Heliyon 10 (2024) e32070

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

In vivo comparison of customized zirconia barriers in guided bone regeneration: An experimental study

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ARTICLE INFO

Keywords:
Bone regeneration
Non-resorbable membranes
Customized CAD/CAM-manufactured membranes
Titanium
Zirconia

ABSTRACT

Objective: This study aims to evaluate the effects of customized zirconia barrier membranes produced for guided bone regeneration (GBR) approaches on bone healing researched with histological and histomorphometric methods.
Methods: The digital modeling was used to create zirconia barrier membranes suitable for the defect on the tibia bone. The membranes were designed using a 3D software system and transferred to the CAD/CAM software system in stl. Afterward, zirconia discs (1400 Mpa) (Aconia BSM- D98 × 16, HT+, Germany) were milled and sintered. Titanium mesh, titanium reinforced d-PTFE, and zirconia barrier membranes were used to cover the defects. As a control group, one defect was left empty. 3 and 6 weeks of the healing term, preparates were obtained from each group after animals were sacrificed. New bone formation, amount of the remaining grafts and tissue response parameters were analyzed histomorphometrically and histologically.
Results: The highest percentage of newly formed bone in the early period was observed in the titanium mesh membrane group (26.39 ± 5.38); In the late period, this rate was highest in the zirconia group (64.42 ± 9.95). However, no statistically significant difference was found in both periods between the groups. The amount of residual graft progressed at a low level in both periods without any difference in the other groups except the control group. In the 3rd and 6th weeks, the amount of new bone formation was the lowest in the control group. No foreign body reaction or necrosis was observed in any of the defects.
Conclusion: With the limitation of the study, it has been concluded that effective results can be obtained with customized zirconia barrier membranes in GBR procedures.

1. Introduction

Recently, dental implant treatment has been used as a therapeutic option to maintain functionality, stability and aesthetics in patients who are partially or completely edentulous [1]. For maintenance of the successful implant therapy, adequate bone volume is

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https://doi.org/10.1016/j.heliyon.2024.e32070
Received 24 November 2023; Received in revised form 9 May 2024; Accepted 28 May 2024
Available online 3 June 2024

essential. Several methods like, GBR, inlay and onlay bone grafting and osteodistraction have been improved to ensure the formation of new bone and obtain osseointegration in large-size defects. GBR is the augmentation procedure applied with graft materials and barrier membranes in the presence of insufficient residual alveolar crest vertically or horizontally or in bone defects around the implant. In GBR, preventing soft tissues from growing into the bone defect and only allowing hard tissue cells to grow into the defect is the biological rationality and it is necessary that the graft material does not displace against intraoral loads and maintain the desired volume during the healing [2–4]. Various techniques and biomaterial options have been evolved to achieve these conditions in bone augmentation over the years. In order to achieve both aesthetic and masticatory results, it is very important to use a membrane that will optimally complete tissue regeneration in GBR. Often confronted problems in GBR include membrane exposures due to soft tissue dehiscence and partially or complete collapse of the membrane. Membrane exposure and soft tissue damages usually end with infection and insufficient augmentation of bone volumes. To overcome post-operative complications and achieve more predictable treatment results, many membranes with different properties have been introduced. Resorbable and non-resorbable membranes, such as; polytetrafluoroethylene (PTFE), expanded PTFE (e-PTFE), high-density PTFE (d-PTFE), titanium mesh, collagen, polylactic acid, polyglycolic acid and their co-polymers have been tested in various clinical and experimental studies [4]. Recently, customized titanium mesh and d-PTFE membrane are frequently preferred for GBR [5,6,7]. However, studies with customized titanium mesh in guided bone regeneration procedures have reported that membrane exposure is still a most common complication [8]. Titanium mesh and d-PTFE membrane exposures during the early or late bone healing process may result in bone resorption. Thus, exposure and/or infection could disrupt the new bone formation and the outcome of the guided bone regeneration procedure [9,10].

Barrier membranes customized with computer-aided design and computer-aided manufacturing (CAD/CAM) are becoming a valid and effective method in guided bone regeneration [8,11]. CAD/CAM technology and three dimensional production establish a patient specific non-resorbable membrane to simplify and decrease surgery time preoperatively [6,7]. In recent years, zirconia has been produced with CAD/CAM technology and three dimensional production and could be used as a membrane material in GBR procedures [8,12]. Due to the biocompatibility and aesthetic properties of zirconia, the frequency of use has increased considerably in recent years. The fact that zirconia has bioinert properties, integration in soft tissue, reduce inflammatory response, and less biofilm adhesion compared to titanium makes it an alternative and innovative material in studies [13,14,15].

Customized zirconia barrier membranes produced with digital modeling and 3D computer technology can achieve the ideal adaptation between the barrier membrane and the bone defect. Since manual shaping and cutting processes will be avoided during the surgery, the operation time is shortened, thus the risk of complications is also reduced. It is reported that the use of zirconia as a customized barrier membrane in GBR procedures can be considered to contribute to new bone formation [8,16,15]. Furthermore, related research numbers in the present literature are quite limited. This in vivo study was designed to determine histomorphometrically and histologically the effect of customized zirconia barrier membranes on new bone formation, amount of residual bone, as well as inflammation density in GBR processes, and also to determine whether zirconia can be used as an alternative material for nonresorbable membranes.

2. Methods

2.1. Animal model and experimental groups

The study was authorized by the Ethical Committee of Animal Experiments in Bursa Uludag University (No. 2022-07/01). Animals were housed and operated in the Department of Surgery, Bursa Uludag University Faculty of Veterinary. 2–3 years old between 40 and 50 kg weights six adult male sheep, were included. The same number of animals (n = 3) were separated into early and late term healing groups. Using 2 tibias of each animal, 4 standardized defects were created surgically, all four were filled with autogenous bone chips mixed with xenograft (Ubgen, Re-Bone, Italia) at a 1:1 ratio. Defects were randomly covered with a customized zirconia barrier, titanium mesh, and titanium-reinforced d-PTFE membranes (Cytoplast, Osteogenics, Lubbock, Texas) and fixed using screws. (1.5 × 3.0 mm, Profix, Ac Dental, Istanbul, Turkey) One defect was covered with no membrane as a control group.

2.2. Preparation of biomaterials

Zirconia barrier membranes were custom-made for the defect size and modeling of the experimental animal tibia bone sample. A 0.6 mm-thick barrier was designed. An “stl” file was assembled and forwarded to CAD/CAM software. Afterward, milling and sintering processes were applied to 1400 Mpa zirconia discs (Aconia BSM- D98 × 16, HT+, Germany). After milling and sintering, zirconia barriers were disinfected and sterilization procedures were followed [8].

2.3. Surgical procedure

All surgical approaches were made with general anesthesia under sterilized conditions. First, the surgical area was shaved and disinfected with %10 povidone-iodine (Poviiodeks Batikon, Kim-Pa, Istanbul, Turkey). Premedication was provided by administering Xylazine (Rompun, Bayer, 0.2–0.5 ml/kg, i.m., Bayer, Istanbul, Turkey). Then, induction was supplied by intravenous application of ketamine hydrochloride (Alfamine, 5 mg, i.v., Atafen) after angioket administration from V. jugularis. General anesthesia was accomplished and sustained during the full operation time with ketamine (7.5 mg/kg) and diazepam (0.5 mg/kg). The skin and periosteal incisions were made and the tibia was uncovered with the help of a periosteal elevator. Standardized defects were created under saline irrigation (Isotonic Nacl, Eczacıbaşı-Baxter, Istanbul, Turkey) with a physio dispenser at 750–800 rpm with a 6 mm

internal diameter trephine bur (Hu-Friedy, Steris, US). The anterior wall of the defects was created with a micro-saw (Frios, Dentsply Sirona, New York) at a vertical height of 5 mm. Autogenous graft particles were collected from the tibia bone distant from the defect areas with a bone scraper (SafeScraper, META)(Fig. 1a and b)Biomaterials were randomly applied to the bone defects (Fig. 2a)After all biomaterial applications the periosteum was sutured with No.0 vicryl (Ethicon Coated Vicryl®, Johnson&Johnson), and primary wound closure was sutured with No.0 trophilen (Dogsan, Turkey). Then, tibias were stabilized with a polyvinylchloride splint, which was shaped by taking the leg measurements of each experimental animal, in a way that would not restrict the animal's movements and wrapped with a self-adhesive bandage for reducing the fracture ris (Fig. 2b).

2.4. Post-operative process and sacrifice

The sutures were removed after 10 days. To reduce the risk of infection, a single dose of 20 mg/kg ceftriaxone (Novosef 500 mg IM, Zentiva, Istanbul, Turkey) was applied for 5 days via intramuscular (i.m.) injection. A single dose of 0.5 mg/kg meloxicam containing NSAI (Melox 15 mg/1.5 ml IM, Nobel, Istanbul, Turkey) analgesic was applied for 5 days via i.m. injection to reduce postoperative pain. Animals were fed a standard diet throughout the healing period. During the hospitalization period, the general health status of the experimental animals was checked daily and reported weekly. After the surgery, follow up demonstrated an uneventful healing process and no postoperative infection related to the surgical procedure. Completing the healing period without any complications experimental animals were sacrificed at the end of the 3rd and 6th weeks based on the two groups separated previously. Tibias were extracted and after then fixed with 10 % pH 7.0 neutral buffered formalin solution at room temperature for histomorphometric and histologic analysis.

2.5. Histologic and histomorphometric analyses

After the fixation, all specimens were decalcified at room temperature in the solution prepared by taking one measure each of 20 % sodium citrate and 50 % formic acid solution and embedded in paraffin as blocks. 5–7 μm thick sections were obtained from each specimen block using a microtome (Thermo Fisher Scientific, HM 430). Sections stained with hematoxylin & eosin (HE) and Masson Trichrome (MT) were taken to evaluate new bone formation, presence and degree of inflammation, scales of necrosis, fibrosis, and foreign body reaction, and residual graft amount. Sections were examined using a light microscope (Olympus® CX41, Tokyo, Japan). The photomicrographs for each section were captured with the help of a digital camera connected to the microscope and computer (Olympus® DP72 Hyper Crystal LCD, Tokyo, Japan). Defect areas which include new bone formation were calculated with MT-stained sections. Using the analysis program (DP2-BSW, Olympus, Japan)for images brightfield sections were quantitatively analyzed. Afterward, the number of pixels stated as the new bone area was divided by the total number of pixels stated as the complete defect area and converted to μm2. The same processing cycle was generated to calculate residual bone graft amounts. The acquired values are stated as a percentage (%). The presence of tissue response (inflammation), necrosis, fibrosis, and foreign body reaction scales were evaluated using a semi-quantitative tissue response (inflammation) score. For each sample, by examining the inflammatory cell infiltration, the severity of the inflammation score was determined (Table 1) [17].

2.6. Statistical analysis

SPSS 23 software (IBM SPSS 2023, New York, USA) program was used for statistical analysis. Statistical descriptions such as "standard deviation, mean, median, frequency, ratio, minimum and maximum" were used while evaluating the data of the study. For comparisons of groups of three and over without normal distribution "The 'Kruskal Wallis Test" was utilized and the "Mann-Whitney *U* Test" was utilized for binary comparisons. The "Fisher Freeman- Halton Test" was used to compare the qualitative variables. Statistical significance was accepted as $p < 0.05$.

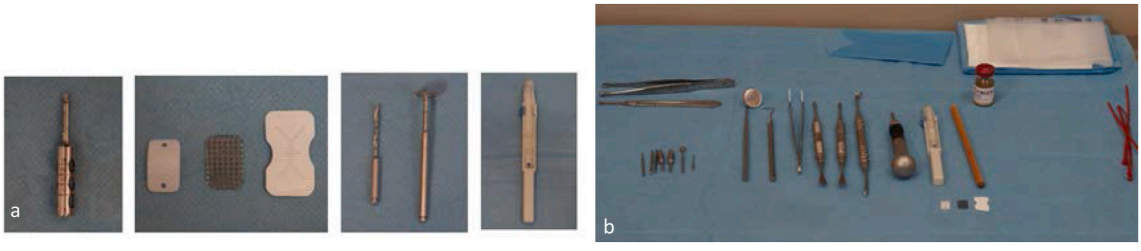

Fig. 1. a) Trephine bur (Hu-Friedy, Steris, US), membranes, micro-saw (Frios, Dentsply Sirona, New York) and bone scraper (SafeScraper, META). b) Surgical preparation.

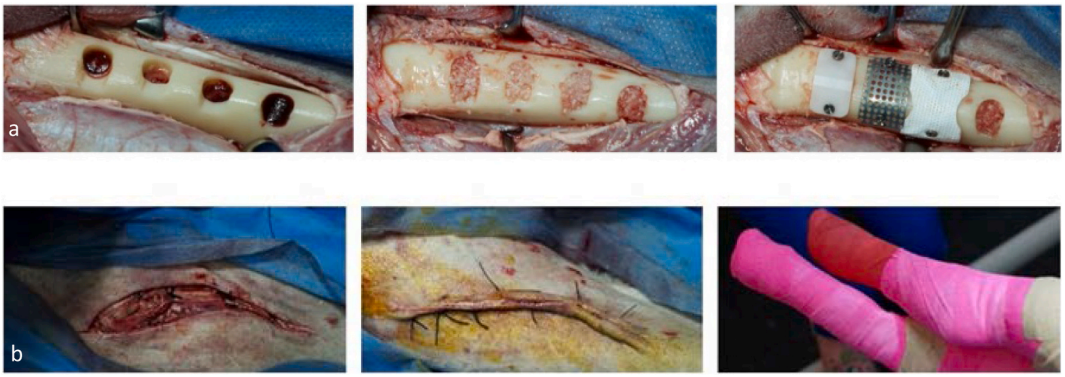

Fig. 2. a) Standardized defects of 6 mm diameter and 5 mm depth, the anterior walls of the defect were removed with a micro-saw (Frios, Dentsply Sirona, New York). A distance of 5 mm was left between the defects. Biomaterials were randomly applied to the bone defects. b) Sutures and stabilized tibias.

Table 1
Scoring table of tissue response.

Score	*0*	*1*	*2*	*3*	*4*
Inflammatory Cellular Infiltration	*No difference from normal control tissue; no presence of macrophages, foreign body cells, lymphocytes, eosinophils, or neutrophils at or around the implant site*	*Presence of a few lymphocytes or macrophages; no presence of foreign body giant cells, eosinophils, or neutrophils*	*Presence of several lymphocytes and macrophages with a few foreign body giant cells and a small foci of neutrophils*	*Presence of large numbers of lymphocytes, macrophages and foreign body giant cells; notable presence of eosinophils and neutrophils*	*Severe cellular infiltrate response to implant or tissue necrosis at or around the site*

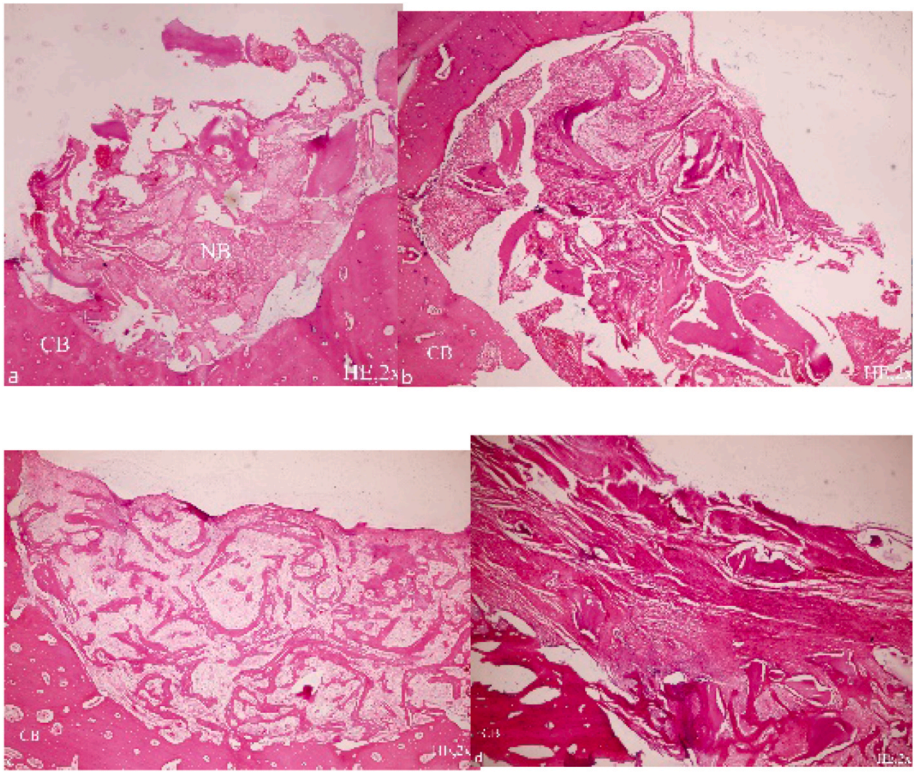

Fig. 3. Histological images of a) customized zirconia barrier, b) titanium mesh, c) d-PTFE, d) control groups at the 3-week healing term. Hematoxylin and eosin staining. CB, cortical bone; CT, connective tissue; NB, newly formed bone.

3. Results

3.1. New bone formation

In the early healing period, peripheral parts of the defect areas (cortical edges), new bone formation areas were generally detected that could not reach the center of the defect area. In the histological sections taken, it was observed that there were areas of fibrosis between dense graft particles. Fibrosis (fibrous callus, connective tissue) detected in the sections contains precursor bone cells, new blood vessels, and fibers and leads to the formation of new trabecular bone through intramembranous ossification. No foreign body reaction or necrosis was detected in any of the defects (Fig. 3).

The amount of new bone formation in the 3rd week differed statistically significantly between the groups ($p < 0.05$). According to the paired comparisons; the newly formed bone amounts of the zirconia barrier membrane ($p = 0.004$), titanium membrane ($p = 0.012$), and d-PTFE membrane ($p = 0.02$) groups were significantly higher than the control group ($p < 0.05$) (Fig. 4) No statistically significant difference was observed in the amount of newly formed bone in the early period between the zirconia barrier membrane and the titanium and d-PTFE membrane groups. When comparing the titanium membrane group and the d-PTFE membrane group, no statistically significant difference was discovered between the amount of newly formed bone in the early period ($p > 0.05$) (Table 2).

According to the results of the sections taken after the late healing term (6th week), remodeling of the newly formed bone is observed in the defect area sections of all membrane groups (Fig. 5). At the end of the 6th week, it is observed that approximately ¾ of the defect area ossifies in the zirconia barrier membrane group (Fig. 5a). The amount of new bone formation in the 6th week showed a statistically significant difference according to the groups ($p < 0.05$). According to the paired comparisons; the newly formed bone amounts of the zirconia barrier membrane ($p = 0.04$), titanium membrane ($p = 0.004$), and d-PTFE membrane ($p = 0.004$) groups were significantly higher than the control group ($p < 0.05$) (Fig. 6)There was no statistically significant difference between the amount of newly formed bone between the zirconia barrier membrane and the titanium membrane ($p = 0.053$). The amount of new bone formation in the late period was significantly higher in the zirconia barrier membrane group than in the d-PTFE membrane group ($p = 0.013$). When comparing the titanium and the d-PTFE membrane group, no statistically significant difference was discovered between the amount of newly formed bone in the late period ($p = 0.07$) ($p > 0.05$) (Table 3).

3.2. Residual bone graft ratios

According to the histological section data taken at the end of the 3rd week, it was determined that some of the bone graft particles were degraded and altered by irregular tight connective tissue or trabecular new bone within the bone defect (Fig. 3) In the 3rd week,

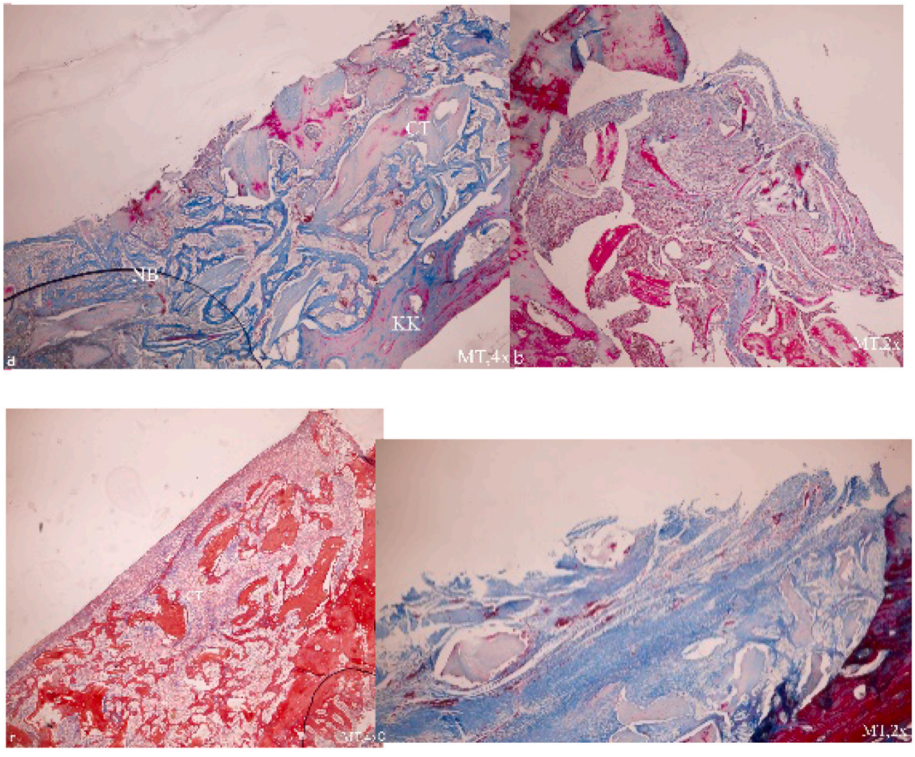

Fig. 4. Histological images of a) customized zirconia barrier, b) titanium mesh, c) d-PTFE, d)control groups at the 3-week healing term. Masson's trichrome staining. CB, cortical bone; CT, connective tissue; NB, newly formed bone.

Table 2
Percentage of new bone formation in all groups at 3 Weeks (%).

GROUP	n	Min- Max (Median)	Mean ± SD	p	p
Control (Empty)[1]	6	10.94–16.77 (16.5)	14.48 ± 2.25	0.05[a]	
d-PTFE[2]	6	16.85–33.41 [11]	26.02 ± 6.41		
Titanium[3]	6	17.33–32.14 [12]	26.39 ± 5.38		
Customized zirconia[4]	6	18.86–38.01 (20.50)	24.42 ± 7.4		2.3.4 > 1[b]

[a] Kruskal Wallis Test. $p < 0.05$.
[b] Mann Whitney *U* Test.

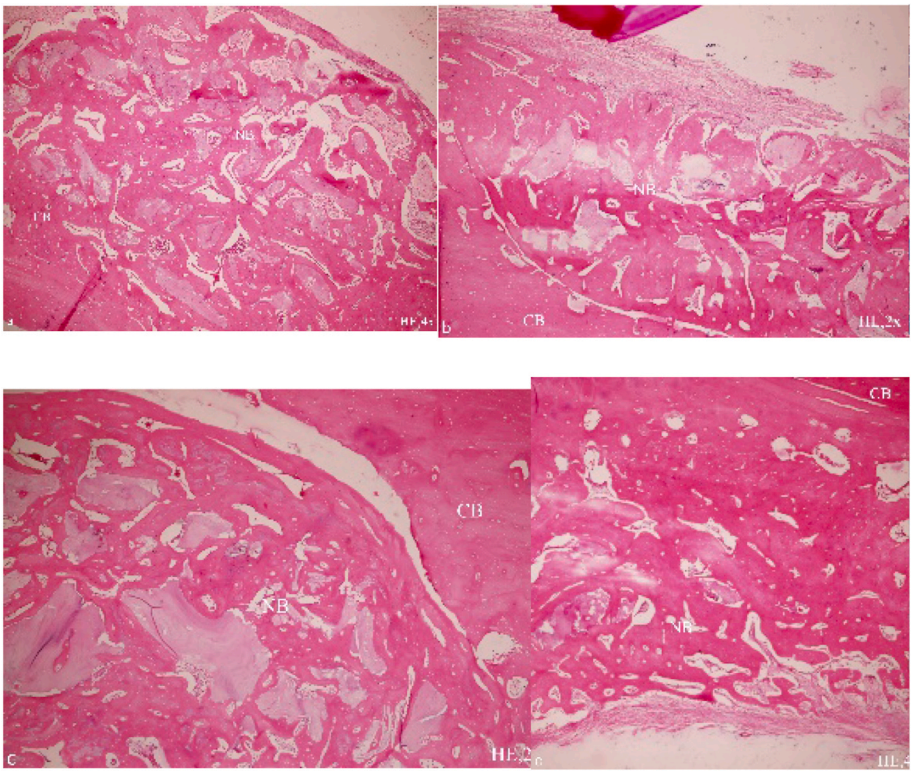

Fig. 5. Histological images of a) customized zirconia barrier, b) titanium mesh, c) d-PTFE, d) control groups at the 6-week healing term. Hematoxylin and eosin staining. CB, cortical bone; CT, connective tissue; NB, newly formed bone.

the amount of residual bone grafts differed significantly between the groups ($p < 0.05$). According to the paired comparisons; The residual bone graft amounts of the zirconia barrier membrane ($p = 0.005$), titanium membrane ($p = 0.013$), and d-PTFE membrane ($p = 0.03$) groups were significantly lower than the control group ($p < 0.05$) (Fig. 4) There was no statistically significant difference between the residual bone graft amounts of the other groups ($p > 0.05$). It was determined that the bone graft particles degraded to a large extent at the end of the 6th week, which is the late healing period (Fig. 5) The amount of residual bone grafts differed statistically significantly between the groups ($p = 0.047$; $p < 0.05$). According to the comparisons made; The residual bone graft amounts of the zirconia barrier membrane ($p = 0.03$), titanium membrane ($p = 0.04$), and d-PTFE membrane ($p = 0.04$) groups were significantly lower than the control group ($p < 0.05$) (Fig. 6)The amount of residual bone graft in the zirconia barrier membrane group was not significantly different from the titanium ($p = 0.054$) and d-PTFE ($p = 0.064$) membrane groups ($p > 0.05$). There was no statistically significant difference in residual bone graft amounts between the titanium membrane and d-PTFE membrane groups ($p = 0.52$) ($p > 0.05$) (Table 4).

3.3. Inflammation intensity

Tissue response to biomaterials was insignificant and inflammation scores were low. In the early and late term of healing, there was no significant difference between the inflammation severity parameters of the groups. No tissue necrosis was observed. As well as no side effects such as membrane exposure were observed in the groups compared to the control group (Table 5).

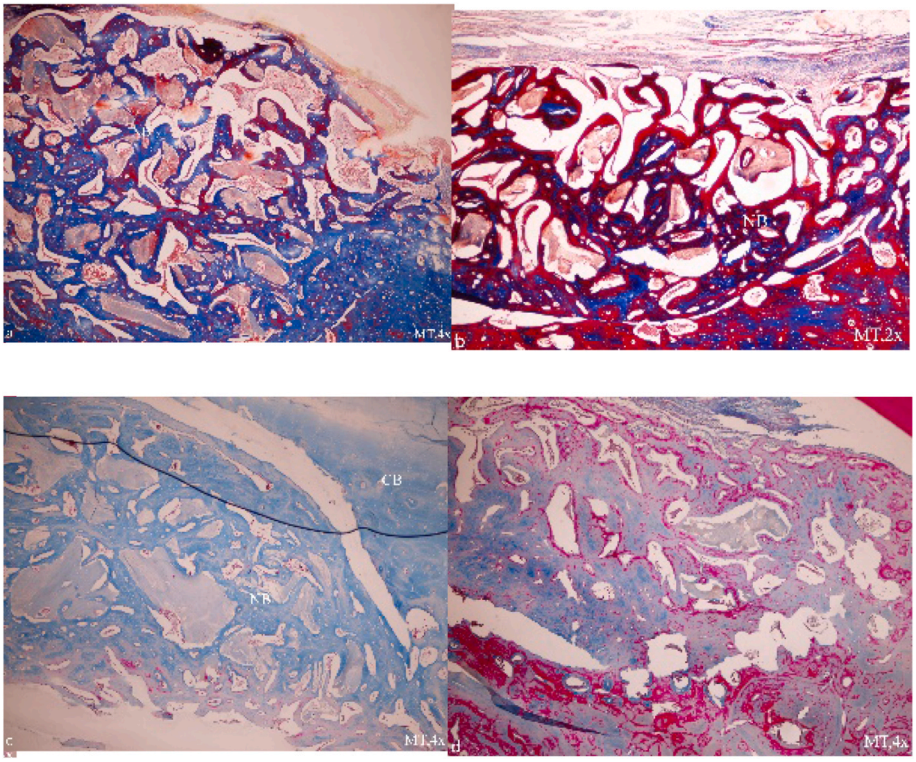

Fig. 6. Histological images of a) customized zirconia barrier, b) titanium mesh, c) d-PTFE, d) control groups at the 6-week healing term. Masson's trichrome staining. CB, cortical bone; CT, connective tissue; NB, newly formed bone.

Table 3
Percentage of new bone formation in all groups at 6 Weeks (%).

GROUP	n	Min- Max (Median)	Mean ± SD	p	p
Control (Empty)[1]	6	12.12–28.96 (15.5)	17.98 ± 6.3	0.05[a]	
d-PTFE[2]	6	35.95–58.82 (46)	47.11 ± 8.95		
Titanium[3]	6	49.05–60.20 (55.5)	55.20 ± 5.41		
Customized zirconia[4]	6	52.12–79.51 (62)	64.42 ± 9.95		2.3.4 > 1[b]

[a] Kruskal Wallis Test. $p < 0.05$.
[b] Mann Whitney *U* Test.

Table 4
Percentage of residual graft material ratios after 3 and 6-week healing terms (%).

GROUP	n	Min- Max (Median)/3-Week	Min- Max (Median)/6-Week	Mean ± SD/3-Week	Mean ± SD/6-Week	p	p
Control (Empty)[1]	6	22.81-40.84 (26.67)	12.75–35.42 (23.63)	32.78 ± 3.26	24.88 ± 7.01	0.05[a]	
d-PTFE[2]	6	15.44–45.06 (33.41)	7.51–17.51 (13.17)	31.70 ± 18.6	12.55 ± 8.4		
Titanium[3]	6	22.47–35.07 (33.63)	3.11–11.09 (7.65)	30.34 ± 11.86	7.61 ± 7.18		
Customized zirconia[4]	6	6.75–44.13 (28.62)	2.38–9.67 (7.74)	23.18 ± 4.98	7.20 ± 7.5		2.3.4 > 1[b]

[a] Kruskal Wallis Test. $p < 0.05$.
[b] Mann Whitney *U* Test.

4. Discussion

In GBR procedures, optimum regeneration of large bone defects associated with tissue loss remains a challenge today. First of all, flap closure should acquire passively during the surgery and the wound closure must remain throughout the postoperative healing period. Any barrier membrane resorbable or non-resorbable, needs to be sealed primarily with soft tissue to prevent inflammations and microbial contamination that could constrain the procedure [18,19]. In order to avoid these difficulties, new materials are introduced and developed every day in the treatment of bone deficiencies.

Table 5
Evaluation of tissue responses after 3 and 6-week healing terms (%).

GROUP	Tissue Response (0–4 Score) n (%)				[a]p
	Score 0 3-Week	Score 1 3-Week	Score 0 6-Week	Score 1 6-Week	
Control (Empty)	5 (83.3)	1 (16.7)	4 (66.7)	2 (33.3)	0.054
d-PTFE	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	
Titanium	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	
Customized zirconia	4 (66.7)	2 (33.3)	5 (83.3)	1 (16.7)	

[a] Fisher- Freeman- Halton Test.

Absorbable membranes, which are preferred in studies due to their ease of tissue compatibility and ease of use, do not have structural properties that will protect the defect area and prevent the collapse of the bone graft, especially in vertical bone regeneration. On the other hand, with rigid materials such as titanium, PTFE membranes and zirconia, protection of the area where new bone will be formed during the healing process and wound stability could be ensured.

Nowadays, d-PTFE membranes and titanium meshes are the most commonly used in large bone defects. These membranes need to be shaped according to the defect during operation. This process is challenging and time-consuming, depending on the operator's skills. Therewithal, the forming process must be carried out properly, otherwise, it could adversely affect the regeneration process. A titanium mesh which has sharp edges and corners could damage soft tissues and could cause complications. A second surgical operation requires the removal of both the non-absorbable membranes and the titanium meshes. Such removal procedures can be invasive due to the ingrowth of fibrous tissue within its micro and macroporosity [19].

With the accelerating importance of digital dentistry, the management of computer-aided design and manufacturing (CAD/CAM) has led researchers to develop a new membrane concept that can be produced according to specific defect morphology [10]. With CAD-CAM technology, based on CBCT (cone-beam computed tomography) scan data of the bone defect and a digital workflow system, a customized barrier could produce to help regenerate new bone volume. With customized meshes, additional time is saved in guided bone regeneration procedures, because the prosthetic plan, implant placements and new bone volume calculations are made before the surgery. This procedure is considered as an advantage in terms of shortening the surgical operation time and thus reducing the risk of complications. The adjustable thickness and pore sizes of titanium meshes with computer-aided designs, increase the frequency of production in customized systems. But membrane exposures have been encountered in studies conducted with the customized production of titanium barrier membranes. If the membrane exposure occurs early, titanium mesh often has to be removed and prolonged healing processes, which should be expected in some cases with large bone defects, are adversely affected if membrane exposure is observed.

Membrane exposure is reported as the most common complication in the use of PTFE and titanium barrier membranes in vertical and horizontal bone regeneration. It is very important for the predictability of the treatment that tissue and bone regeneration develop together during the treatment process of patients with multiple edentulousness. Zirconia produces a better fibroblast response compared to titanium, as well as less biofilm adhesion and less inflammatory response. It has been stated in studies that it responds better than titanium material in terms of biological properties. Also as a barrier membrane zirconia can be used as a safe and effective material for GBR relating to its exact fit to bone defects, reduce intraoperative time, biocompatibility, decrease soft tissue dehiscence and allow predictability [9,10,20,21]. The production of customized zirconia barrier membranes is provided in the clinical environment with the support of three-dimensional computer technology and CAD/CAM software. Only a few clinical and pre-clinical studies have been conducted to examine how effective the customized zirconia barrier membrane is in guided bone regeneration. This study is unique in that it compares the customized zirconia barrier membrane to both titanium mesh and titanium reinforced d-PTFE membrane in the same experimental model.

In the present study, the efficacy of a customized zirconia barrier membrane on bone regeneration was evaluated and the effects of a customized zirconia barrier membrane were compared with titanium mesh and d- PTFE membranes in experimentally created critical-size defects. Sheep tibia was used previously for evaluating the newly formed bone in experimental in vivo studies for GBR [22, 17]. Sheep due to their validity, easy management, body weights, anatomy, metabolism and also bone remodeling being similar to humans make them suitable for medical research as a large animal [23,24]. Nowadays, the use of sheep models as experimental animals in studies has increased, mainly because of the similarity of their structure, weight, and joints to human bone and similarities in bone regeneration. As per the 3Rs Program, this animal model permits the testing of dental implant systems and bone regeneration procedures without the need for euthanasia, thereby safeguarding the animal's life. Additionally, the Biocompatibility Evaluation of Medical Devices-ISO standard (ISO 10993–6 2007) specifies that up to six materials and/or implants can be installed on each leg of a sheep's long bones (femur and tibia), making it feasible to test twelve samples in a single animal [25]. In our study, the power analysis was kept at the optimum level (80 %) and the minimum number of samples was determined based on the sample literature. Based on these data, it was calculated that a total of 48 defects should be created. It was determined that a total of 6 animals were needed as the number of samples could be reached by halving the number of animals when 8 defects in total, 4 of which were opened in each animal, in the right and left tibia bones. Thus, 6 experimental animals were included in our study. A significant difference was found between the biomaterials used in the study; therefore, it has been proven that the minimum sample number determined before the study was calculated correctly. During the study, all surgeries were performed without any complications and the healing period went smoothly. The animals were euthanized at the end of the study in accordance with the 3Rs Program guidelines (Reduction, Refinement, and

Replacement) as outlined by the NC3Rs Reporting Guidelines Working Group in 2010. Histomorphometric and histologic analyses were utilized together with the inflammation intensity parameters. The guided bone regeneration was extensively studied in experimental models such as rabbits, rats, and dogs. The present results should interpret the fact that metabolism and healing are slower in sheep than the other presented animals but faster than the human [26]. The studies indicate that the volume of the cortical bone and the density of the sheep tibia bone should be higher than the dog and human alveolus [27]. The study identified two time periods for assessing early and late-term healing. As sheep have a shorter recovery period, sacrifices were carried out on the third and sixth weeks. According to the literature, it was emphasized that the bone defect that should be created in experimental animal models should not be less than the critical size, that the defect should include both cortical and cancellous bone, and that the defect areas should be kept at an appropriate distance so that the biomaterials used do not affect each other. In the study, it was observed that the size of the critical defect in the tibia bone was determined as '>5 mm' in the sample studies [28–30]. In line with this information, standardized defects of 6 mm diameter and 5 mm depth were created in the right and left tibia bones of each animal. In order to examine the vertical bone augmentation, the anterior walls of the defect were removed with a micro-saw (Frios, Dentsply Sirona, New York). A distance of 5 mm was left between the defects. When interpreting these data, it is crucial to consider the clinical significance of using an experimentally induced critical-sized defect model. This is because such a model does not exhibit any inherent inclination toward spontaneous regeneration and recovery. As a result, it more accurately reflects the compromised state of a jawbone configuration in a biological context. Antibiotic use is recommended to prevent post-operative infection in bone augmentation applications [20]. In this study, antibiotics were administered post-operatively once a day for 5 days in order not to adversely affect wound healing due to infection (Novosef 500 mg i.m. Vial, 20 mg/kg). No side effects or complications were encountered during the recovery period. It was observed that the tibia bones obtained after sacrification were not exposed to infection and preserved their macroscopic integrity. In the study, the percentage of new bone formation was found to be significantly higher and fibrosis was found to be significantly lower in the membrane-applied groups, both in the early and late healing periods compared to the control group. The control group was the group with the lowest values in terms of both new bone formation and fibrosis. The results were consistent with previous studies confirming that the barrier membrane should prevent non-osteogenic cells from participating in regeneration and that in new bone regeneration, the defect should be filled with new bone by targeting osteogenic and angiogenic cells and specifically bone cells.

The purpose of this study was to evaluate the impact of using zirconia as a non-resorbable barrier membrane material in GBR procedures. Based on the current data, it has been observed that zirconia performs better than titanium mesh and d-PTFE membranes in promoting new bone formation in critical-sized defects. In the 3-week healing, customized zirconia barrier means new bone formation% was higher than the control group and the differences were statistically significant. It can be concluded that the customized zirconia barrier membranes are capable of achieving a higher new bone formation level compared with the titanium and d-PTFE groups, although these groups have shown no statistical differences in the early weeks of healing. In the 6-week healing, there were no statistically significant differences observed between the groups of titanium and zirconia membranes. But customized zirconia barrier means new bone formation% was higher than the d-PTFE and the control group. According to the present results, this difference was evident for an efficient material for GBR in the later term of healing. This result supports the findings of Anderud et al. who also demonstrated a higher bone modulation and calcifications in 12-week healing in their study of vertical bone augmentation procedures in rabbit calvaria. Mean bone volume in the 12-week healing rabbit calvarias for zirconia and microporous hydroxyapatite was between 12.2 and 18.9 % (median 16.0 %), and 6.6–16.7 % (median 11.6 %), respectively, and the differences were statistically significant ($p < 0.005$). Another study performed on rat femur demonstrated vertical bone augmentation in 2,4 and 8th week healing terms [15]. Radiographic and bone mineral density analyses were applied to the obtained bone samples. Immunofluorescence, immunohistochemistry, and histology/morphometry were analyzed for osteogenesis and angiogenesis evaluations. According to the results of histomorphometric analyses, the dome with the lowest height within the zirconia barriers had a significantly higher percentage of new bone ($70 \pm 5{,}50$ %) compared to those with medium height (47.40 ± 4.51) and maximum height (34.40 ± 4.16 %) was found to have. In histological analyzes, the presence of osteoprogenitor cells was detected, and it was stated that the new tissue formation beneath the zirconia barrier membrane showed a progressive rise in the expression level from the 2 nd week to the 4th week after surgery. ($p < 0.05$) This level remained high until the 8th week. As a result, the researchers reported that the zirconia barrier could act as an effective and useful material in guided bone regeneration processes depending on the volume of the GBR area and the vertical and horizontal bone height gain and assessments of osteogenic capacity. According to the results of these experimental studies, the Zr barrier played a significant role in the GBR process, contributing to the gain of new bone, and positively affecting the osteogenesis with its biocompatibility properties.

In the present study, no statistically significant residual graft% was found between customized zirconia, titanium, and d-PTFE groups after 3 weeks and 6 weeks of healing. In the 3-week and 6-week healing terms, the customized zirconia barrier and all the other groups of materials residual graft% was lower than the control group and there was a significant statistical difference. These results have similar amounts likewise for the studies has been done. For example, Arca et al. reported 42.6 % new bone formation and 5.17 % residual bone graft in their clinical study. They aimed to determine the regeneration properties of the Zr barrier membrane produced specifically for the patient in the application of vertical bone augmentation. Based on CBCT data, a zirconia barrier membrane was designed and manufactured using CAD/CAM technology after a detailed anamnesis and intraoral examination for a patient who is forty-six years old and applied to the clinic with advanced bone resorption. After a 7-month healing period, the Zr membrane was removed and new bone formation was observed in the augmentation area. Similar histological and clinical results were detected in the clinical study of Mandelli et al. Histological examination revealed a dense network of vascularization, new bone cells, areas of mineralization, and osteocyte cells. While zirconia barrier membranes gave successful results clinically and histologically, to confirm the predictability of the zirconia, more long-term prospective studies with a larger number of patients are required. In the clinical study of Mandelli et al. although exposure was observed in one case, no signs of infection were found. Mandelli et al. suggested that the

exposure might have been caused by a design flaw, where sharp edges were present beneath thin soft tissues. In our study, it was examined there were no signs of soft tissue dehiscence, inflammation, wound complications or Zr barrier membrane exposure. Same clinical outcomes as noted in Heikal et al. and Hofferber et al. studies. Heikal et al. identified patient groups with bilaterally highly resorbed posterior alveolar bone in the mandible and used customized Zr membranes that they produced three-dimensionally in their new bone regeneration processes. Hofferber et al. reported on the successful use of personalized zirconia ridge augmentation matrices to enhance deficient alveolar ridges prior to dental implant placement through case reports. Both studies have reported that no infection, soft tissue dehiscence, indications of inflammation or exposure of Zr membrane were observed. The current study found notable variations in average vertical and horizontal bone heights and bone density measurements, which align with the outcomes and findings of previous research series.

5. Conclusion

With the limitation of the present study, it seems that customized zirconia barrier membrane is capable of achieving statistically significant new bone formation in GBR procedures. In the present study, it is determined that the membrane is fully compatible with bone defects. No necrosis, inflammation, or foreign body reaction was observed, indicating good biocompatibility. Further clinical and experimental studies are needed to maintain precise results for customized zirconia barrier membrane in GBR, which will help demonstrate their efficacy.

Ethics statement

The present study was authorized by the Ethical Committee of Animal Experiments in Bursa Uludag University (No. 2022-07/01).

Data availability statement

Data included in article/supplementary material/referenced in article.

Funding statement

The present study was supported by the Scientific Research Projects Coordination and Fund Executive Unit of Istanbul University (Project No: 38892, Start Date: August 10, 2022).

CRediT authorship contribution statement

Zeynep Tuncludemir: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Data curation, Conceptualization. **Ihsan Caglar Cinar:** Writing – review & editing, Project administration, Data curation, Conceptualization. **Zehra Avcı Kupeli:** Methodology, Data curation. **Elif Unlu:** Resources, Investigation. **Serdar Yalcin:** Supervision, Resources, Project administration.

Declaration of competing interest

The authors report no conflicts of interest.

Acknowledgements

Authors express their gratitude to Ph.D. students Elif Unlu (Department of Surgery, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa) and Zehra Avcı Kupeli (Department of Pathology, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa) for their assistance with the surgical procedure and histological analyses of the study.

Abbreviations

GBR	Guided Bone Regeneration
CAD/CAM	Computer-aided design/Computer-aided manufacturing
PTFE	Polytetrafluoroethylene
d-PTFE	dense- Polytetrafluoroethylene
e-PTFE	expanded- Polytetrafluoroethylene
NSAI	Non- Steroidal Anti- Inflammatory
HE	Hematoxylin & Eosin
MT	Masson Trichrome
SPSS	Statistical Package for the Social Sciences
CBCT	Cone-beam Computed Tomography
3Rs	Reduction, Refinement, and Replacement

Zr Zirconia

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32070.

References

[1] C.H.F. Hämmerle, D. Tarnow, The etiology of hard- and soft-tissue deficiencies at dental implants: a narrative review, J. Periodontol. 89 (2018) S291–S303.
[2] R.K. Schenk, D. Buser, W.R. Hardwick, et al., Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible, Int. J. Oral Maxillofac. Implants 9 (1994) 13–29.
[3] I.A. Urban, A. Monje, Guided bone regeneration in alveolar bone reconstruction, Oral Maxillofac. Surg. Clin. North Am. 31 (2019) 331–338.
[4] Y.D. Rakhmatia, Y. Ayukawa, A. Furuhashi, K. Koyano, Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications, J. Prosthodont. Res. 57 (2013) 3–14, https://doi.org/10.1016/j.jpor.2012.12.001.
[5] C.H. Hämmerle, T. Karring, Guided bone regeneration at oral implant sites, Periodontol 17 (2000. 1998 Jun) 151–175, https://doi.org/10.1111/j.1600-0757.1998.tb00132.x. PMID: 10337322.
[6] Hartmann Amely, Hildebrandt Helmut, Jörg U. Schmohl, W. KämmererPeer, Evaluation of risk parameters in bone regeneration using a customized titanium mesh: results of a clinical study, Implant Dent. 28 (6) (December 2019) 543–550, https://doi.org/10.1097/ID.0000000000000933.
[7] G.U. Jung, J.Y. Jeon, K.G. Hwang, C.J. Park, Preliminary evaluation of a three-dimensional, customized, and preformed titanium mesh in peri-implant alveolar bone regeneration, J Korean Assoc Oral Maxillofac Surg 40 (4) (2014 Aug) 181–187, https://doi.org/10.5125/jkaoms.2014.40.4.181. Epub 2014 Aug 26. PMID: 25247148; PMCID: PMC4170664.
[8] F. Mandelli, T. Traini, P. Ghensi, Customized-3D zirconia barriers for guided bone regeneration (GBR): clinical and histological findings from a proof-of-concept case series, J. Dent. 114 (2021 Nov) 103780, https://doi.org/10.1016/j.jdent.2021.103780. Epub 2021 Aug 13. PMID: 34400253.
[9] N.K. Soldatos, P. Stylianou, V.P. Koidou, N. Angelov, R. Yukna, G.E. Romanos, Limitations and options using resorbable versus nonresorbable membranes for successful guided bone regeneration, Quintessence Int. 48 (2) (2017) 131–147, https://doi.org/10.3290/j.qi.a37133. PMID: 27834419.
[10] K. Sagheb, E. Schiegnitz, M. Moergel, C. Walter, B. Al-Nawas, W. Wagner, Clinical outcome of alveolar ridge augmentation with individualized CAD-CAM-produced titanium mesh, Int. J. Implant Dent. 3 (2017) 36.
[11] J. Malmström, J. Anderud, P. Abrahamsson, D. Wälivaara, S.G. Isaksson, E. Adolfsson, Guided bone regeneration using individualized ceramic sheets, Int. J. Oral Maxillofac. Surg. 45 (10) (2016) 1246–1252.
[12] S. Arca, A. Olaechea, N. Martin-Morales, F.O. Valle, G. Mendoza-Azpur, Personalized bone regeneration with a novel zirconia membrane: a case report, Int J Periodontics Restorative Dent. 41 (3) (2021 May-Jun) 391–395, https://doi.org/10.11607/prd.4658.
[13] L. Rimondini, L. Cerroni, Torriceni P. CarrasiA, Bacterial colonization of zirconia ceramic surfaces: an in vitro and in vivo study, Int. J. Oral Maxillofac. Implants 17 (2002) 793–798.
[14] A.S. Al-Radha, D. Dymock, C. Younes, D. O'Sullivan, Surface properties of titanium and zirconia dental implant materials and their effect on bacterial adhesion, J. Dent. 40 (2) (2012 Feb) 146–153, https://doi.org/10.1016/j.jdent.2011.12.006. Epub 2011 Dec 9. PMID: 22182466.
[15] A. Petre, C. Balta, H. Herman, S. Gharbia, A. Codreanu, B. Onita-Mladin, N. Anghel-Zurbau, A.G. Hermenean, S.R. Ignat, S. Dinescu, I. Urzica, S. Drafta, L. Oancea, A. Hermenean, A novel experimental approach to evaluate guided bone regeneration (GBR) in the rat femur using a 3D-printed CAD/CAM zirconia space-maintaining barrier, J. Adv. Res. 28 (2020 Jul 23) 221–229, https://doi.org/10.1016/j.jare.2020.07.012. PMID: 33364058; PMCID: PMC7753221.
[16] J. Anderud, P. Abrahamsson, R. Jimbo, S.G. Isaksson, E. Adolfsson, J. Malmström, A. Wennerberg, Guided bone augmentation using ceramic space-maintaining devices: the impact of chemistry, Clin. Cosmet. Invest. Dent. 45 (2015), https://doi.org/10.2147/ccides.78589.
[17] S. Cakir, B.A. Gultekin, M. Karabagli, T.E. Yilmaz, E. Cakir, E.E. Guzel, S. Yalcin, C. Mortellaro, E. Mijiritsky, Histological evaluation of the effects of growth factors in a fibrin network on bone regeneration, J. Craniofac. Surg. 30 (4) (2019 Jun) 1078–1084, https://doi.org/10.1097/SCS.0000000000005339. PMID: 30839463.
[18] J. Kleinheinz, A. Buchter, B. Kruse-Losler, D. Weingart, U. Joos, Incision design in implant dentistry based on vascularization of the mucosa, Clin. Oral Implants Res. 16 (5) (2005) 518–523. PubMed PMID: 16164456 34.
[19] L. Ciocca, G. Lizio, P. Baldissara, A. Sambuco, R. Scotti, G. Corinaldesi, Prosthetically CAD-CAM-guided bone augmentation of atrophic jaws using customized titanium mesh: preliminary results of an open prospective study, J. Oral Implantol. 44 (2) (2018) 131–137.
[20] L.J. Peterson, Antibiotic prophylaxis against wound infections in oral and maxillofacial surgery, J. Oral Maxillofac. Surg. 48 (1990) 617–620.
[21] S. Roehling, M. Astasov-Frauenhoffer, I. Hauser-Gerspach, O. Braissant, H. Woelfler, T. Waltimo, H. Kniha, M. Gahlert, In vitro biofilm formation on titanium and zirconia implant surfaces, J. Periodontol. 88 (3) (2017 Mar) 298–307, https://doi.org/10.1902/jop.2016.160245. Epub 2016 Oct 7. PMID: 27712464.
[22] R. Rodriguez y Baena, D. Zaffe, U.E. Pazzaglia, S. Rizzo, Morphology of peri-implant regenerated bone, in sheep's tibia, by means of guided tissue regeneration, Minerva Stomatol. 47 (12) (1998 Dec) 673–687. PMID: 10207303.
[23] M. Egermann, J. Goldhahn, E. Schneider, Animal models for fracture treatment in osteoporosis, Osteoporos. Int. 16 (Suppl 2) (2005) S129–S138, https://doi.org/10.1007/s00198-005-1859-7.
[24] A. Cipitria, J.C. Reichert, D.R. Epari, S. Saifzadeh, A. Berner, H. Schell, M. Mehta, M.A. Schuetz, G.N. Duda, D.W. Hutmacher, Polycaprolactone scaffold and reduced rhBMP-7 dose for the regeneration of critical-sized defects in sheep tibiae, Biomaterials 34 (38) (2013) 9960–9968, https://doi.org/10.1016/j.biomaterials.2013.09.011.
[25] M.D. Calasans-Maia, M.L. Monteiro, F.O. Áscoli, J.M. Granjeiro, The rabbit as an animal model for experimental surgery, Acta Cir. Bras. 24 (4) (2009 July-Aug).
[26] M.L. Hillier, L.S. Bell, Differentiating human bone from animal bone: a review of histological methods, J. Forensic Sci. 52 (2) (2007 Mar) 249–263, https://doi.org/10.1111/j.1556-4029.2006.00368.x. PMID: 17316219.
[27] J. Abdel-Haq, C.Z. Karabuda, V. Arısan, Z. Mutlu, M. Kürkçü, Osseointegration and stability of a modified sand-blasted acid-etched implant: an experimental pilot study in sheep, Clin. Oral Implants Res. 22 (3) (2011 Mar) 265–274, https://doi.org/10.1111/j.1600-0501.2010.01990.x. Epub 2010 Oct 13. PMID: 20946211.
[28] C. Dellavia, E. Canciani, G. Pellegrini, G. Tommasato, D. Graziano, M. Chiapasco, Histological assessment of mandibular bone tissue after guided bone regeneration with customized computer-aided design/computer-assisted manufacture titanium mesh in humans: a cohort study, Clin. Implant Dent. Relat. Res. 23 (4) (2021 Aug) 600–611, https://doi.org/10.1111/cid.13025. Epub 2021 Jun 17. PMID: 34139056.
[29] J.W. Frame, A convenient animal model for testing bone substitute materials, J. Oral Surg. 38 (1980) 176.
[30] T.A. Najjar, D. Kahn, Comparative study of healing and remodeling in various bones, J. Oral Surg. 35 (375) (1977) 109.