately postoperative, and six and twelve weeks postoperatively.

STUDY SAMPLE

Sample size estimation was executed with G*Power software hand-selected for this purpose. The analysis was based on a significance level of $\alpha = 0.05$, a power of 80%, and a

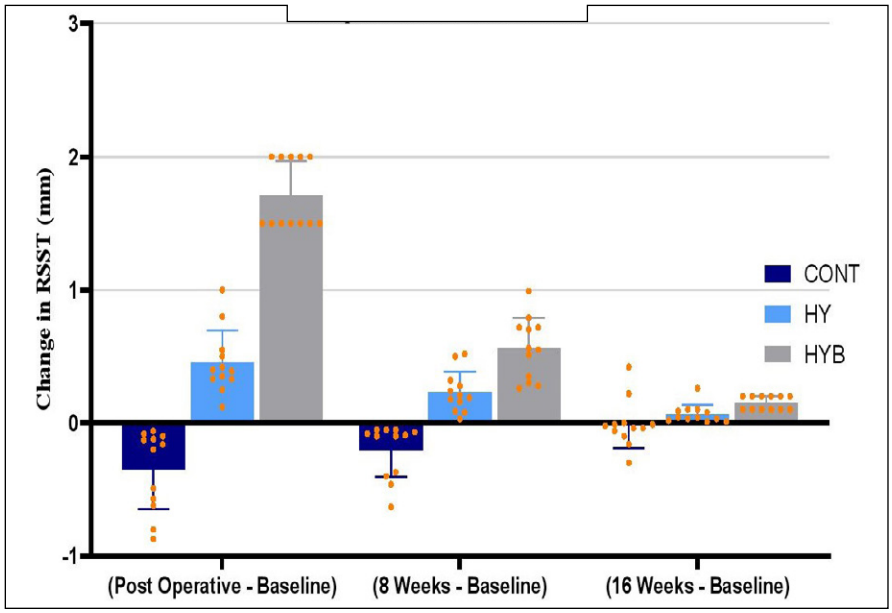


Fig. 3: Mean Change in Radiographic Soft Tissue Thickness (RSTT) with Individual Case Distribution across Study Groups at Different Time Intervals
Source: Own materials

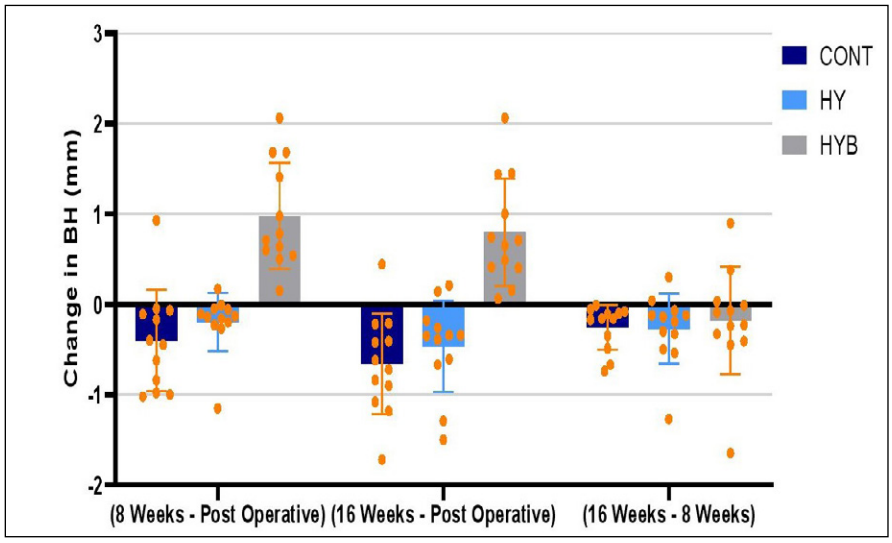


Fig. 4: demonstrates the mean change in bone height (BH) with individual case distribution across study groups at different time intervals
Source: Own materials

medium effect size according to Cohen's conventions (f = 0.25) for a three-group repeated measures design. Estimated 12 cases per group (total 36 with dropout 20(%.

ELIGIBILITY CRITERIA

Patients meeting the inclusion and exclusion criteria were recruited. Inclusion criteria for the study were: male and female individuals over 18 years of age having partially edentulous spaces in the posterior area of either jaw with appropriate bone volume for implant insertion. The participants required to show the best possible dental health, measured by the gingival bleeding index (≤10%) and O'Leary plaque index (<10%). The study excluded patients with systemic conditions affecting bone healing, patients on medications affecting bone metabolism, patients with periodontal disease, pregnant and lactating women, patients with poor oral hygiene and patients unable to attend follow-up appointments.

SAMPLING AND GROUPING

Candidate patients are randomly (sequentially numbered) selected throughout the course of the routine dental check-ups to allocate the 36 implant sites into three equal groups (1:1:1 allocation ratio). Some participants who satisfied the screening criteria were recruited until the desired sample size was met. Each participant had been randomly assigned to one of three groups:

- **Group (HA) (n=12 implants):** Patients in this group received a hyaluronic acid gel applied to the implant site during implant placement.
- **Group (HAB) (n=12 implants):** Patients in this group received hyaluronic acid gel and particulate bone graft applied to the implant site during implant placement.
- **Control Group (CONT) (n=12 implants):** Patients in this group underwent implant placement only without the use of hyaluronic acid gel aznd particulate bone graft.

Table 1. Comparisons of the socio-demographic characteristics among the studied groups (n=12)

Socio-demographic characteristics		CONT	HY	HYB	Total	p-value
		No. (%)	No. (%)	No. (%)		
Age groups (years)	≤30	1(8.3)	2(16.7)	1(8.3)	4	0.658*
	31-40	3(25.0)	1(8.3)	7(58.3)	11	
	41-50	3(25.0)	5(41.7)	2(16.7)	10	
	≥51	5(41.7)	4(33.3)	2(16.7)	11	
Gender	Males	4(33.3)	5(41.7)	5(41.7)	14	0.890†
	Females	8(66.7)	7(58.3)	7(58.3)	22	

*Freeman-Halton Exact test; †Chi square test
Source: Own materials

Table 2. Comparisons of the location characteristics among the studied groups (n=12)

Lesion characteristics		CONT	HY	HYB	Total	p-value
		No. (%)	No. (%)	No. (%)		
Jaw	Maxilla	5(41.7)	7(58.3)	8(66.7)	20	0.455†
	Mandible	7(58.3)	5(41.7)	4(33.3)	16	

†Chi square test
Source: Own materials

Table 3. Comparison of the RSST among the studied groups (n=12)

Radiographic Thickness	CONT	HY	HYB	p-value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Baseline	$1.91 \pm 0.40^{a,A}$	$1.85 \pm 0.36^{a,A}$	$2.36 \pm 0.62^{b,A}$	0.024
Post-Op	$1.56 \pm 0.27^{a,B}$	$2.31 \pm 0.24^{b,B}$	$4.07 \pm 0.62^{c,B}$	<0.001
8 Weeks	$1.70 \pm 0.33^{c,C}$	$2.09 \pm 0.27^{b,C}$	$2.92 \pm 0.56^{c,C}$	<0.001
16 Weeks	$1.90 \pm 0.39^{a,A}$	$1.92 \pm 0.35^{a,A}$	$2.51 \pm 0.63^{b,A}$	0.004
p-value†	<0.001	<0.001	<0.001	

*One-Way ANOVA test; ^{a}, ^{b}, ^{c}: Different lowercase superscript letters within a row indicate significant differences between groups at the same time point (Tukey post-hoc test, P<0.05).
†General Linear Model (Repeated Measures): ^{A}, ^{B}, ^{C}: Different uppercase superscript letters within a column indicate significant differences within the same group over different time points P<0.05.
Source: Own materials

Table 4. Comparison of the bone height among the studied groups (n=12)

Bone Height	CONT	HY	HYB	p-value*
	Mean ± SD	Mean ± SD	Mean ± SD	
Post-Op	$4.23 \pm 0.85^{a,A}$	$4.85 \pm 1.31^{a,A}$	$3.90 \pm 0.79^{a,A}$	0.077
8 Weeks	$3.84 \pm 0.84^{a,B}$	$4.66 \pm 1.38^{ab,AB}$	$4,87 \pm 0.83^{b,B}$	0.050
16 Weeks	$3.58 \pm 0.83^{a,B}$	$4.39 \pm 1.27^{ab,B}$	$4.69 \pm 0.49^{b,C}$	0.016
p-value†	<0.001	0.003	<0.001	

*One-Way ANOVA test; ^{a}, ^{b}: Different lowercase superscript letters within a row indicate significant differences between groups (Tukey post-hoc test).
†General linear Model (Repeated Measures); ^{A}, ^{B}, ^{C}: Different uppercase superscript letters within a column indicate significant differences over time (Repeated Measures ANOVA).
Source: Own materials

PREOPERATIVE PHASE

PATIENT PREPARATION

Before starting the trial, each participant's teeth were scaled and polished, and home care was established and encouraged. All potential implant sites were examined clinically by evaluation of soft tissue condition, ridge morphology, and space availability. Mesiodistal and interocclusal spacing was assessed to ensure sufficient clearance from adjacent teeth or implants. These findings were complemented

radiographically by Cone Beam Computed Tomography (CBCT) to ensure eligibility, check bone quality, and to make the best treatment decision regarding the implant size and placement angulation.

SURGICAL PHASE

HYALURONIC ACID PLACEMENT

Preparation and loading of hyaluronic acid

The haluronic acid (Ricerfarma S.R.L, Italy, and Milan) was prepared for clinical application using a standardized delivery method to ensure precise volumetric administration. The following protocol was adopted.

Standardization of the delivery vehicle

A sterile 1 ml insulin syringe (Changzhou Jifeng Medical Equipment Co., Changzhou, China) was modified by removing its needle-bearing using a sterile surgical blade (Fig.1).

This modification results in a large bore delivery channel allowing consistent discharge of the viscous gel and consistency with bone graft delivery technique.

Volumetric measurement

The modified syringe was filled with a precise volume of 0.2 ml hyaluronic acid.

Aseptic handling

The loading process was carried out under strict aseptic conditions immediately before surgical intervention to preserve the bioactivity of the high molecular weight HA

BONE GRAFT AND HYALURONIC ACID PLACEMENT

Preparation of the HA-impregnated sticky bone composite

The preparation of a cohesive and bioactive graft material was carried out using a protocol (Fig.2).

Standardize mixing

A volume of 0.25 cc of cortical cancellous allograft powder (particle size 1000-2000 µm) was transferred into a sterile dappen dish.

Incorporation of hyaluronic acid

Subsequently, 0.2 ml of Hyaluronic acid was added to the bone particles.

Formation of the cohesive mass

The components were thoroughly mixed using a sterile spatula until the graft reached a sticky and manageable consistency. This combination transforms the loose bone particles into a cohesive bioactive composite (Sticky Bone Analogue), which facilitate stabilization of the graft at the surgical site and prevent particles migration.

Loading into delivery system

The formed sticky bone composite was carefully loaded into a modified 1 ml insulin syringe (needle bearing removed). This custom delivery system facilitated volumetric precision delivery and controlled condensation of the graft into the peri-implant area.

IMPLANTATION PROCEDURE

After all measurements were recorded, sufficient anesthesia was administrated to the surgical site to get complete analgesia to initiate the surgical procedure. The participants were asked to rinse with 0.2 chorhexidine mouthwash for one minute to reduce the bacterial load intraoral before the surgical procedure. After achieving adequate anesthesia of the surgical site, making all the preoperative measurements a complete-thickness mucoperiosteal flap was raised utilizing periosteal reflection instruments. Then drilling was performed to construct the osteotomy site according to the determined sequence of recommended implant system manufacture. Once the osteotomy preparation was finished, the implant was placed in the created socket and then the cover screw was placed, a periosteal releasing incision at the base of the buccal flap was performed. This allowed for tension-free wound closure (Fig.3).

Following the successful placement of the dental implants, the following standardize clinical protocols were implemented:

SUTURE PREPARATION (PRE-LOADING)

Prior to the application of the bioactive materials, a horizontal mattress suture (using 4-0 nylon) was preplaced across the surgical site but left untied. This step was crucial to facilitate immediate and stable primary closure once the materials were in place, preventing material displacement.

GROUP (HA) HYALURONIC ACID APPLICATION

A volume of 0.2 ml of HA was measured using a modified insulin syringe. The gel was delivered from the buccal aspect extending towards the mid-crestal region of the ridge, and similarly from the lingual aspect extending towards the mid-crestal region of ridge, ensuring complete circumferential coverage around the implant platform.

GROUP (HAB) HA-IMPREGNATED STICKY BONE APPLICATION

A volume of 0.2 ml of HA was mixed with 0.25 cc of cortical cancellous allograft powder (particle size 1000-2000 µm) in a sterile container until a sticky bone consistency was achieved. This composite was

loaded into a modified insulin syringe and applied over implant site. The composite was delivered from the buccal aspect extending towards the mid-crestal region of the ridge, and similarly from the lingual aspect extending towards the mid-crestal region of ridge, ensuring complete circumferential coverage around the implant platform.

GROUP (CONT) CONTROL GROUP

In this group no bioactive materials or bone grafts were applied. The surgical site was managed by allowing natural blood clot formation around the implant platform. This group served as baseline to evaluate the natural healing process and soft tissue response without external intervention.

WOUND CLOSURE

Immediately after material placement, the pre-placed horizontal mattress suture was tightened and tied. To ensure complete wound closure two additional interrupted sutures were placed mesially and distally to the implant site. All participant were asked to return for suture removal. Each participant was recalled to remove the suture from the site and to check if any complications in the healing process that might not have been noticed by the patient are present.

RADIOGRAPHIC EVALUATION

Bite-wings were used to estimate the radiographic soft tissue thickness (RSTT) at the region of the missing tooth. All the radiographs were taken with a digital intraoral sensor (RVG 5200; Carestream Dental, Atlanta, GA, USA). To obtain a high reproducibility and consistency of the alignment, paralleling approach was performed with customized sensor holder (YP Dental, Anyang City, China), (Fig1A). To calibrate the dimensions, a metal sphere of known diameter was attached to the next tooth with a heavy-body condensation silicone substance (Fig1B). Image processing and measurements were performed with Image J software (National Institutes of Health, Bethesda, MD, USA) with the calibration of the spatial scale (pixels to millimeters) using the metal sphere. The vertical distance from the alveolar crest of the bone to the gingival edge was then calculated (Fig.2). The mucosal thickness was consistently measured at a reference position 5 mm distal to the height of contour on the mesial face of the neighboring tooth. A digital intraoral bite-wing radiograph was taken immediately after the implant insertion process to determine the marginal bone height. An intraoral digital sensor (RVG 5200; Carestream Dental, Atlanta, GA, USA) was positioned using a special alignment device (YP Dental, Anyang City, China). The cone of the X-ray tube was positioned parallel to the guiding ring of the positioning device for standardization. The vertical bone height (mm) was measured from the second thread (serration) on the mesial aspect of the implant fixture to the crestal bone level using Image J software (National Institute of Health, Bethesda, MD, USA). Follow-up radiographs were taken at scheduled intervals: immediately postoperatively, at 8 weeks and at 16 weeks. In these follow-up appointments, the same standardized radiography methodology with the reference metal sphere in position was used. All linear measurements on the computer were made by the calibrated image analysis program.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Version 26.0. The normality of the data was tested, and parametric tests were used. Frequencies, means, and standard deviations were calculated. Chi-square test was used for nominal data, and the Freeman-Halton Exact test was used instead of Chi-square when any cell had an expected value less than 5 for tables larger than 2 by 2. Numerical data were compared using one-way ANOVA for mean differences among multiple groups (more than two groups) under the study, with a post hoc test to find significant differences. Paired t-test was performed for the numerical data of two paired groups, and the general linear model for repeated measures was used to find differences among paired data of more than two groups. A P-value ≤ 0.05 was considered statistically significant.

ETHICAL APPROVAL

The study received approval from the Scientific Research Committee, Department of Oral Surgery and Periodontology, College of Dentistry, Mustansiriyah University (No. MUOSU/POST-202615) and registered in clinical trial number ClinicalTrials.gov NCT07665281

RESULTS

Numbers examined for the primary outcome and numbers of participants randomly allocated and receiving intended therapy for each group. Recruitment started in December 2025 and was finished in March 2026. Follow-up assessments for the last individual recruited were conducted in July 2026.

Table 1 presents the comparisons of the socio-demographic characteristics across the analyzed groups. There was no statistically significant difference between the groups in terms of age and gender.

Comparisons of the RSST in the studied groups demonstrated statistically significant differences for the baseline period (P = 0.024), post-operative period P<0.001, at 8 weeks P<0.001 and 16 weeks P=0.004. It also found highly statistically significant differences during the observation periods in the CONT group P<0.001, the HY group P<0.001 and the HYB group P<0.001 when comparing each study group with respect to time (Table 3).

The average bone height after surgery was not significantly different between groups (p=0.077). There were statistically significant differences at 8 weeks and 16 weeks, p=0.050 and p=0.016, respectively as time went on. However, the significant differences across these periods were between the HAB and the CONT group and there were no significant differences between the HA and the CONT group or between the HAB and the HA group. Comparisons within groups over time showed a significant statistical change in bone height (P<0.05) for all groups studied. In particular, the CONT group and HA group showed a continuous decrease over time (from Post-Op to 8 weeks, remaining lower at 16 weeks), whereas the HAB group showed a significant increase in bone height at 8 weeks compared to post-operative stage with a small reduction at 16 weeks but still significantly higher than post-operative level, (Table 4).

The mean change in bone height (BH) with individual case distribution across study groups at different time intervals has been shown in figure 4.

DISCUSSION

This study evaluated the impact of hyaluronic acid (HA) and bone grafts on peri-implant tissue phenotype, particularly focusing on bone stability and soft tissue response, important for implant longevity and aesthetics. The results indicated a notable increase in soft tissue thickness and stability in the HA group, especially in combination with the bone graft compared to the control. This study highlights the synergistic regenerative potential of HA and bone grafting for modifying soft tissue biotype and makes a significant contribution to clinical comparisons of these interventions.

Baseline demographic and anatomic characteristics

Statistical analysis showed no significant differences in age distribution, gender, and jaw position among the CONT, HA and HAB groups at baseline, indicating well-matched demographics and baseline anatomy. This enhances the reliability that observed clinical and radiographic outcomes are attributed to Hyaluronic Acid treatments rather than patient characteristics. The uniformity in demographic features and surgical sites allows for a clear correlation between soft tissue biotype changes and the interventions applied, minimizing variability from anatomical differences.

RADIOGRAPHIC SOFT TISSUE THICKNESS

The HAB group showed the highest values of radiographic soft tissue thickness at all evaluation periods (postoperative, 8 and 16 weeks) followed by the HA group and the control group had the lowest values. This larger dimension observed in the HAB group is a synergistic net effect resulting from the biological advantages of hyaluronic acid and the mechanical space maintaining capability of the bone graft. The present findings are in line with previous studies that showed that particulate bone grafts indirectly contribute to preservation of the alveolar ridge contour and the peri-implant soft tissue profile by protecting the underlying hard tissue architecture and avoiding collapse of the horizontal ridge, and not by directly stimulating soft tissue proliferation [15-18]. Furthermore, the positive biological effects of the HA group are consistent with the literature that described the direct effects of hyaluronic acid on early soft tissue repair due to its hydrophobicity, faster angiogenesis, fibroblast proliferation, and extracellular matrix synthesis [19, 20]. In contrast, the radiographic soft tissue thickness was also higher in the HA group than in the control group, but less so than in the HAB group. This finding is more likely related to biological properties of hyaluronic acid, including its hydrophilic nature, promoting angiogenesis, fibroblast proliferation and extracellular matrix synthesis, which enhance early soft tissue healing. These biological effects have been extensively documented in both experimental and clinical studies [19, 20].

RADIOGRAPHIC BONE HEIGHT

Bone height scores analysis between the study groups showed significant differences at immediate post-op, 8-week and 16-week periods. The HAB group showed significantly better preservation of the vertical bone levels than the control group at all evaluation time points, while the HA group exhibited an intermediate behavior without significant differences to either group. These results are consistent with the observations of [21] and [22] who found significantly better bone fill, superior volume retention, and less marginal bone loss around implant sites when a bovine bone substitute was used in combination with hyaluronate. The main biological mechanism for such activity is based on the synergistic action of the mineral matrix and HA that supports early neo-vascularization, HA that promotes early neo-vascularization, osteoblast development and collagen matrix mineralization during the crucial early phases of healing as described by [23, 24]. However, some studies using non-cross-linked HA alone have not shown any significant difference in long term marginal bone loss compared to non-treated conventional sites, a nuance that justifies the inability of our pure HY group to reach statistical superiority over the control in time [25].

STUDY LIMITATIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

The study presents favorable findings but acknowledges several limitations. The small sample size may limit the statistical power and generalizability of results, necessitating larger cohorts for future research. The 16-week follow-up is brief, lacking assessments of long-term tissue maturation and stability, as well as adequate histological support. Although the study examined the standalone hyaluronic acid (HA) and its combination with a mineralized bone graft (HAB) further research should investigate various combinations of hyaluronic acid with different scaffolds and graft ratios. Comparative studies with established soft tissue procedures will help clarify the role of HY composite formulations in clinical scenarios.

CONCLUSIONS

The additional use of hyaluronic acid with a particulate bone graft (HAB) significantly enhances peri-implant tissue healing. This combination leverages the biological effects of hyaluronic acid along with the space-maintaining properties of the graft material, resulting in improved dimensional stability. While hyaluronic acid alone (HA) facilitates soft tissue augmentation through its hydrophilic characteristics that stimulate extracellular matrix formation, angiogenesis, and collagen synthesis, it falls short in maintaining horizontal ridge dimensions during healing without graft support. In contrast, the HAB method demonstrates superior preservation of horizontal bone width over a 16-week healing period and promotes favorable soft tissue characteristics. Findings indicate that integrating hyaluronic acid with a particulate bone graft not only supports peri-implant soft tissue augmentation but also effectively maintains the dimensions of both hard and soft tissues. Overall, the HAB approach proves to be more predictable for simultaneous enhancement of soft tissue quality and maintenance of hard tissue architecture compared to using hyaluronic acid alone.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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