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# Evaluation of hyaluronic matrix efficacy in sinus augmentation: a randomized-controlled histomorphometric and micro–computed tomography analysis

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*Abstract.* The objective of the present study was to test the hypothesis that the addition of hyaluronic acid–based matrix to collagenated heterologous bone graft for sinus augmentation would enhance bone formation compared to collagenated heterologous bone graft alone in the early healing period, by micro−computed tomography and histomorphometry. Thirteen systemically healthy patients requiring bilateral two-stage maxillary sinus augmentation (residual crest height $\leq$ 4 mm) were enrolled in this split-mouth prospective randomized controlled study. One sinus side as a control group was grafted with only collagenated heterologous bone graft; the other region as a test group was grafted with hyaluronic matrix and collagenated heterologous bone graft. Bone biopsy samples were taken after 4 months during the dental implant surgery and analyzed using micro−computed tomography and histomorphometric parameters. According to the micro−computed tomography and histomorphometric results, a significantly higher percentage of new bone was observed in the test group when compared to the control group after 4 months of healing.

This study confirmed the hypothesis that the addition of hyaluronic matrix to collagenated heterologous bone graft for sinus augmentation enhances bone formation compared to collagenated heterologous bone graft alone in the early healing period.

Key words: maxillary sinus augmentation; dental implant; hyaluronic matrix; histomorphometry; micro computed tomography.

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The rehabilitation of partially or completely edentulous patients with implant-supported prostheses has been a routine treatment in recent decades, with reliable outcomes[1]. The long-term stability of dental implants in the function and prognosis of implant-supported prostheses are directly associated with the quality and quantity of the available bone for implant placement[2].

Clinicians usually have to deal with insufficient bone height in the posterior maxilla due to alveolar bone atrophy and pneumatization of the maxillary sinus after teeth loss. Maxillary sinus augmentation procedures have been used to obtain sufficient bone quantity and quality to allow implant placement. Since Tatum[3] first described a maxillary sinus augmentation procedure using the lateral window technique, various grafting materials have been used for this purpose[4,5]. These include autografts, allografts, xenografts, alloplasts, and combinations of these in various forms[6].

Autograft is believed to be the gold standard in augmentation procedures due to its osteogenic, osteoinductive, and osteoconductive properties; however; it has main disadvantages in sinus grafting such as the availability of only limited quantities, the need to include additional surgical sites, donor site morbidity, and a tendency towards resorption which may compromise long-term implant stability[7,8]. Recent studies have shown higher implant survival/success rates with xenografts than with autografts in sinus augmented areas[9]. In addition to bovine bone being used frequently for sinus augmentation, a collagenated heterologous bone graft (CHBG) has been used recently in augmentation procedures[10]. CHBG is similar to human bone in that it is osteoconductive and integrates well at host sites. Although many studies have evaluated the suitability of materials of different origins, it still remains unknown which is the most convenient graft material for maxillary sinus augmentation[11].

To obtain successful sinus augmentation outcomes, regeneration of well-vascularized, healthy bone is critical[12]. Other than the type of graft material used, the duration between sinus augmentation and implant placement influences regenerative outcomes in maxillary sinus augmentation[13–15].

Longer healing periods increase the amount of newly formed bone. However, for patient comfort and quality of life, shortening the length of surgical treatment time is an important issue[1].

The application of exogenous hyaluronic acid and hyaluronic acid–based materials have provided good results in manipulating and accelerating wound the healing process in a large number of medical disciplines, as evident in ophthalmology, dermatology, dentistry, and rheumatology[16]. Hyaluronic acid is a naturally occurring, nonsulphated glycosaminoglycan that is normally present in great quantities in extracellular matrixes such as basal laminae, connective matrixes, and synovial fluid[17]. Through its complex interactions with matrix components and cells, hyaluronic acid has multifaceted roles in biology using both its physicochemical and biological properties[18]. It plays a predominant role in tissue morphogenesis, cell migration, differentiation, and adhesion[19]. Hyaluronic acid also has osteoconductive properties and accelerates bone regeneration by means of chemotaxis, proliferation, and successive differentiation of mesenchymal cells[20].

According to the literature, there is a limited number of studies using hyaluronic acid for sinus augmentation. Schwartz et al.[21] reported the use of hyaluronic acid as a carrier material with demineralized bone allograft (DFDBA) for sinus augmentation in human patients. Their results showed that hyaluronic acid can be used as a carrier for DFDBA without reducing the clinical effectiveness of the graft.

Butz et al.[22] evaluated the time-dependent efficacy of bovine hydroxyapatite/synthetic peptide in a sodium hyaluronate carrier (PepGen P-15 Putty) for maxillary sinus augmentation. Emam et al.[23] also aimed to test the efficacy of PepGen P-15 Putty as a sole graft material for sinus augmentation.

Imaging techniques such as micro–computed tomography (micro-CT) have made it possible to obtain high-resolution three-dimensional images to directly examine the bone architecture. With this technique, no specimen preparation is required, and testing is nondestructive compared to conventional histomorphometry[24]. This method allows evaluation of the three-dimensional architecture of grafted bone after a period of bone healing. However, despite improvements in micro-CT, the histomorphometric techniques still remain a gold standard for analysing bone formation and allow more accurate evaluation of the association between graft particles, newly formed bone, and the cellular characterization[25].

This clinical study aimed to testing the hypothesis that the addition of hyaluronic matrix to CHBG for sinus augmentation would enhance bone formation in the early healing period compared to CHBG alone, using micro-CT and histomorphometric evaluation.

## Materials and Methods

Thirteen systemically healthy patients requiring bilateral maxillary sinus augmentation (residual crest height $\leq$ 4 mm) were included in this prospective randomized controlled study.

Eight female and five male patients (mean age, 0 years; range, 33–69 years) were enrolled between September 2013 and June 2015. The exclusion criteria were advanced systemic diseases, chronic medication use, maxillary sinus disease, current pregnancy or lactation, and smoking habit. At baseline, a comprehensive oral examination, panoramic radiographs, and cone beam computed tomography (CBCT) scans were performed. CBCT scans were analyzed for residual crest height, residual crest width, intrasinusal pathologies, and morphology of the bony walls. Patients with good oral hygiene and no active periodontitis underwent two-stage maxillary sinus augmentation.

The study was performed according to the Declaration of Helsinki as revised in 2001[26,27]. The study protocol was approved by the clinical research ethics board of the university (2014/08 - 16 (KA-14030)). The patients were fully informed about the procedures and could terminate their participation in the study at any time. All patients provided written informed consent.

The present study has been registered to the clinicaltrials.gov system as a randomized controlled trial with identifier number NCT02692261.

All patients received bilateral sinus augmentation via a lateral window approach as described by Tatum[3]. After local anesthesia, this approach began with a crestal incision on the top of the alveolar ridge, which was supplemented by two releasing incisions at the anterior and posterior extent of the crestal incision. A full-thickness mucoperiosteal flap was raised, and a small buccal window was then created using a round bur under sterile saline irrigation on the lateral wall of the sinus until the bluish hue of the sinus membrane was visible. The Schneiderian membrane was elevated from the bony floor with sinus elevation curettes freely anteriorly, posteriorly, and medially to ensure tension-free elevation. The space created below the membrane was available for graft placement. If the sinus membrane was inadvertently perforated, collagen membrane was applied to seal the opening and to ensure the confinement of the graft material. As larger perforations occurred, the augmentation procedure was postponed and patient was excluded from the study.

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In this split-mouth study design, the selection of which side (right or left maxillary sinus) would receive only CHBG (1 g) (Apatos mix, Osteobiol®, Italy) as a control group or hyaluronic matrix (Hyaloss™ matrix, ANIKA Therapeutics, Italy) in addition to CHBG (1 g) as a test group was performed by a coin-toss randomization process at the time of the surgery. Hyaluronic matrix is composed entirely of an ester of bacterial hyaluronic acid with benzyl alcohol, a concentration ranging from 20 to 60 mg/ml. It is a product manufactured as a solid in the form of fibers that form a gel when hydrated, releasing pure hyaluronic acid. In the test group, according to the manufacturer's instructions, each 0.5-cc CHBG was mixed with two bundles of hyaluronic matrix and a few drops of sterile saline solution.

Following graft placement, all lateral windows were covered with collagen membrane (Evolution Std, Osteobiol®, Italy). The flaps were sutured, and the sutures were removed 10 days after surgery. Postoperatively, patients received antibiotics (amoxicillin-clavulanic acid, 875/125 mg 3 times daily for 7 days; or clindamycin, 300 mg 3 times daily for 7 days in patients allergic to β-lactamicus), and nonsteroidal anti-inflammatory drugs (flurbiprofen, 100 mg twice daily for 5 days). Additionally, patients were advised to rinse twice daily with chlorhexidine (0.2%) for 10 days and to refrain from brushing or flossing the surgical sites until sutures were removed. Placing a cold compress superficially on the skin overlying the site for the first 24 hours and maintaining a soft diet to avoid trauma were also recommended. Instructions to avoid smoking and any sinus pressure–inducing actions (e.g., use of straws, nose blowing) were given to the patients.

Patients were recalled for postoperative follow-up at 1 and 3 months until bone biopsy samples were obtained at 4 months following sinus augmentation. A total of 26 bone biopsy samples were harvested from the grafted areas using a trephine bur with an internal diameter of 2 mm at the time of implant insertion (Fig. 1). A total of 41 implants were placed at the augmented sinuses for all included patients. The biopsy samples were gently removed from the trephine bur and immediately immersed in 10% neutral buffered formalin and prepared for micro-CT and histomorphometric analysis. All bone biopsies were obtained by an experienced periodontist (E.D.).

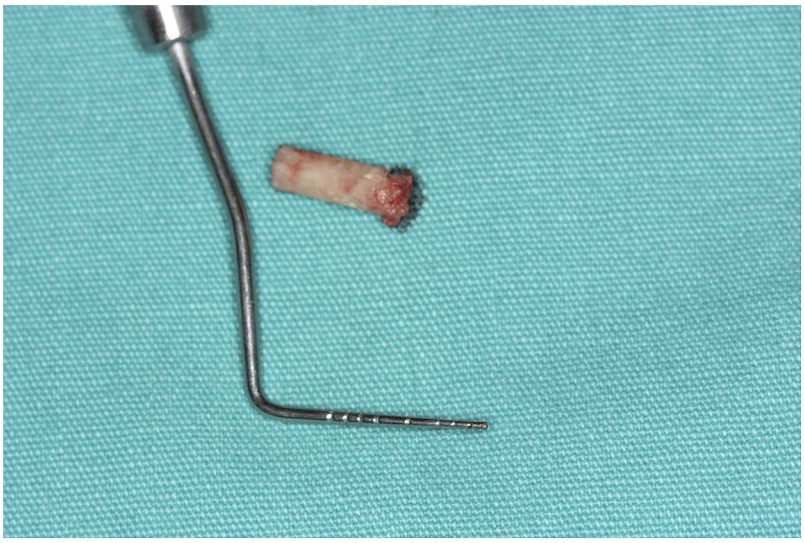

*Fig. 1.* Photograph of a bone biopsy sample taken from the implant socket using a trephine bur.

### Micro-CT evaluation

The specimens were scanned with a high-resolution micro-CT system (Skyscan 1174; Skyscan, Kontich, Belgium) in 180 degrees of rotation in 0.7-degree steps with a 40.89-μm pixel size. Digital micro-CT images were acquired at 50 kV/800 μA with an exposure time of 2.3 seconds. Three-dimensional (3D) reconstruction of raw data obtained through scanning was performed by NRecon (NRecon version 1.6.9.4, Skyscan, Kontich, Belgium) software, provided by the manufacturer. Following reconstruction, regions of interest (ROIs) were drawn within the sample to analyze the 3D structure of the sample. To distinguish grafted bone and newly formed bone from original bone, the Multi-level Otsu Method was used[28]. This is a thresholding method selecting two threshold values that maximize the between-class variances; essentially, in this way, within-class variances are minimized[29].

Percentages of newly formed bone, graft, and connective tissue, the gray level of newly formed bone and graft, and the structural model index (SMI) were evaluated on the 3D images performed by CTAn (version 1.13.5.1) software. SMI is a relative index which was derived according to the method of Hildebrand and Ruegsegger[30]. This variable determines the presence of either plate-like or rod-like trabeculae[31]. It is defined in a range of 0 to 3, where closer to 0 corresponds to an ideal plate and 3 to an ideal cylinder. Therefore, any value in this range indicates how plate-like or rod-like a structure is. Plate-like trabecula is associated with a higher osseous stiffness.

### Histologic processing and histomorphometric analysis

Following micro-CT scanning, bone biopsy samples were decalcified in De Castro solution (chloral hydrate, nitric acid, distilled water) and embedded in paraffin by using an automated tissue processor with vacuum. Serial sections 3- to 5-μm thick were stained with hematoxylin and eosin (HE) and Masson's trichrome (MT). Photomicrographs of each section were generated by a light microscope (Leica DMR) attached to a computerized digital camera (Model DFC 480, Leica Westlar Germany). The entire section was visible at the lowest magnification. Bright-field images were captured and analyzed quantitatively by an image processing program (LAS and Qwin Plus, Leica Inc. Westlar Germany).

Histologically, bone trabeculae that surround the graft material are accepted as newly formed bone. New bone trabeculae have osteocytes with centrally localized larger nuclei accepted as new bone, and bone trabeculae osteocytes have smaller nuclei with reversel line accepted as host bone. The host bone site is accepted as the coronal site of the bone biopsy sample.

The number of pixels corresponding to the newly formed bone and connective tissue area in each image was quantified, divided by the total number of pixels corresponding to total bone and connective tissue area, respectively. The number of pixels corresponding to the graft area was also quantified and divided by the total number of pixels corresponding to the total tissue area. All measurements were converted to square micrometers ($\mu m^2$) in each specimen; the final percent-

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*Table 1*. Micro-CT imaging findings (mean ± SD).

| | CHBG | CHBG+ hyaluronic matrix | *P* value |
|---|---|---|---|
| Newly formed bone (%) | 24.24 ± 1.26 | 30.99 ± 1.54 | 0.003* |
| Residual graft material (%) | 12.07 ± 0.57 | 13.15 ± 0.29 | 0.003* |
| Connective tissue (%) | 55.67 ± 1.19 | 47.85 ± 1.48 | 0.003* |
| Gray value of graft | 62.09 ± 0.31 | 76.27 ± 1.55 | 0.003* |
| Gray value of newly formed bone | 123.26 ± 2.49 | 135.22 ± 2.90 | 0.003* |
| SMI | 2.49 ± 0.02 | 2.85 ± 0.03 | 0.003* |

CHBG, collagenated heterologous bone graft; CT, computed tomography; SD, standard deviation; SMI, structural model index.
* Statistically significant.

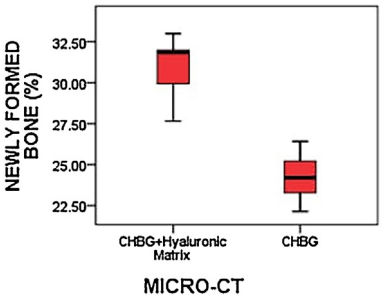


*Fig. 2*. Percentage of newly formed bone analyzed by micro-CT.

age of newly formed bone, graft, and connective tissue was noted[21,22,32].

### Statistical analysis

Statistical evaluations were made using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Dependent variables were test and control groups in which different graft materials were used, using histomorphometric and micro-CT measurements. The normality of distribution and the homogeneity of variances of the sample were established using the Shapiro–Wilk test. All parameters were analyzed by nonparametric Wilcoxon signed-rank test. Statistical significance was set at $P < 0.05$.

## Results

In this split-mouth study, a two-stage maxillary sinus augmentation was performed in 26 maxillary sinuses in 13 patients. Postoperative healing was uneventful in all patients. Minimal swelling, as expected, was observed at the surgical sites postoperatively and subsided by the end of the first week. No clinical signs of infection were seen in any patients. Preoperative CT scans revealed that there was no statistically significant difference in residual crest height ($P = 1.00$) and residual crest width ($P = 0.622$) between the test and control groups. The biopsy specimens were obtained at an average of 4.09 ± 0.20 months for the control group and 4.13 ± 0.23 months for the test group at implant placement. No statistically significant difference was found in the healing period between the groups ($P = 0.564$).

At follow-up appointments, panoramic radiographs were taken. It was noted that all implants were successfully osseointegrated, and prostheses were delivered. No implant was lost during the study period.

### Micro-CT imaging findings

Table 1 presents the results of micro-CT analysis of the biopsy specimens. The average percentage of newly formed bone for the CHBG and the CHBG + hyaluronic matrix group were 24.24% ± 1.26% and 30.99% ± 1.54%, respectively. A significantly higher percentage of new bone was observed in the test group when compared to control group ($P = 0.003$) (Fig. 2).

### Histologic and histomorphometric findings

Osteogenesis was evidenced by areas of secreted osteoid originating from adjacent osteoblasts. Woven bone or lamellar bone formation, seen in close contact with graft particles, were representative of new bone trabecula. In the CHBG + hyaluronic matrix group, more new bone trabeculae and connective tissue and more blood vessels were detected (Fig. 3). Lymphocytes, macrophages, and multinucleated cells invading graft material and fibrous encapsulation were seen in the study groups. The materials used in this study were biocompatible and did not elicit any foreign-body reaction.

Table 2 summarizes histomorphometric findings of 26 bone biopsy samples. Histomorphometric analysis showed that average percentage of newly formed bone was 19.07% ± 1.75% and 24.05% ± 2.97% for the control and test group, respectively (Fig. 4). Statistically significant differences between the groups were found in regard to newly formed bone ($P = 0.004$).

## Discussion

The present study aimed to assess the effect of hyaluronic acid–based matrix on short-term bone regeneration in augmented maxillary sinus. We hypothesized that hyaluronic acid–based matrix would ensure faster bone regeneration and contribute higher new bone formation in

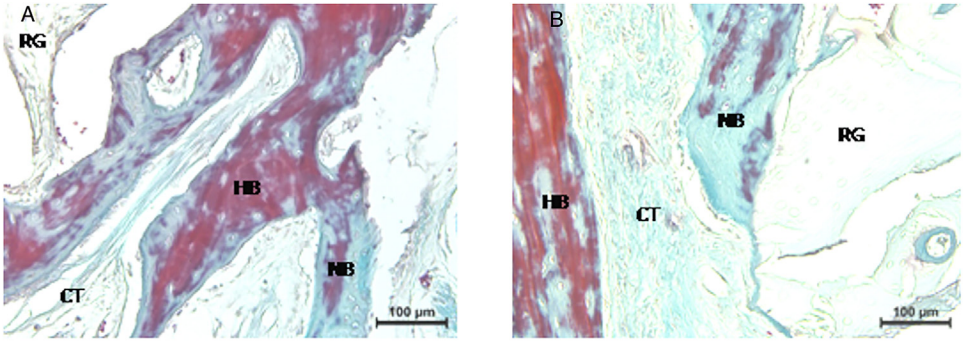


*Fig. 3*. Masson's Trichrome (MT x 200). More new bone trabecula and more connective tissue abundant with blood vessels were seen in the CHBG + hyaluronic matrix group. (A) Photomicrograph of control group specimen. (B) Photomicrograph of CHBG + hyaluronic matrix group specimen. NB, newly formed bone; RG, residual graft material; HB, host bone; CT, connective tissue.

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*Table 2*. Histomorphometric results (mean ± SD).

| | CHBG | CHBG+ hyaluronic matrix | *P* value |
|---|---|---|---|
| Newly formed bone (%) | 19.07 ± 1.75 | 24.05 ± 2.97 | 0.004* |
| Residual graft material (%) | 8.36 ± 1.92 | 10.46 ± 2.19 | 0.075 |
| Connective tissue (%) | 39.16 ± 7.12 | 42.26 ± 7.64 | 0.328 |

CHBG, collagenated heterologous bone graft; SD, standard deviation.
* Statistically significant.

augmented maxillary sinus. To test the aforementioned hypothesis, the study design chosen was a randomized, controlled, split-mouth study in patients. One sinus side was augmented with CHBG + hyaluronic matrix, and the contralateral sinus was augmented with CHBG alone; the bone formation process was screened by micro-CT and histomorphometry after a 4-month healing period. Micro-CT and histomorphometric analysis showed a significant higher percentage of newly formed bone in the CHBG+ hyaluronic matrix group.

Long-term success of two-stage maxillary sinus augmentation is substantially dependent upon regeneration of vital and well-vascularized bone[33]. Although higher new bone formation is time-dependent, a shorter healing period providing shorter treatment time is desirable from the viewpoint of patient comfort. After using bone graft substitutes, investigators have described healing periods of 6 to 8 months[34]. In this study, a shorter healing period of 4 months was preferred to reduce treatment time compared with that that associated with other bone graft substitutes. This healing time is almost comparable with that of autologous bone grafting, which is known to have a revascularization of 3 to 4 months[35].

After this healing period, micro-CT and histomorphometry analysis revealed that when hyaluronic matrix was used with CHBG for sinus augmentation, new bone formation was higher than that with CHBG use only. According to the literature, there are a limited number of studies conducted with early bone healing after sinus augmentation is present. Butz et al.[22] stated that PepGen P-15 Putty can be used successfully for sinus augmentation and implant placement as soon as 2 months after augmentation is achieved.

Emam et al.[23] used also PepGen P-15 Putty for sinus augmentation and attributed the considerable amount and the pattern of newly formed bone to the cell-binding potential of the PepGen P-15 particles and the presence of sodium hyaluronate as a carrier. They stated the ability of sodium hyaluronate to accelerate new bone formation via mesenchymal cell differentiation and to facilitate the mineralization of the calcifying matrix. The authors also reported histologic results similar to those of the present study, in that sodium hyaluronate provided considerable spacing between graft particles, helping to lower the packing density to allow vascular and cellular invasion to the grafted area. This hypothesis was supported by the abundant vascular spaces as demonstrated in the present study's histologic outcomes.

In a controlled study using a murine model, the test side sinonasal cavity was packed with hyaluronic acid and the other cavity was left untreated[36]. New bone regeneration was significantly higher on the hyaluronic acid–treated side than the control side. Mendes et al.[37] evaluated the effects of sodium hyaluronate in the healing process of the tooth sockets of rats in an immunohistochemistry study. The data showed that hyaluronic acid treatment induced earlier trabecular bone deposition, resulting in a more organized bone matrix at 7 and 21 days after tooth extraction. According to their results, hyaluronic acid also stimulated the expression of osteogenic proteins such as bone morphogenetic protein 2 and osteopontin. According to Baldini et al.[38], from a histological point of view, hyaluronic acid–derived matrix enabled new bone formation after a shorter period when used with autologous bone. Faster bone healing provides important benefits for the clinical situation, allowing the reduction of the healing time maintained after bone grafts. In this socket preservation study, it was concluded that the use of hyaluronic acid permits a better and faster bone healing process.

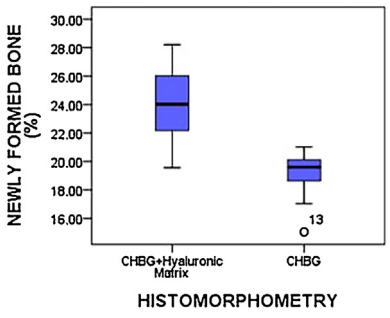


*Fig. 4*. Histomorphometric analysis of percentage of newly formed bone.

Schwartz et al.[21] reported the use of hyaluronic acid as a carrier material with DFDBA for sinus augmentation in humans. According to their histomorphometric results, even though hyaluronic acid carrier reduced the absolute amount of DFDBA to 16%, DFDBA with hyaluronic acid carrier was found to be as effective as DFDBA in combination with bovine hydroxyapatite. Most of the histologic sections obtained in their study showed very dense, newly formed bone that resembled cortical bone. In accordance with this study's clinical results, Schwartz et al. also indicated that DFDBA with hyaluronic acid carrier exhibited good handling characteristics and sufficient body to fill the sinus space without sagging.

According to the micro-CT results, SMI showed values closer to 3 for both groups. In parallel with our findings, Huang et al.[39] also found values closer to 3 for grafted bone samples retrieved from maxillary sinuses. It can be concluded that rod-like trabeculae may be indicative of newly formed bone.

The average percentage of newly formed bone obtained in the present research demonstrated results comparable to those of other studies of bone graft substitutes in maxillary sinus augmentation. From the results of this study, it can be concluded that the use of hyaluronic acid–based matrix with CHBG for sinus augmentation offers a reliable outcome, permitting implant placement after a 4-month healing period. This study confirmed the hypothesis that the addition of hyaluronic acid–based matrix to CHBG for sinus augmentation enhances bone formation compared to CHBG alone in the early healing period. However, further studies are needed to evaluate hyaluronic acid efficacy for sinus augmentation, with a larger number of samples and longer-term implant survival data.

## Declarations

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**Competing Interests:** The authors report no conflict of interest related to this study. None of the authors has any financial or personal relationships with other people or organizations that may inappropriately influence their actions.

**Ethical Approval:** The study protocol was approved by Clinical Research Ethics Board of Hacettepe University (2014/08 – 16 [KA-14030]).

**Patient Consent:** Written informed consent has been obtained from participants to publish clinical photographs.

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