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Evaluation of Xenogeneic Bone Graft and Bone Marrow Mesenchymal Stem Cells Mixture in Promoting Osteogenesis of Jaw Bone Defects (Clinical and Radiographic study)

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ABSTRACT

Aim: The aim of this work was to evaluate bone regeneration capacity of Xenogeneic bone graft and Bone marrow mesenchymal stem cells (BMSCs) mixture in Osseous Jaw defects. **Subjects and Methods**: This study was carried out on 10 patients, aging from 15 to 50 years old, who had osseous defects due to benign tumor or large cyst ≥3cm^3 by clinical and radiographic examination. All Defects were grafted with a mixture of BMSCs seeded into collagen sponge and xenogeneic bone graft. All patients were subjected to a panoramic radiograph, cone-beam computed tomography (CBCT) to assess jaw bony defect size, bone volume, and bone density. **Results:** The significance evaluation for volumetric changes for each time interval revealed significant difference ($P < 0.05$) except for immediate postoperative which were insignificant different. The significance evaluation for bone density each time interval revealed insignificant difference except for bone density change percentage which revealed significant difference ($P<0.05$). **Conclusion:** The use of autologous BMSCs seeded on gelatin scaffold combined with xenogeneic bone graft was promising for regenerating bone at odontogenic large defects ≥ 3 cm^3.

KEYWORDS

Xenogeneic Bone Graft,
Stem Cells Mixture,
Osteogenesis,
Jaw Bone Defects.

INTRODUCTION

One of major surgical challenges in oral and maxillofacial field had been reconstruction of bony defects. Intraoral defects that resulted from a variety of pathologic processes are common problems encountered by oral and maxillofacial surgeons [1].

Bone grafting represents a frequently performed procedure to enhance bone regeneration at these defects. Autologous bone graft is considered the golden standard bone grafting material. However, its harvesting is associated with donor-site morbidity and restricted availability. Moreover, Alternative use of allografts has high cost, potential immunogenic response by host to foreign tissue, and possibility of disease transmission [2].

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Engineering of bone via genetically modified stem cells has been viewed as a potential alternative to conventional bone grafts for repairing bone defects. Mesenchymal stem cells (MSCs) are multipotent stromal cells with ability to undergo self-renewal and multi-lineage differentiation, can additionally exert a biologically supportive function through paracrine actions and trophic factors [3].

Bone marrow mesenchymal stem cells (BMSCs) are readily available and possess a high osteogenic capacity. As a result, they are considered to be one of the most appropriate stem cell populations for bone regeneration and are thus widely used for efficiency comparison purposes with other cell sources as combined with suitable scaffolds are more effective in promoting new bone formation than scaffolds alone in animal bone defect models [3].

Absorbable collagen sponge has been used for enhancement of bone healing at many bony defects. Collagen sponge advantages include; being a natural scaffold, their ability to enable blood clot formation, their adaptability, their ability to resorb totally and rapidly over time and their low cost. Collagen sponge soaked with bone marrow aspirate was reported as a method for repairing human bone defect[4].

Xenogeneic biomaterials display a similar morphology as human bone and have the potential of being resorbed. They have the potential to be a viable substitute to autograft and allograft. Xenograft materials of porcine origin have provoked a great deal of research to assess their potential as a substitute for osseous grafts. It provides biocompatible, bioabsorbable, and osteoconductive matrix [5].

The aim of this work was to evaluate bone regeneration capacity of Xenogeneic bone graft and BMSCs mixture in Osseous Jaw defects.

The primary outcome was to evaluate bone regeneration gain and bone density, using cone-beam computed tomography (CBCT), and the secondary outcome was to evaluate bone defect volume, using CBCT

MATERIAL AND METHODS

This current study was carried out on 10 patients (5 females and 5 males) with age ranging from 15 to 50 years old who had osseous defects due to benign tumor or large cyst ≥3cm^3 by clinical and radiographic examination and were medically free without any systemic illness or under any medication that interfere with normal bone healing selected from attending outpatient clinics of Oral Maxillofacial Surgery Department, Faculty of Dental Medicine, Al Azhar University (Assiut), Egypt.

This study was approved by the Ethical Committee of Faculty of Dental Medicine, Al Azhar University (Assiut) number: AUAREC202300010-3 from February 2021 to December 2023. Informed written consents were given to the patients after informing them about the operation and risks.

Exclusion criteria were malignant tumors, small defects < 3 cm^3, heavy smokers (> 20 cigarettes/day), disabilities, systemic disease or administrating any medications to retard bone healing, lack of compliance, or patients who were not suitable for general anesthesia.

Pre-operative assessment included history taking, intraoral and extraoral clinical examination, patient photographs from various views, and after clinical examination, all enrolled patients in the study were subjected to a radiographic screening included a panoramic radiograph as a primary survey, CBCT to assess jaw bony defect and determine its size. Patient's preoperative readings of bone density and bone volume was recorded.

All patients were prescribed Augmentin (Amoxicillin/Clavulanate potassium (gsK (GlaxoSmithKline)) 1gm twice daily before surgery, Investigations were required to prepare patients for general anesthesia.

Preparation for Surgical Procedure:

Patients were prepared for surgery with basic traditional method. Procedure performed under general anesthesia with nasal intubation. One case was

done with an extraoral submandibular approach due to lesion extension and the rest of the cases were done with intraoral vestibular incision. Preparation of recipient sites were done to receive graft and closure was done with collagen membrane and sutured it tightly by 3-0 vicryl sutures with interrupted sutures to obliterate lesion completely.

Surgical procedures

Patients were prepared under general anaesthesia for draping and disinfectant of iliac crest to harvest BMMNCs. Iliac crest bone marrow aspirate was primarily collected, using a 13-gauge bone marrow trocar, a puncture was made penetrating anterior superior iliac spine with a watch wind movement. 20ml of bone marrow aspirate was obtained in a heparin-treated 50ml syringe. A minor repositioning of trocar was done for each 10ml to access different areas of cancellous bone marrow through the same cortical access hole.

Bottle was inverted several times to ensure thorough mixing. Iliac crest bone marrow aspirate was then diluted (1ml for each 5ml aspirate) with saline or Ringer lactate and then carefully layered very slowly onto lymphocyte separating medium (Pancoll® Paque Plus, PAN Biotech, Buckinghamshire, UK)* in silicon falcon tube and avoiding mixing the two solutions (6ml diluted aspirate on 3ml Pancoll at the tube).

Bone marrow aspirate was diluted and then carefully layered onto the pancoll at silicon tube then centrifuged at 2000 rpm for 20 minutes at room temperature using a multispeed 4000 rpm vertical rotor. The upper layer containing plasma and platelets was then collected using a sterile pipette leaving mononuclear cell layer undisturbed at the interface then carefully transferred to a sterile tube and centrifuged at 2000 rpm for 10 min. Cell isolate was then washed using balanced salt solution, supernatant was then removed, and cell pellet was resuspended at 2ml of previously obtained platelet poor plasma containing gentamycin and dexamethasone. Then seeded into collagen sponge and mixed with xenogeneic bone graft.

Postoperative assessment:

Postoperative medications and instructions will be described for all patients to avoid postoperative complications such as infection and edema.

Each patient received intravenous Cefotax (Cefotaxime 1gm/12 hours (EGYPTIAN PHARMACEUTICAL INDUSTRIES CO (E.I.P.I.CO)) for one day postoperatively followed by Amoxicillin/ Clavulanate potassium (Augmentin) 1gm twice daily for the next 5 days. Analgesic anti-inflammatory drug was received in the form of Voltaren (Diclofenac sodium 75mg vial (Novartis CO)) till the second postoperative day followed by Cataflam (Diclofenac potassium 50mg tablets (Novartis CO)) three times daily and instructed to avoid smoking, patients were closely observed daily in the first week and then asked to attend (follow-up atone week, 3 – 6 months postoperatively).

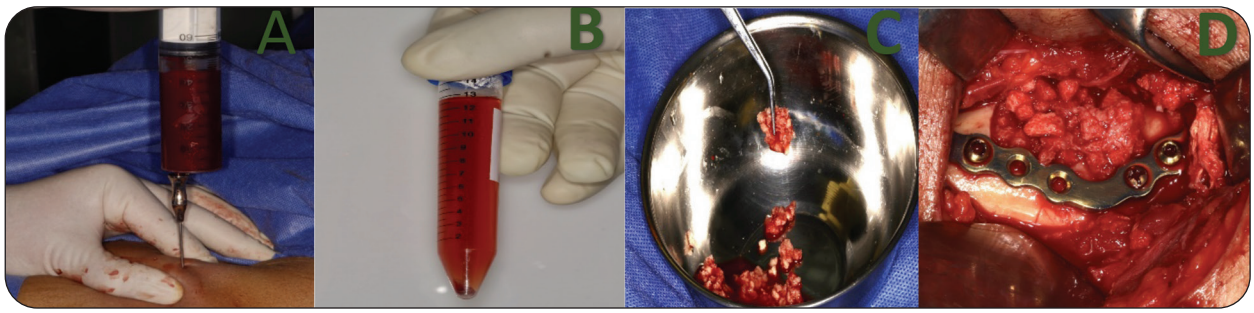

Fig. (1) Photographs illustrating Surgical Procedure, (A): collection of BMMNCs using a 13-gauge trocar from anterior iliac crest, (B): isolation of BMMNCs, (C) xenobone graft after mixing with BMMNCs, (D) placement of graft mixture at mandibular defect.

Postoperative clinical evaluation involved observation of wound examination for suture breakdown, dehiscence, swelling, infection, edema, hematoma, graft rejection, presence of oronasal fistula, nasal regurgitation, pain.

Radiographic evaluation:

Preoperative radiographic examinations had included CBCT scans that was carried out for each patient for determination of lesion volume on CBCT (axial sections) (Fig.2). The volume of defect remaining around newly formed bone was calculated and then subtracted from total measured lesion volume to have volume of newly formed bone at 0, 3- and 6-months post-operative. Analysis was done using a ProMax three-dimensional model CBCT (Planmeca, Finland, 2009).

The exposure parameters had included a field view of 90×100 mm, voxel size of 200 µm, X-ray tube kilovoltage of 88 kVp, and 8 mA. An oral and maxillofacial radiologist had evaluated and superimposed images using Romexis software package (version 3.4.4). Planmeca, Finland, 2009.

At first step, CBCT images of T0 and T1 were superimposed in common sites with least changes over time, such as skull base and orbit in coronal, axial, and sagittal planes.

After superimposition of midsagittal planes of two CBCTs by operator, the software automatically fitted them to make the best superimposition. Bone density was evaluated using Hounsfield unit (HU) in a qualitative analysis. In order to evaluate bone density of graft, common graft areas in both images of each patient were diagnosed as grafted bone was fully distinguished from adjacent bones, a circle was drawn by software in equal dimensions just in graft area for purpose of density measurement.

Manual segmentation of the bony defect using manual segmentation tool in romexis software version 5.3.4.39 Manual segmentation was made by tracing of bony defect layer by layer in sagittal cross section until the last layer.

Statistical analysis

Statistical analysis was performed with SPSS 20® (Statistical Package for Social Science, IBM, USA), Graph Pad Prism® (Graph Pad Technologies, USA) and Microsoft Excel 2016 (Microsoft Co-operation, USA). Qualitative data as age were evaluated for significance using Chi Square test. While for quantitative data, they were presented as means and standard deviation (SD) values. Defect site changes had been recorded at fixed time intervals (Preoperative, Immediate Postoperative, Three Months Postoperative and Six Months Postoperative). One-way analysis of variance (ANOVA) Followed by Tukey`s post hoc test was performed to evaluate effect of time on bone changes during this study for all groups. In addition, percentage of change were calculated fur further statistical analysis, according to the following formula. The significant level was set at $P \leq 0.05$.

$$\frac{(\text{Base Line}) - (\text{Postoperative})}{(\text{Base Line})} \text{ X } 100$$

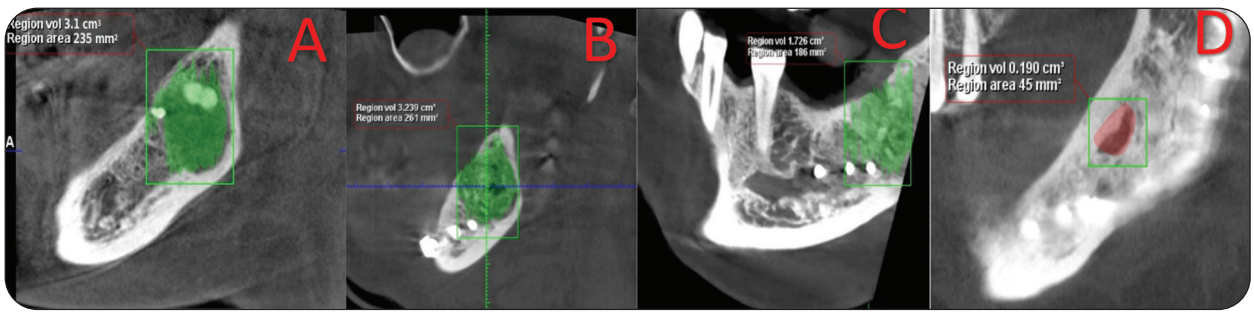

Fig. (2) Photo radiograph for determination of lesion volume on CBCT (axial sections), (A) Preoperative, (B) Immediate Postoperative, (C) 3-months Postoperative, (D) 6-months Postoperative

RESULTS

Gender, pain and clinical evaluation (suture break, dehiscence, swelling and edema, infection, and graft rejection) were insignificantly different among the studied groups.

Regarding the time effect on volumetric changes, the significance evaluation between time intervals revealed insignificant difference as P-value > 0.05 except for three and six months postoperative which revealed significant difference as P-value <0.05 with (-72.62%±22.2) change. The significance evaluation for volumetric changes for each time interval revealed significant difference as P-value <0.05 except for immediate postoperative which were insignificant different as P-value >0.05 (Table 1) (Fig. 3).

Table (1) *One Way ANOVA Analysis of Time Effect on Volumetric Changes*

	Preoperative	Immediate Postoperative	Three Months Postoperative	Six Months Postoperative	% change	P-value
Group (I)	1.68±0.98 [a]	1.99±6.10 [a]	0.91±0.54 [b]	0.46±0.23 [b]	-72.62%±22.2	**0.0004***

M; Mean, SD; Standard Deviation, CI; Confidence Interval, % change; Percentage of Change, P; Probability Level.
Means with same superscript letter in same row were insignificant different.
Means with different superscript letter in same row were significant different.
**; Significant Difference.*

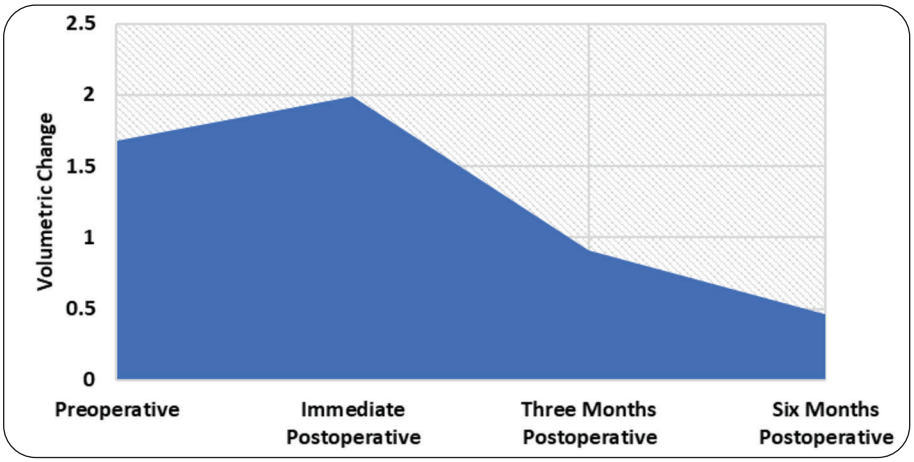

Fig. (3) Area Chart showing Time Effect on Volumetric Changes

Regarding bone density, the significance evaluation between time intervals revealed significant difference as P-value <0.05 with (458.8%±140.5) change. The significance evaluation for bone density each time interval revealed insignificant difference as P-value >0.05 except for bone density change percentage which revealed significant difference as P-value < 0.05.

DISCUSSION

Autologous bone grafting is regarded as the gold standard for treating critical-size bone defects. Although autogenous grafts are effective, they often require an additional surgical site to harvest the bone graft (with potential surgical complications) and the amount of autologous bone may be limited in quantity and quality. Although artificial bone may

be sufficient in quantity, it lacks viable cells and active growth factors and is incapable of inducing bone healing of critical size defects [7].

MSC based therapy is a relatively new method for bone regeneration and has gained more attention in recent years. MSC-based therapy possesses multiple advantages over autologous bone grafting. First, MSC-based therapy is less invasive as it does not require an additional open wound to harvest bone graft, and therefore, decreases the risk of potential complications. Second, since the cells and the scaffolds are both scalable in vitro, MSC-based therapy is less likely to be constrained by the size and quantity of implants to fill large bone defects. Third, once the process is optimized, MSC-based therapy may have the potential to regenerate bone even faster and more efficiently than autologous bone grafting [8].

Recent advances in the field of tissue engineering have increased the probabilities of promoting and accelerating the natural healing process. The use of osteoprogenitor cells, growth factors, and osteoconductive scaffolds have been shown to promote optimal bone healing in critical patients while minimizing the short comings of conventional treatments [9].

Bone marrow has two different types of stem cells: hematopoietic cells and MSCs [10]. The bone marrow concentrates were reported to successfully regenerate segmental mandibular defects up to 15 cm in length [11].

Our aim was to evaluate bone regeneration capacity of Xenogeneic bone graft and Stem Cells mixture at Osseous Jaw defects, 10 patients had Osseous Jaw defects equal or more than 3 cm^3 due to benign tumour or large cyst, were enrolled at this study, medically free without any systemic illness or under any medication, good general health that could interfere with normal bone healing.

There is no one standard definition of a critical-sized defect. Defects may be evaluated both in relative and absolute terms and vary whether considering animal models or humans. In general, a "critically-sized" defect is regarded as one that would not heal spontaneously despite surgical stabilization and requires further surgical intervention. The precise size or volume of bone that comprises a critical-size bone defect was not defined. General guidelines that had been suggested defect length greater than 2-2.5 cm and greater than 50% loss of circumference of bone [12].

Age range of the patients in our study was any age from 15-55 that were suitable for general anaesthesia. It was in agreement with Leskela HV et al. [13], who had stated that osteogenic potential of bone marrow MSCs did not decrease with aging, and thus can be used to accelerate bone healing if augmented with proper growth factors and osteoconductive scaffolds.

In the present study, participant baseline characteristics such as gender distribution did not affect outcome of study, as baseline characteristics were equally distributed between three groups and showed no statistically significant findings.

In terms of pain severity, xenograft and BMSCs, had reported a pain range from mild to moderate, with an average pain level categorized as moderate and a moderate level of variability, with a middle-of- road pain level.

When comparing the findings of this study to other research in the field, it's essential to consider that pain assessment results had showed no significant differences. This lack of significant difference contrasts with some other studies that had been reported varying levels of pain [14-19].

In some prior studies, inclusion of stem cells had been associated with a reduction in post-operative pain compared to control groups. However, these differences in pain levels had not always reached statistical significance [20, 21].

CBCT was done immediate post-operative, 3 month and 6 months later to evaluate volumetric

changes and bone density between study groups. Manual segmentation of bony defect using manual segmentation tool at romexis software version 5.3.4.39. Manual segmentation was made by tracing of bony defect layer by layer at sagittal cross section until the last layer. Sagittal view showing completed volume segmentation and its volume at mm3. Computed tomography and cone beam computed tomography were commonly utilized as diagnostic and planning aids due to their accuracy. They were used to determine bone density, locate essential structures, diagnose pathology, and plan any augmentation treatments that may be required [22].

In the present study, xenograft and stem cell had showed 72.62%±22.2 decrease at volumetric changes at 6 months, this result revealed significant difference as P-value < 0.05.

In the present study, xenograft and BMSCs had experienced substantial positive volumetric changes, representing a significant increase. These findings had demonstrated significant variations at volumetric changes, with statistical significance indicated by a P-value < 0.05.

In contrast to some previous studies that had reported varying degrees of volumetric changes following similar interventions, the present study had indicated that augmenting with both xenograft and BMSCs resulted in the most substantial positive volumetric changes. This finding had suggested that the combination of xenograft and stem cells had a more pronounced impact on volumetric changes compared to other treatment modalities.

Usually, after small cyst enucleation (>3cm), complete bone regeneration of the defect took place about 12 months. For larger defects, complete bone healing may take up to 24 months. However, bone healing could be delayed at patients with diabetes and systemic lupus erythematosus [23].

The bone defect was critical in size > 3 cm, had showed successful bone regeneration after only 6 months postoperatively. In a previous study [24], have been used by themselves without any fillers inside large maxillary cystic defects and shown to accelerate healing and bone regeneration, these results coincide with ours.

Gelatin scaffolds support osteoblast activities and permit cell proliferation and migration [25]. In the present study, the gelatin scaffold was modified by xenograft to induce osteogenic differentiation of seeded BMSCs.

In our study, Patients showed formation of bone at 3 months according to CBCT and almost 72.62% of lesion defect was filled with bone at 6 months, it was due to mesenchymal stem cells found in bone marrow so differentiating begins immediately after graft stabilization. It was in agreement with study done by Rickert et al. [26], as he had demonstrated significantly more bone formation in a mixture of bovine bone material with a concentrate (BMAC) of mononuclear cell (MNC) (17.7 7.3%) compared with control group with a mixture of natural bone mineral and autogenous bone (12.0 6.6%) after a healing time of 4 months. In another study in a sheep model, Gutwald et al. [27] could also show higher bone regeneration values when BMSCs was combined with PANCOLL-extracted MNCS (29%) compared to autogenous bone as a control group (16%) after 4 months.

In conclusion, clinical application of autologous bone marrow concentrates with xenograft), after enucleation of jaw pathologic lesions, had accelerated bone regeneration and improved density of regenerated bone. The relatively new autografting biotechnology used in present study was cost effective; requiring no additional graft materials, and safe; with minimal risks involved. Further studies with larger sample sizes were required to confirm these results and support the current evidences. The development of controlled delivery systems for prolonged exposure to these autografts may improve bone regeneration even further.

CONCLUSION

The use of autologous BMSCs seeded on gelatin scaffold combined with xenogeneic bone graft was a promising treatment option for regenerating bone at odontogenic large defects ≥ 3 cm^3. Applying BMSCs may favor formation of new bone. A long-term study is proposed to evaluate findings and explore full potential of suggested therapy for bone defect reconstruction.

Conflict of interest

Authors hereby declare no conflict of interest.

Funding of the study

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تقييم الكسب غير المشروع للعظام وخليط الخلايا الجذعية الوسيطة لنخاع العظم في تعزيز تكون العظم في عيوب عظام الفك (دراسة سريرية وشعاعية)

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الملخص :

الهدف: كان الهدف من هذا العمل هو تقييم قدرة تجديد العظام في الكسب غير المشروع للعظام وخليط الخلايا الجذعية الوسيطة لنخاع العظم (BMSCS) في عيوب الفك العظمية.

المواد والاساليب: أجريت هذه الدراسة على 10 مرضى تتراوح أعمارهم بين 15 إلى 50 سنة، والذين لديهم عيوب عظمية بسبب ورم حميد أو كيس كبير ≤3 سم3 عن طريق الفحص السريري والشعاعي. تم تطعيم جميع العيوب بمزيج من BMSCS المصنف في إسفنجة الكولاجين والكسب غير المشروع للعظام. تم إخضاع جميع المرضى لتصوير شعاعي بانورامي، والتصوير المقطعي المحوسب بالحزمة المخروطية (CBCT) لتقييم حجم عيب عظم الفك، وحجم العظام، وكثافة العظام

النتائج: كشف تقييم الأهمية للتغيرات الحجمية لكل فاصل زمني عن اختلاف كبير ($P<0.05$) باستثناء فترة ما بعد الجراحة المباشرة التي كانت مختلفة بشكل ضئيل. كشف تقييم الأهمية لكثافة العظام في كل فترة زمنية عن اختلاف غير مهم باستثناء نسبة تغير كثافة العظام التي كشفت عن اختلاف كبير ($P<0.05$).

الخلاصه: كان استخدام BMSCS الذاتي المصنف على سقالة الجيلاتين مع الكسب غير المشروع للعظام واعدًا لتجديد العظام في العيوب السنية الكبيرة ≤ 3 سم 3.

الكلمات المفتاحية: طعم العظام الحيوانية، خليط الخلايا الجذعية، تكون العظم، عيوب عظام الفك