Original Article

Efficacy of Spongy Xenogeneic Scaffold Loaded with Simvastatin in the Treatment of Severe Alveolar Horizontal Defect: A Clinical and Histological Study

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Received: 02-Feb-2022;
Revision: 15-May-2022;
Accepted: 12-Dec-2022;
Published: 15-May-2023

Abstract

Background: Bone tissue engineering offers several advantages for repairing skeletal defects. In this study, we designed and fabricated a scaffold for bone tissue engineering in patients with horizontal alveolar defect. **Aim:** The items included in the fabrication of the scaffold were xenogenic bone graft, gelatin as a substrate to improve the physical integrity of scaffold, and simvastatin to stimulate osteogenesis (10 mg per 1 g of xenograft). **Methods:** Fourteen patients with a horizontal defect in the alveolar ridge were enrolled in the study. Seven patients underwent routinely guided bone regeneration (GBR) using xenogenic bone graft plus collagenous membrane, and seven patients were treated with the scaffolds. After four months of follow-up after surgery, both the scaffold and GBR groups were examined for changes in the width of alveolar ridge and histologically for the quantity of newly produced bone. **Results:** The newly designed scaffold showed superior osteoconduction characteristics to routine GBR materials, which were used in this study. The difference in the quantity of the newly produced bone between the scaffold group and GBR group was significant and higher for the scaffold group. Regarding newly produced bone percentage, the scaffold group showed a mean of 20.93 and the GBR group presented a mean of 13.25% (P = 0.004). Also, the mean value for the duration of surgery for GBR was 45 minutes and for scaffold was 22 minutes, which was significantly lower in the scaffold group (P < 0.001). **Conclusions:** The newly designed scaffold is a suitable treatment modality for bone tissue engineering.

Keywords: *Horizontal alveolar defect, simvastatin, tissue engineering*

Introduction

Ridge resorption caused by tooth loss mostly compromises proper implant placement. Therefore, in such situations, to provide adequate ridge, width and height bone augmentation is suggested. In general, guided bone regeneration (GBR), ridge splitting, distraction osteogenesis, or block bone graft is used and is associated with promising outcomes.[1-3] Autogenous block graft is used mostly as the gold standard to reconstruct the severe resorbed alveolar ridge.[4] Nonetheless, its disadvantages are increased surgical cost and time and restrictions in the quantity of obtainable bone. Also, the morbidity of donor site can cause changes in appearance, impaired wound healing, temporary loss of function, pain, and iatrogenic injury.[5] GBR is another reliable option to reconstruct an atrophic ridge. However, the major drawback of GBR is the poor mechanical properties of particulate bone grafting material and collagen membrane against structural collapse.[2,6] Compressive forces result in the displacement of some parts of the grafting materials

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_86_22

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How to cite this article: Esmaeili V, Boostani H, Ahmadpour F. Efficacy of spongy xenogeneic scaffold loaded with simvastatin in the treatment of severe alveolar horizontal defect: A clinical and histological study. Niger J Clin Pract 2023;26:369-75.

and membrane collapse, leading to compromising regeneration outcomes, which can occur while suturing the soft-tissue flap or throughout the healing phase of GBR.[6] Also, xenograft or allograft material alone is not favorable for inducing alveolar bone generation compared with an empty control at the histological level.[7] More fibrous tissue, less bone volume, and foreign body reaction have been reported as important microscopic results around allograft or xenograft materials than an empty control; however, the clinical gross examination indicates dense new hard tissue generation.[7]

This study reported a novel method for the application of bone tissue engineering at the recipient site. Regenerative medicine besides tissue engineering is used to overcome the abovementioned obstacles in bone regeneration. Tissue scaffolds and healing-promoting factors are major categories in tissue engineering and regenerative medicine.[8] For increasing osteoconduction and osteoinduction, a favorable bioactive scaffold is needed to be bioefficient, biocompatible, and biodegradable.[9] To fabricate the scaffold in this study with these characteristics, we used gelatin and particulate deproteinized bovine bone mineral. Gelatin as a hydrolyzed type of collagen causes collagen formation during bone healing. Gelatin possesses tissue conductive effects and can increase tissue regeneration.[10] Particulate deproteinized bovine bone mineral is also one of the best documented and most widely used bone substitutes to augment peri-implant alveolar bone defect.[6] As healing-promoting factors, we chose simvastatin, which is a synthetic statin having many activities, such as bone resorption prevention and anabolic activity on bone. These effects of simvastatin result from increased Bone morphogenetic protein 2 (BMP2) inhibition of osteoclasts through the Transforming growth factor beta (TGF-beta) and Mothers against decapentaplegic homolog 3 (Smad3) pathway and promoted osteoblastic differentiation by the elevated expression of vascular endothelial growth factor in osteoblasts.[8,11]

So, in this study we designed a flexible three-dimensional xenogeneic scaffold to avoid the grafting material collapse during and after surgery, decrease the duration of surgery, and provide an optimal 3D scaffold for osteogenesis with the benefit of delivering drug locally.

Methods

Patient enrollment protocol

The present research was performed from November 2019 to April 2021 in the Department of Oral Implantology of Dentistry Faculty. Conscious consent was obtained from all patients. Fourteen patients (seven patients who had undergone routine GBR and seven patients treated with the scaffolds) were included. The inclusion criterion was patients with a horizontal defect in the alveolar ridge area of less than 4 mm thick, who were a candidate for dental implant placement. However, the exclusion criteria were as follows:

- Patients with systemic disease that impaired the tissue repair process (connective tissue diseases, kidney failure, liver failure, diabetes, etc.)
- Heavy smokers
- Patients on anti-osteoporosis drugs
- Pregnancy
- Patients with psychological problems.

Fabrication of simvastatin-loaded scaffolds

We used the mineralized, bovine cancellous bone particulates (Particle size: 150-1000 micrometers; Cancellous bone, NovaTeb Inc., Rsasht, IR) for augmentation. The graft material underwent solvent preservation instead of sintering for more perseverance of the trabecular pattern and osteoconductive features of the bone.[12]

100 mg of simvastatin ethanol (Sigma-Aldrich, USA (was dissolved in ethanol (2.5 ml). After that, the solution was used using a dropper to the bone particulates; thus, each gram of xenograft contained 10 mg of simvastatin; then, ethanol was evaporated completely. The whole procedure was performed in a laminar flow hood to make sure of complete sterile conditions. 9.53% (95.3 mg/mL) gelatin solution (type B; bovine skin type; Sigma-Aldrich) was obtained by dissolving the gelatin powder in deionized water at a temperature of 40°C via stirring at 100 RPM. Then, 1 mL of the obtained gelatin solution was mixed with 0.5 ml of prepared xenograft while stirring continued at 100 RPM. In the first step, the mixture was poured into the custom-made rectangular dish (10 mm*10 mm*5 mm) up to 2 mm height of the dish. In the next step, the remaining 3 mm of dish was filled with gelatin solution to form a gelatin sponge, which acts as a barrier against non-osteoblastic cells. Then, the composite suspension was refrigerated (4 C) for one night to form a gel. Then, freeing of gel composites was carried out slowly at -15 overnight, -20 C, and -70 C for 4 h. The composites of the frozen gel were lyophilized for 24 h for obtaining the porous sponges.[8] The cross-linking of the side of scaffold facing the elevated periosteum was conducted through exposure to UV light by UVP UV Crosslinker (CL-1000 Series, Ultra-Violet Products Ltd., UK;[13] UV irradiation with k = 254 nm; t = 120 min at an intensity of 0.96 J/cm2). Sterilizing the bioimplants was carried out with Cobalt-60 gamma irradiation (25 kGy)[14] [Figure 1].

Graft site preparation

Conservative surgical incisions were considered for minimizing vascular network disruption of the soft tissue and preserving adjacent soft-tissue papillae. A sulcus flap with no vertical releasing incisions was employed. We made a wide subperiosteal reflection for exposing two to three times the treatment region, and the papilla was reflected on the edentulous site mesial side.

Seven patients received the newly designed scaffold, which was fixed by a resorbable suture to the periosteum. Also, other seven patients were treated with the routine GBR technique (xenogenic bone particles and collagen membrane).

Tension-free closure around the grafted site was achieved by releasing incisions on the periosteum, and then, the soft tissue was released and advanced. Closing of the wounds was carried out using the horizontal mattress and interrupted 4-0 resorbable sutures (Vicryl Rapide, Supa, Iran).

The surgeries were performed by one surgeon. The patient visited weekly in the first month and then monthly to record the soft-tissue status of the area. The patients received oral rinsing with 0.12% chlorhexidine gluconate (Sina Darou, Iran) immediately before surgery and two times a day for seven days after surgery. Penicillin 500 mg or clindamycin 300 mg was postoperative medication.

Radiographic examination

All subjects were subjected to cone-beam computed tomography (CBCT) scanning before surgery (Planmeca ProMax 3D, Planmeca Oy). The following technique parameters were used: Field of View height, 5.6 cm; FOV diameter, 10 cm; beam currency, 8.0 mA; acceleration voltage, 90 KV; and voxel size, 0.2 mm.

Histological examination

During implant placement, the bone required for the histological examination features was harvested with the help of a fine osteotome or a fine trephine. All surgeries are performed by the same surgeon. Then, specimens were fixed using 10% paraformaldehyde, decalcified using formate sodium, and embedded using paraffin.

From each specimen, three pieces of histological sections were chosen at random. After H and E staining, light microscopy (magnification x40, Olympus, Tokyo, Japan) was used to observe each section. Through the image analytical software (Image-Pro Plus 6.0; Media Cybernetics Inc., USA), the quantity of newly produced bone was introduced as the percentage of the newly produced bone area in the original drill defect area.

Statistical analysis

Newly formed bone areas and duration of surgery were assessed through a one-way analysis of variance (ANOVA). Data analysis was carried out by SPSS 15. A $P < 0.05$ was regarded as significant.

Results

Fourteen patients participated in this study: Seven patients involved in the control group (three females and four males with a mean age of 43.5 years) and seven patients involved in the test group (three females

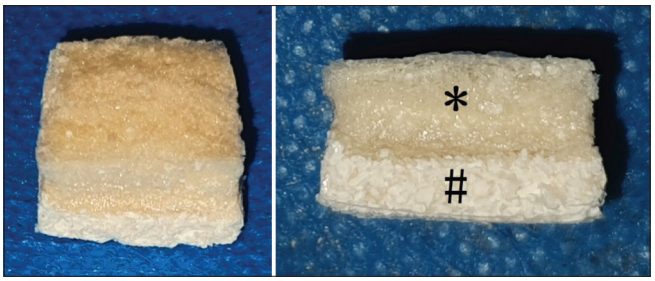

Figure 1: Hybrid spongy block loaded with 10 mg/1g simvastatin. *UV cross-linked compressible gelatin sponge acting like a barrier against migration of non-osteoblastic cells (3 mm). #xenograft particulate graft stabilized with gelatin matrix (2 mm)

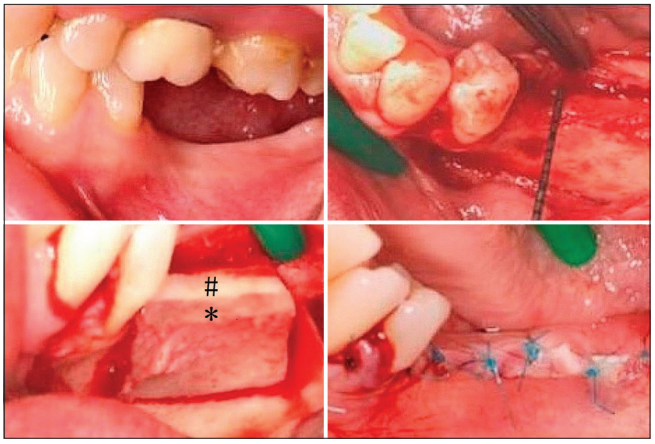

Figure 2: Alveolar ridge reconstruction surgery procedure with the newly designed 3D scaffold. The prefabricated scaffold helped to reduce surgery duration significantly. *The gelatinous sponge side faced the soft tissue to act as a barrier

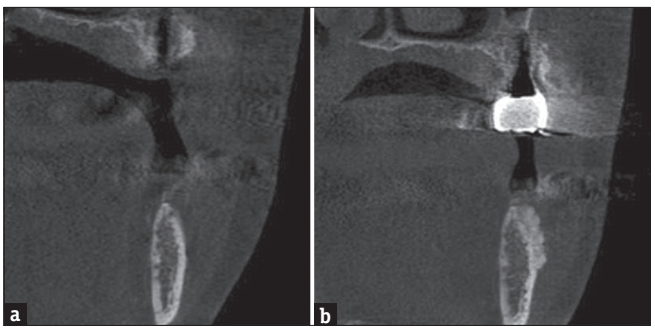

Figure 3: (a) Preoperative. (b) 4 months postoperative radiography alveolar ridge reconstruction with the newly designed 3D scaffold

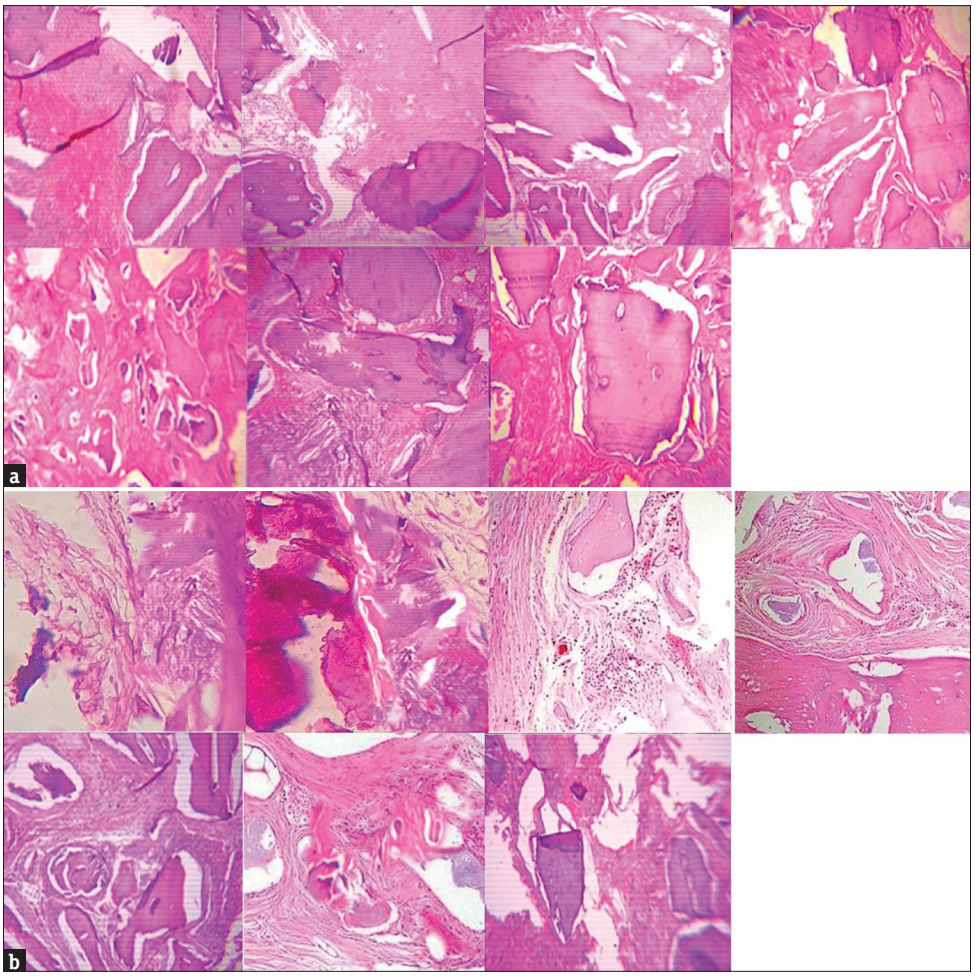

Figure 4: Histological view of hematoxylin and eosin staining of samples collected from patients 4 months after reconstruction of alveolar defect during implant surgery. Xenogenic particulate grafts represented in purple color in histological view. (a) Scaffold group. (b) GBR group

Table 1: Patient data and follow-up duration

Patient number	Intervention	Gender	Age	Follow-up duration (days)
1	Scaffold	M	35	125
2	Scaffold	M	48	117
3	Scaffold	M	55	134
4	Scaffold	M	39	140
5	Scaffold	F	44	120
6	Scaffold	F	45	124
7	Scaffold	F	56	137
8	GBR	M	37	120
9	GBR	M	47	123
10	GBR	M	60	140
11	GBR	M	33	127
12	GBR	F	40	115
13	GBR	F	50	116
14	GBR	F	38	110
	Mean	SD	Mean	SD
GBR	43.57	9.32	121.57	9.84
	Mean	SD	Mean	SD
Scaffold	46	7.74	128.14	8.85

and four males with a mean age of 46 years) [Table 1]. All subjects were found with uneventful healing; no symptoms of soft-tissue dehiscence, infection, or secondary healing were found during a 4-month follow-up [Figure 2]. The grafting material showed healing and fusing with the native alveolar bone according to the clinical examination during implant surgery and periapical radiography 4 months post-surgery [Figure 3].

Table 2 represents the mean value of the width of the ridge, measured clinically after retracting the flap during the surgery, immediately after reconstruction, and about 4 months after surgery in the patients who were treated with scaffold. The mean value for ridge of "scaffold group" measured clinically at the uppermost part of the ridge before reconstruction was 3.04 mm (SD = 0.22). An increase in width was found for the ridge of "scaffold group" with mean values of 7.94 mm (SD = 0.21) immediately after surgery and 6.02 mm (SD = 0.53)

Table 2: Width of the ridge before and after reconstruction measured clinically (in millimeters)

Patient number	Intervention	Width of ridge (preoperative)	Width of ridge immediately after surgery	Width of ridge after 4 months	Percentage of change in width of ridge
1	Scaffold	2.7	8	6.1	23.75%
2	Scaffold	3.1	7.9	6.8	13.92%
3	Scaffold	3	8.1	6.1	24.69%
4	Scaffold	3.3	7.7	5.5	28.57%
5	Scaffold	2.8	7.7	5.4	29.87%
6	Scaffold	3.2	7.9	5.7	27.84%
7	Scaffold	3.2	8.3	6.6	20.48%
		Mean SD	Mean SD	Mean SD	Mean SD
		3.04 0.22	7.94 0.21	6.02 0.53	24.16% 5.54

Table 3: Percentage of newly formed vital bone

Patient number	Intervention	Vital bone	
1	Scaffold	15,54%	
2	Scaffold	21,60%	
3	Scaffold	25,60%	
4	Scaffold	19,39%	
5	Scaffold	20,30%	
6	Scaffold	26,22%	
7	Scaffold	17,90%	
8	GBR	17,20%	
9	GBR	15,40%	
10	GBR	8,20%	
11	GBR	17,73%	
12	GBR	8,17%	
13	GBR	10,81%	
14	GBR	15,28%	
	n	Mean	SD
GBR	7	13.25%	4.11
Scaffold	7	20.93%	3.89
Significance level	*P*=0.004		

Table 4: Duration of surgery

Patient number	Intervention	Duration of surgery (min)	
1	Scaffold	20	
2	Scaffold	25	
3	Scaffold	17	
4	Scaffold	21	
5	Scaffold	20	
6	Scaffold	30	
7	Scaffold	25	
8	GBR	45	
9	GBR	47	
10	GBR	43	
11	GBR	53	
12	GBR	49	
13	GBR	39	
14	GBR	42	
	n	Mean	SD
GBR	7	45 min	4.68
Scaffold	7	22 min	4.35
Significance level	*P*<0.001		

months after surgery during implant placement. The data show that after about 4 months there was about 24.16% (SD = 5.54) decrease in the graft volume. These results show that this procedure ended up achieving the appropriate ridge width (≥5 mm) for the placement of implant.

Histological analysis

Regarding histological characteristics, all subjects exhibited the presence of residual graft particles, new bone produced, and connective tissue in lesser or greater quantities. The newly produced bone with direct contact with residual particles of each bone substitute material showed the proper osteoconductive capacity. The percentage of new bone produced for each analyzed sample is shown in Table 3.

Regarding newly produced bone percentage, the scaffold group showed a mean of 20.93% (SD = 3.89) and the GBR group presented a mean of 13.25% (SD = 4.11), which is confirmed by previous studies. There was a significant difference in the newly produced bone percentage between the scaffold and GBR groups with a higher value for the scaffold group, which represents better osteoconductive quality (P = 0.004). Figure 4 shows the histological view of grafted ridge with scaffold and GBR.

Duration of surgery

According to previous studies, reducing the duration of surgery has a significant effect on reducing pain and swelling after surgery.[15] Therefore, in this study, we decided to evaluate the effect of using the prefabricated scaffold over the duration of surgery. After injecting the local anesthesia and ensuring complete anesthesia, surgery was performed according to the mentioned steps. The duration of the surgery was measured accurately by an observer using a chronometer. The start of the measurements was considered when the surgeon started to make incisions. Measurements are performed in both control and intervention groups, and finishing

the suturing procedure was considered the time of completion of surgery.

The mean value for the duration of surgery for GBR was 45 minutes and for scaffold was 22 minutes, which was significantly lower than the scaffold group ($P < 0.001$) [Table 4].

Discussion

To better maintain the space and let the bone repopulate the graft and recreate a bone volume similar to the pre-extraction size, we designed a 3D scaffold containing bovine particulate graft embedded in a gelatin matrix, which allowed primary stability of the graft and local delivery of simvastatin.

Here are the reasons to fabricate such a scaffold:

1. Deproteinized bovine bone graft material has been widely used clinically for repairing osseous defects. Many clinical studies on humans have been conducted, and long-term data on the outcome of bone grafting procedures have been declared. For example, Lei *et al*. assessed 20 patients treated with xenogenic bone graft for four years and reported that an organic bovine bone is osteoconductive and can promote the successful long-term outcome of bone grafting.[12]
2. Gelatin sponge offers a proper environment for the migration and proliferation of preosteoblasts. Hemostatic gelatin sponge is associated with several advantages to be used in tissue engineering because it is easy to obtain, cost-effective, biocompatible, biodegradable, and does not induce allergic responses or other undesirable side effects. The gelatin sponge caused no inflammatory reaction during degradation while using scaffold for chondrocyte growth and cartilage tissue engineering in a rabbit model.[10] We also chose UV to cross-link the scaffold. Davidenko *et al*. showed that UV could be utilized for cross-linking with no effect on integrin a2b1-mediated cell attachment, proliferation spreading, or coverage.[13] The freeze-drying technique helped the fabrication of 3D scaffolds with an interconnected inner architecture and a porosity of nearly 99%, a value that falls within the favorable range efficient for cell infiltration.[16,17]
3. Simvastatin was utilized for osteopromotive and anti-inflammatory purposes. Simvastatin accelerated bone regeneration and soft-tissue healing by an increase in osteoblastic differentiation and stimulation of neovascularization through its effect on endothelial growth factor and bone morphogenetic proteins.[18,19] Simvastatin also showed an antibacterial effect with minimal inhibitory concentration (MIC) that ranged from 0.062 to 0.25 mg mL−1 against Methicillin-resistant Staphylococcus aureus (MRSA), which is a critical factor in successful bone regeneration.[20] Simvastatin triggers angiogenesis and angio-maturation and could develop the generation of large blood vessels surrounding the ectopic bone, which can be due to its role in enhancing the expression of BMP-2 and VEGF.[11,21] It can promote the absorption of the regional and inflammatory mesenchymal cells to scaffold architecture. The osteoinductive effects of simvastatin led to the differentiation of the mesenchymal cells directly to the mature osteoblasts and chondrocytes, resulting in periosteal bud generation soon following surgery. Also, osteoclast suppression due to simvastatin application can be the consequence of RANKL depression.[22]

Conclusion

According to the histological observations, the newly designed scaffold offered superior osteoconduction leading to a higher amount of vital bone apposition in direct contact with grafting materials. Microscopic investigation of histological samples showed a significant difference in the amount of new bone formation between the GBR group and the scaffold group. The scaffold showed a markedly higher quantity of new bone compared with the GBR group, indicating that the gelatin scaffold containing simvastatin could promote osteoblast differentiation and stimulation and osteoclast suppression.[22] Therefore, this scaffold can be considered an appropriate option for bone augmentation and reconstruction due to its better osteoconduction.

The materials also are readily available, relatively inexpensive, do not transfer pathologic conditions, and are highly biocompatible. Also, in this study we showed that by using these prefabricated scaffolds the duration of surgery was significantly decreased, which reduced chair time significantly. Reducing the duration of surgery can reduce postoperative pain and swelling, increase patient comfort and satisfaction, and make the procedure as possible as minimally invasive. Also, the amount of width gained in the alveolar ridge after reconstruction surgery was adequate for implant placement in all patients, and due to the spongy form of these scaffolds, they could easily adapt the shape of the alveolar ridge while maintaining their integrity. In conclusion, these qualities help these scaffolds to be more user-friendly and decrease the complexity of reconstructive surgery.

In this study, we described the osteoconductive potential of a gelatin and xenogenic bone scaffold loaded with simvastatin in bone regeneration. The results showed the favorable biocompatibility, biodegradability, and

superior osteoconduction of this scaffold, which make it a considerable choice over other routine surgical options for alveolar horizontal ridge augmentation. However, further studies are recommended to investigate the efficiency of this scaffold in other reconstructive surgeries, for example, sinus lift, socket preservation, and vertical alveolar ridge defects.

Ethical approval

This study has the approval of the ethics committee with reference number IR.AJAUMS.REC.1399.038.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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