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Journal of
Biomaterials and Tissue Engineering
Vol. 12, 1–7, 2022

Comparison of the Efficacy of HYAFF11 Mesh with Collagen Membranes in Guided Bone Regeneration

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This study aimed to evaluate the efficacy of HYAFF11-based meshes in guided bone regeneration (GBR). Two defects of 5-mm width were prepared in the calvaria of 36 rats. The rats were divided into the following groups: empty defect (group E); HYAFF11-based mesh (group HY), collagen membrane (group CM), xenograft (group X), xenograft + HYAFF11 mesh (group X + HY), and xenograft + collagen membrane (group X + CM). Sacrifications were performed at the third and sixth post-surgical weeks, followed by histological evaluation. The highest amount of new bone (NB) formation in the third week was observed in group X + CM (0.94 ± 0.40 mm^2), followed by group X + HY (0.80 ± 0.21 mm^2). The highest amount of NB formation in the sixth week was observed in group X + CM (1.57 ± 0.66 mm^2), followed by groups X and X + HY (1.39 ± 0.48 mm^2 and 1.34 ± 0.44 mm^2, respectively). There was no statistically significant difference in the amount of residual graft between groups X + HY, X + CM, and X at the third and sixth weeks. Considering the close values observed between the groups, it could be concluded that HYAFF11 may be an alternative biomaterial for use in GBR. Further studies using different graft materials and defect sizes are required to evaluate the effectiveness of HYAFF11 in GBR.

Keywords:

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1. INTRODUCTION

Guided bone regeneration (GBR) is a technique developed in the late 70's to treat local defects in jawbones. The GBR technique promotes the proliferation of osteoblasts, the new bone-forming cells, in the defect area by applying a selective barrier membrane between the bone defect and the periost, while preventing the rapid proliferation and migration of the epithelium to the defect area.[1,2] Different types of membranes have been produced over the years, as they are one of the main biomaterials used in GBR.[3] The biomaterials used in GBR must be biocompatible, clinically applicable, space-maintaining with sufficient mechanical strength, and have an ideal pore width that allows for supply of the defect area while also preventing the migration of epithelium.[4] Nonresorbable polytetrafluoroethylene (PTFE) and its expanded form, e-PTFE, are first-generation membranes. These membranes are biocompatible materials with a high level of space-maintaining capability. However, they have some drawbacks, such as the frequent occurrence of complications, including openings in soft tissue during the recovery period and requiring an additional surgical operation to remove the membrane following the recovery period. This led to the development of resorbable second-generation membranes. Collagen membranes are frequently used in GBR as naturally derived, resorbable membranes. Collagen membranes are easy to apply and promote platelet aggregation, fibroblast attraction, and wound healing; however, they have their own drawbacks.[5,6] They are not rigid and have a resorption period shorter than the time required for bone regeneration. Graft materials with different attributes, such as autogenous, allogenic, alloplastic, and xenograft materials, are also used with these membranes. Deproteinized bovine bone-derived xenografts are frequently used for bone augmentation. Xenografts are natural biomaterials with physicochemical properties similar to those of human bone. They are osteoconductive, that is, they act as scaffolds for the growth of osteoblasts. They have a slow resorption time, which allows them to support the membranes with which they are applied.[7–9]

Recently, degradable materials based on hyaluronic acid (HA, also called hyaluronate or hyaluronan) have been used in dental practice.[10,11] HA, discovered in 1934, is a naturally occurring glycosaminoglycan consisting of N-acetyl-D-glucosamine and D-glucuronic acid. It is found in many types of bodily fluids, organs, and tissues (such as

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synovial fluid, saliva, serum, gingival crevicular fluid, periodontal tissues, eye, cartilage, brain, muscles, lung, kidney, and brain).[12–16] HA is reported to have bacteriostatic, anti-inflammatory, fungistatic, antiedematous, osteoinductive, and viscoelastic properties.[12–16] The pure form of HA has disadvantages such as rapid dissolution in water and fast resorption time. Therefore, semi-synthetic polymers have been developed by modifying the physicochemical structure of HA. HA-based biomaterials are especially used in regenerative procedures because HA connects to proteins that play a role in wound healing, namely fibrin, fibrinogen, collagen, and fibronectin, and it has an effect on bone morphogenesis and osteogenesis during the early wound healing period.[17,18] Cross-linking and coupling reactions are two methods used to obtain modified forms of HA. Through coupling reactions, specific functional groups of polysaccharides (e.g., hydroxyl and carboxyl) are changed by chemical reactions such as esterification, sulfatation, and amidation. With the stabilization of the polymer by esterifying the free carboxyl group of glucuronic acid, which is frequently repeated along the HA chain, HYAFF is created.[19,20] This chemical modification decreases the hydrophilic properties and increases the hydrophobic properties. The biodegradation of HYAFF has been studied both *in vitro* and *in vivo.*[19] Oligosaccharides, which are degradation products of HYAFF, have been reported to be involved in many biological events such as angiogenesis, morphogenesis, maturation of mesenchymal progenitor cell differentiation, and migration.[19] HYAFF11 fibers were constructed through complete benzyl-esterification of the carboxyl groups of the D-glucuronic acid unit of HA. HYAFF11 has been widely used in both clinical products and experimental studies in a variety of forms such as nonwoven fleeces, membranes, sponges, cylinders, and tubes. A knitted, resorbable, suturable, biocompatible mesh composed of HYAFF11 fibers was recently developed specifically for periost regeneration in orthopedic surgeries (Hyalonect, Fidia Advanced Biopolymers SRL, Albano Terme, Italy). Circular weft-knitted fibers composed of microfilaments of 15-mm diameter were used to create a 150-mm thick suturable elastic mesh with approximately 500-mm void spaces between the loops.[19]

This experimental study aimed to evaluate and compare the effectiveness of mesh produced from HYAFF11 fibers in bone regeneration when used alone or in combination with xenografts to that of collagen membrane with and without xenografts, which is frequently used in clinical practice. The null hypothesis was that HYAFF11-based meshes have no effect on early and late period bone healing.

2. EXPERIMENTAL DETAILS

2.1. Study Design

The study protocol was approved by the Istanbul University Animal Experimentation Local Ethics Committee (approval no. 2021/02). All procedures were conducted in accordance with international ethical guidelines for the treatment and welfare of experimental animals.

Male Sprague Dawley rats aged 8–12 weeks and weighing 250 ± 20 g were used in this study. The animals were kept in a room with 55% relative humidity at 21 ± 1 °C, automated light for 12 h (lights were turned on daily at 07:00 h), silence, ventilation, and heating. The experimental animals were fed standard feed and tap water ad libitum. Each animal was kept under observation in an individual cage. The six experimental groups in this study were as follows:

Group 1: Empty defect (group E)

Group 2: Empty defects covered with collagen membrane (group CM) (Bio-Gide, Geistlich Pharma, Wolhusen, Switzerland)

Group 3: Empty defects covered with HYAFF11-based mesh (group HY) (Hyalonect, Fidia Advanced Biopolymers SRL, Albano Terme, Italy)

Group 4: Defects filled with xenografts (group X) (Bio-Oss, Geistlich Pharma, Wolhusen, Switzerland)

Group 5: Xenograft-applied defects covered with collagen membrane (group X + CM)

Group 6: Xenograft-applied defects covered with HYAFF11-based mesh (group X + HY)

2.2. Sample Size Calculation

G*Power (v3.1.7) software was used to perform power analysis to determine the number of samples. The power of a study is stated as 1-β (β = Type II error probability), and studies are generally required to have an 80% power. According to a study by Yılmaz et al., the clinical difference in new bone formation measurements between the groups was found to be an average of at least 6.33 units, with an effect size of $d = 2.191$.[21] The sample size was calculated to be five per group in order to achieve 80% power with a level of $\alpha = 0.05$. Six samples were planned for each group to compensate for possible losses. A total of 72 defects in 36 rats were included in this study; 18 experimental animals (36 defects) were sacrificed in the third and sixth postoperative weeks.

2.3. Surgical Procedures

The experimental animals were intraperitoneally administered 50 mg/kg ketamine HCl (Ketalar®, Pfizer, Turkey) and 15 mg/kg xylazine hydrochloride (Rompun® Flakon, Bayer Turk Kimya Sanayi Ltd., Sti, Turkey) to achieve anesthesia. The hairs on the vertex of the rats' heads were shaved and wiped with povidone-iodine solution (Poviseptin, Mertsel Tic. AS., Turkey) to achieve asepsis. In the center line, on top of the vertex, a 2.5-cm anteroposterior skin incision was made from the nasofrontal area to the external occipital protuberance. The cranial bone was exposed by lifting the skin and periosteum. Two full-thickness bone defects were carefully prepared on the

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right and left sides of the center line of each parietal bone to avoid damaging the dura mater. The defects were prepared using a trephine bur (Salvin Dental Specialties, Inc., Charlotte, NC, USA) with a 5-mm inner diameter mounted on a surgical drill connected to a physiodispenser at 500 rpm with constant saline irrigation. The same type of experimental material was applied to both defects after the surgical area was cleaned with saline solution. The periost and scalp were sutured with 4–0 resorbable sutures (Vicryl, Ethicon, Johnson & Johnson, NJ, USA), and primary wound closure was achieved. The sutured area was covered with air-permeable surgical plaster (Nexcare First Aid Tape, 3M, USA).

2.4. Post-Operative Care

After surgery, the rats were intraperitoneally administered 3 mg/kg gentamicin (20 mg; Kocak Farma Ilaç ve Kimya Sanayi A.S., Turkey) twice daily for 3 days postoperatively as prophylaxis against infection. The animals were also intraperitoneally administered 5 mg/kg tramadol HCl (Tramadolor 100 mg; Sandoz İlaç San. Tic. A.S., Istanbul, Turkey) twice daily for 3 days postoperatively as a postoperative analgesic. The wound sites of the animals were examined during the administration of analgesics, and the wound dressings were replaced twice a day for the first postoperative week, if necessary.

2.5. Sacrification

Experimental animals were sacrificed with 120 mg/kg sodium pentotalin in the third and sixth weeks after surgery. Following sacrifice, the bone tissues containing the defects were dissected, removed, and transferred to the laboratory for histologic and histomorphometric evaluation.

2.6. Histologic and Histomorphometric Analysis

Histologic and histomorphometric evaluations were conducted by a single-blinded pathologist (MST). All specimens were fixed in 10% buffered formalin for one week. The samples were then decalcified in a 10% formic acid and 10% sodium citrate solution for a month. Paraffin blocks prepared from routinely processed decalcified specimens were cut into 4-μm slices. The sections were evaluated using a light microscope (BX60; Olympus, Tokyo, Japan) attached to a color video camera and connected to a computer. The defect sides were captured using a camera (Olympus E-330; Olympus Corporation, Tokyo, Japan) and displayed on a computer monitor. All measurements were performed using the Olympus Image Analysis System 5 (Olympus Corporation). The slides were evaluated at $\times$40 magnification for new bone formation and the remaining grafts, which were measured in one microscopic field. The proportions of the area occupied by the newly formed bone and the remaining grafts were measured and compared with the total area. In addition, infection, necrosis, and foreign body reactions were evaluated as '$-$' if absent and '$+$' if present.

2.7. Statistical Analysis

The NCSS (Number Cruncher Statistical System) software was used for statistical analyses. To evaluate the results of this study, descriptive statistics (average, standard deviation, median, first quarter, third quarter, frequency, percentage, minimum, and maximum) were used. The consistency of the means of quantitative data with normal distribution was measured using the Shapiro–Wilk test and graphical examinations. Kruskal–Wallis and Dunn–Bonferroni tests were used to compare quantitative variables with abnormal distribution between groups. The Wilcoxon signed-rank test was used to compare quantitative variables with abnormal distribution within groups. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

No complications or dropouts were observed during any stage of the study. Histologic and histomorphometric assessments of 72 defects in 36 rats were performed. No infection, foreign body reaction, inflammation, or necrosis was observed in the groups. Representative images of histological samples in the third and sixth weeks are shown in Figures 1 and 2.

Starting from the defects in the third and sixth weeks, the defect area in the control group was filled with fibrous tissue and small new bone formation. Various amounts of graft particles were observed in the defects of the X, X + CM, and X + HM groups in both the third and sixth weeks. While many residual graft particles of xenografts were observed in the third week, there was a relative decrease in the amount by the sixth week. Areas of significant new bone formation were observed among the graft materials in the sixth week compared to the third week, whereas collagen fibers were evident in the connective tissue surrounding the graft particles in the X, X + CM, and X + HM groups in the third week. In groups CM and HY, bone regeneration was observed on the side sections of the defect areas, and loose connective tissue was observed in the defect areas in the third week, similar to a control group. In the sixth week, fibrosis was partly observed, especially in the central section of the defect, whereas new bone formation was more distinctly observed.

The amount of new bone formation measured in the groups at the third and sixth weeks is shown in Table I. The lowest amount of new bone formation in the third and sixth weeks was observed in group E. The highest amount of new bone formation in the third week was observed in group X + CM (0.94 ± 0.40 mm^2), followed by group X + HY (0.80 ± 0.21 mm^2). However, there was no statistically significant difference between the two groups ($p = 0.805$). Even though a higher amount of new bone formation area was observed in group CM (0.46 ± 0.34 mm^2),

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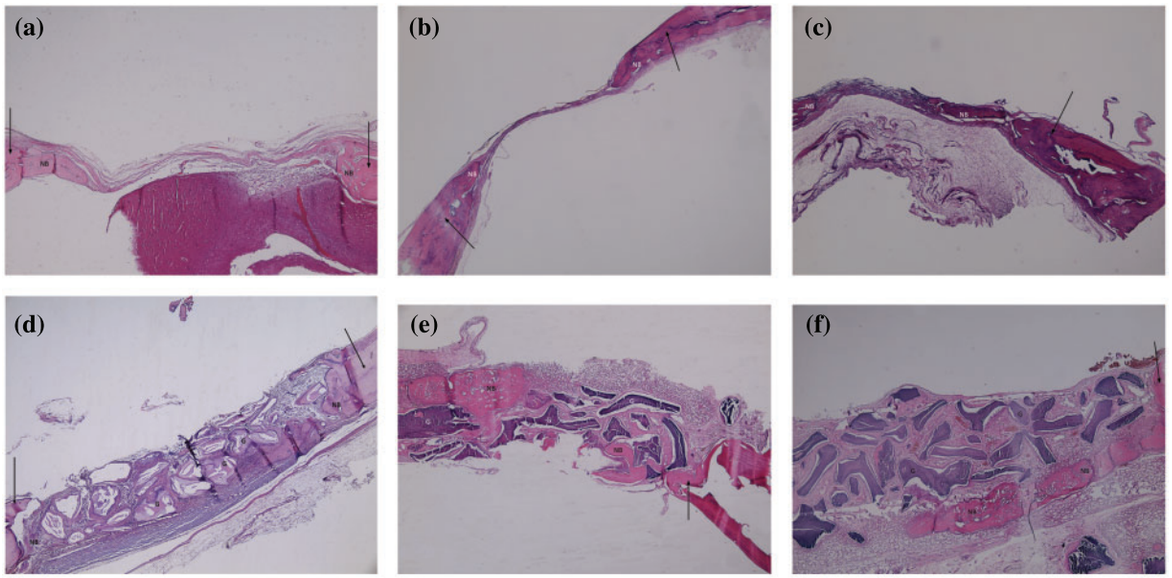


Fig. 1. Histological views of the groups in the third week (H&E ×40) (a) Group E, new bone (NB) formation is seen starting from the defect edges (arrows); (b) Group CM, NB formation is seen between the defect edges (arrows); (c) Group HY, NB formation is seen near the defect edges (arrows); (d) Group X, NB formation and graft particles (GP) are seen between the defect edges (arrows); (e) Group X + CM, mixed NB formation and GP are seen near the defect edge (arrow); (f) Group X + HY, NB formation and GP are seen forming from the defect edge (arrows).

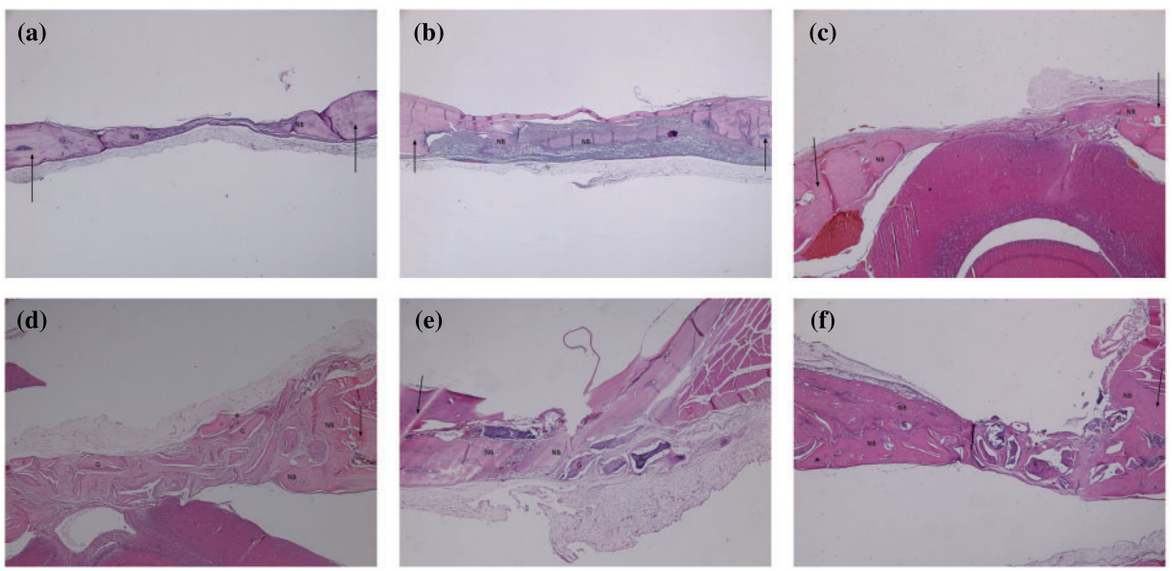


Fig. 2. Histological views of the groups in the sixth week (H&E ×40) (a) Group E, new bone formation (NB) starting from the defect edges (arrows); (b) Group CM, NB formation starting from the defect edges (arrows) and extending to the middle; (c) Group HY, NB forming near the defect edge (arrow); (d) Group X, NB forming from the defect edge (arrow) and graft particles (GP); (e) Group X + CM, NB formation and GP alongside the defect edge (arrow); (f) Group X + HY, mixed GP and large NB formation alongside the defect edge (arrow).

there was no significant difference ($p = 0.557$) with group HY (0.34 ± 0.19 mm^2). Group HY had statistically significantly lower new bone formation than group X (0.34 ± 0.19 mm^2; 0.77 ± 0.22 mm^2; $p = 0.045$). Applying grafts to defects and covering their surfaces with HY (group X + HY) instead of xenografts alone (group X) showed no advantage ($p = 0.848$).

All defects were filled with a higher amount of new bone formation in the sixth week, whereas the lowest amount was again observed in group E. The highest amount of new bone formation in the sixth week was measured in group X + CM (1.57 ± 0.66 mm^2), followed by groups X and X + HY (1.39 ± 0.48 mm^2 and 1.34 ± 0.44 mm^2, respectively). No statistically significant difference

Table I. The amount of new bone formation.

		New bone formation (mm^2)	
		3rd week	6th week
E ($n = 6$)	Avg ± SD	0.16 ± 0.15	0.27 ± 0.16
	Median (Min–Max)	0.11 (0.07–0.45)	0.21 (0.14–0.55)
CM ($n = 6$)	Avg ± SD	0.46 ± 0.34	0.77 ± 0.57
	Median (Min–Max)	0.28 (0.2–0.92)	0.46 (0.34–1.54)
HY ($n = 6$)	Avg ± SD	0.34 ± 0.19	0.51 ± 0.17
	Median (Min–Max)	0.28 (0.16–0.6)	0.54 (0.27–0.7)
X ($n = 6$)	Avg ± SD	0.77 ± 0.22	1.39 ± 0.48
	Median (Min–Max)	0.76 (0.41–1.07)	1.56 (0.55–1.79)
X + CM ($n = 6$)	Avg ± SD	0.94 ± 0.40	1.57 ± 0.66
	Median (Min–Max)	0.90 (0.54–1.41)	1.5 (0.9–2.35)
X + HY ($n = 6$)	Avg ± SD	0.80 ± 0.21	1.34 ± 0.44
	Median (Min–Max)	0.89 (0.5–1.0)	1.45 (0.5–1.72)
	[a]p	[a]0.001**	[a]0.001**
		[b]p	[b]p
E-CM		0.071	0.132
E-HY		0.223	0.244
E-X		0.001**	0.001**
E-X + CM		0.001**	0.001**
E-X + HY		0.001**	0.002**
CM-HY		0.556	0.732
CM-X		0.158	0.064
CM-X + CM		0.064	0.036*
CM-X + HY		0.109	0.109
HY-X		0.045*	0.028*
HY-X + CM		0.015*	0.015*
HY-X + HY		0.028*	0.052
X-X + CM		0.661	0.805
X-X + HY		0.848	0.805
X + HY-X + CM		0.805	0.622

Notes: Avg ± SD = average ± standard deviation; Min = minimum; Max = maximum.

Table II. The amount of residual graft material.

		Residual graft	
		3rd week	6th week
X ($n = 6$)	Avg ± SD	0.59 ± 0.16	0.50 ± 0.11
	Median (Min–Max)	0.62 (0.36–0.75)	0.5 (0.3–0.6)
X + CM ($n = 6$)	Avg ± SD	0.53 ± 0.10	0.39 ± 0.14
	Median (Min–Max)	0.55 (0.41–0.65)	0.35 (0.25–0.62)
X + HY ($n = 6$)	Avg ± SD	0.67 ± 0.12	0.47 ± 0.16
	Median (Min–Max)	0.67 (0.5–0.79)	0.53 (0.19–0.6)
	p	[a]0.226	[a]0.419
X–X + CM		0.515	0.201
X–X + HY		0.290	0.724
X + CM-X + HY		0.088	0.355

Notes: Avg ± SD = average ± standard deviation; Min = minimum; Max = maximum.

was observed between the HY and CM membranes applied to empty defects ($p = 0.064$).

The area of residual graft in groups X, X + CM, and X + HY in the sixth week was smaller than that in the third week. There was no statistically significant difference in the amount of residual graft between the three groups in the third and sixth weeks (Table II).

Different types of graft materials can be used to support membranes in GBR. The amount of new bone formation changes depending on the properties of the graft material used. There are only a few studies in the literature that used the HYAFF11 mesh with graft material. Rhodes et al. prepared defects of 9-mm width and 5-mm depth in the proximal humerus of dogs.[19] Canine demineralized bone matrix (DBM), plain putty, autograft mixed with marrow aspirate, or Allomatrix (DBM/calcium sulphate/CMC putty) was applied to the defects, and the surfaces were covered with HYAFF11 mesh (Hyalonect) and sutured to the adjacent periosteum with 4–0 Vicryl. In the sixth week, a histological evaluation was performed. All defect surfaces were covered with dense, moderately thick fibrous membranes, and there was a mesh in all defects. Neither fibrous tissue nor extravasation of bone filler was observed in any of the defects. The newly formed bone was found to have morphological and qualitative characteristics similar to those of the adjacent solid bone. Ayanoğlu et al. prepared defects in rabbits' metaphyseal tibias.[22] In group I, the defects were left empty; in group II, only the HYAFF11 mesh was applied; in group III, allograft was applied; and in group IV, allograft was applied and the surface was covered with HYAFF11 mesh (Hyalonect). In the third and sixth weeks, sacrification was performed, followed by histologic and radiologic evaluations. Histologically, better bone healing was observed in the HYAFF11 mesh-treated groups than in the other groups. There was no significant difference between groups II and III or groups III and IV in the third and sixth weeks, whereas significantly better bone healing was observed in group IV than in group II. No significant radiologic differences were observed between groups II and III or groups II and IV in the third and sixth weeks ($p > 0.05$). Yılmaz et al. prepared five defects of 10-mm width in the ilium of sheep and left them empty in group E, applied autogenous graft and covered with mesh in group ABG + HY, only applied collagen membrane in group CM or applied autogenous graft and covered with collagen membrane in group ABG + CM, and covered with HYAFF11 mesh (Hyalonect) in group HY.[21] The experimental animals were sacrificed in the third and sixth weeks, and histological evaluation was performed. A higher new bone formation rate was observed in group ABG + CM (52.83 ± 3.06 and 82.33 ± 4.08, respectively) than in groups ABG + HY and HY (46.33 ± 2.25 and 77.17 ± 3.49, respectively) in the third and sixth weeks; the difference was found to be statistically significant in the third week ($p = 0.017$) and insignificant in the sixth week ($p > 0.05$). In terms of membrane success, the CM and

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HY groups had similar new bone formation ratios ($p > 0.05$). In our study, there was no difference in the amount of new bone formation between the third and sixth weeks, similar to the findings of a study by Yilmaz et al. compared to the CM and HY groups.[21] In this study, deproteinized bovine bone mineral-based xenografts were used because of their osteoconductive and non-immunological properties; these xenografts are widely used in clinical and experimental studies.[7–9] Similar to the studies referenced above, the highest amount of new bone formation was observed in graft-treated groups. However, since these studies used different experimental setups and histological evaluation techniques, it was not possible to quantitatively compare the results of the studies. In general, it can be concluded that the use of HYAFF11 together with the graft increases the formation of new bone depending on the characteristics of the graft material used. However, it is necessary to evaluate defect characteristics and histological examination techniques in the same experimental animal to obtain definitive results. Additionally, graft materials have different resorption times. For example, although autogenous grafts are considered the gold standard owing to their osteogenesis, osteoinduction, and osteoconduction properties, they have a rapid resorption time. In contrast, xenografts have osteoconduction and space-maintaining features for a longer time owing to their slower resorption rate.[7] Therefore, it would be more accurate to perform both early and late histological evaluations in experimental studies.

The resorption times were reported to be 40 days for HYAFF11 mesh and 4–6 months for collagen membranes. This study was conducted in rats, and neither mesh nor collagen membrane remnants were observed in the histological evaluations performed at the third and sixth weeks. The reason for the similar results obtained from the histological evaluations performed on sheep in the third and sixth weeks in the study by Yılmaz et al. might be the shorter healing period in experimental animals compared to humans.[21] Histological evaluation of healing at an early period, for example, the first week, could prove to be useful in future experimental studies. It would be beneficial to consider the resorption time in the clinical success evaluation of HYAFF11.

Collapse of membranes due to soft tissue pressure may be observed during the healing period. To prevent these complications, titanium-reinforced membranes, mini screws (to keep the collagen membrane in place), or graft materials could be used. Contrary to the usual membranes, HYAFF11 meshes are easy to manipulate and suture; however, they are not resistant to vertical loads. Neither the collagen membrane nor HYAFF11 was fixed in this study. When the study was completed, similar results were observed for the CM and HY groups. No complications, such as inflammation, necrosis, or openings around the wound area, were observed in either group. Although the amount of new bone formation measured in both groups at the third and sixth weeks was higher than that in group E, the difference was statistically insignificant. In future studies, it would be useful to prefer larger defect sizes to evaluate the groups.

The 'critical size defects' model is often used to evaluate bone regeneration in experimental studies. A critical-size defect is defined as the volume of the bone defect prepared on the subject that could not heal spontaneously during the study period or during the life of the experimental animal.[23, 24] According to a systematic review, the most commonly used diameters for defects were 5 and 8 mm in studies conducted on rats.[25] In this review of 22 studies and 447 defects, only 7 defects (1.6%) of 5-mm diameter showed complete closure histologically in 2 months. In the present study, rats were used as experimental animals. Two 5-mm diameter defects were prepared in the skulls of each rat. The lowest new bone formation rates were observed in group E in the third and sixth weeks after the histological examination. These results show that the diameter of the defect we chose is suitable for evaluating material success.

4. CONCLUSIONS

In this study, we evaluated the efficacy of using HYAFF11 as a membrane in the treatment of intraosseous defects. Similar new bone formation ratios were observed when compared to the collagen membranes commonly used in GBR. These results revealed that HYAFF11-based meshes can be used in GBR. Since the present study was performed on relatively small-diameter defects in rats and the type of graft material used in the GBR technique may affect the healing process, further experimental and clinical studies with different graft materials and defect sizes are required to evaluate the effectiveness of the HYAFF11 mesh in GBR.

Ethical Compliance

The research experiments conducted on animals in this study were approved by the Ethical Committee and responsible authorities of our research organizations, and the experiments were conducted following all guidelines, regulations, and legal and ethical standards required for animals.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments: This study was supported by the Scientific Research Projects Coordination Unit of Istanbul University (grant number: 37784).

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Received: 28 May 2022. Accepted: 21 June 2022.