Received: 11 July 2019 | Revised: 28 December 2019 | Accepted: 8 January 2020

DOI: 10.1002/JPER.19-0399

JOURNAL OF Periodontology

ORIGINAL ARTICLE

Influence of collagen membrane on bone quality in titanium mesh reconstructions—Study in rats

Cristine D'Almeida Borges[1*] | **Paulo Esteves Pinto Faria**[2*] | **Paula Gabriela Faciola Pessôa de Oliveira**[1] | **Mariana Sales de Melo Soares**[1] | **Milla Sprone Tavares Ricoldi**[1] | **Monalisa Sena Costa**[2] | **Arthur Belém Novaes Júnior**[1] | **Paulo Tambasco de Oliveira**[1] | **Mário Taba Júnior**[1]

[1]Department of Oral Surgery and Periodontology, University of São Paulo – Ribeirão Preto School of Dentistry, Ribeirão Preto, SP, Brazil

[2]Department of Dentistry, University of Ribeirão Preto, Ribeirão Preto, SP, Brazil

Correspondence
Dr. Paulo Esteves Pinto Faria, Department of Dentistry, University of Ribeirão Preto, Ribeirão Preto, SP 14096-900, Brazil.
Email: pfaria@unaerp.br

[*]Cristine D'Almeida Borges and Paulo Esteves Pinto Faria contributed equally to this work.

Funding information
Coordenação de Nível Superior—Brasil; National Council for Scientific and Technological Development

Abstract

Background: New bone formation and tissue remodeling are the major challenges in implantology today. Titanium meshes have demonstrated reconstructive potential for vertical bone gain. However, the soft tissue healing is technically sensitive to the surgical procedure. The combined usage of collagen membrane and specification of the meshes may ensure greater predictability. Therefore, this study aims to evaluate the influence of collagen membrane on the quality of the new bone formation in guided bone regeneration (GBR) procedures with different titanium meshes.

Methods: Twenty-eight Wistar rats were randomly allocated into four main experimental groups, according to mesh pore size in μm: Group P300 (titanium meshes, with 0.3-mm thickness and 3-mm pore size; n = 7); Group P175 (titanium meshes, with 0.3-mm thickness and 1.75-mm pore size; n = 7); Group P85: (titanium meshes, with 0.04-mm thickness and 0.85-mm pore size; n = 7); Group P15: (titanium meshes. with 0.04-mm thickness and 0.15-mm pore size; n = 7). The femurs of each animal were subdivided into test and control groups: Test: bovine bone graft associated with porcine collagen and collagen membrane was used; control: bovine bone graft associated with porcine collagen was used without association with collagen membrane. Bone quality evaluation by in vivo microtomography and histologic analysis were performed.

Results: Bone volume formation was similar between groups (P >0.05). However, the titanium meshes with pore size >1 mm demonstrated higher mineral bone density in comparison with meshes with pore size <1 mm (P <0.05), regardless of the combined usage of collagen membrane. All groups showed a spongy bone formation after 30 days.

Conclusions: Combined usage of collagen membrane in GBR procedures with titanium mesh did not show improvements in new bone quality in rat femur model. However, titanium mesh pore size specifications may influence bone quality.

KEYWORDS

bone graft, bone regeneration, guided bone regeneration

1 | INTRODUCTION

Dental implants for tooth replacement have been supported by studies,[1–3] but in some cases, residual bone volume (BV) is often insufficient for optimal rehabilitation. Thus, restoration of has become necessary to achieve success in rehabilitation with implants.[4] One of the techniques that allows gain and maintenance of bone tissue is guided bone regeneration (GBR), in which a mechanical barrier is positioned to prevent rapid fibroblasts proliferation, allowing defect osteoprogenitor cells to repopulate the area, initiating bone formation process.[5–7] GBR is recognized as an effective and predictable method to ensure bone formation and, in many cases, is associated with bone grafts or substitutes, which act as osteoconductors.[8]

Studies comparing the best type of barrier to be used have been published in literature for the past 60 years.[4,6,9–12] Occlusive membranes in association with bone graft material demonstrated a gain of bone tissue in several studies.[11–14] Absorbable membranes, for example, maintain a temporary barrier between 6 and 8 weeks,[15] and eliminate need for further surgical procedure for removal. However, it has been shown that occlusive membranes without titanium reinforcement, made from soft materials such as collagen membranes, tend to collapse in large reconstructions because they do not have adequate resistance to space maintenance,[9] which is essential in vertical bone reconstructions.[10]

Titanium mesh barriers have been shown that BV to be created can be planned before the surgical procedure and mesh can be molded to maintain volume during healing period without graft compression by the flap.[16] The presence of pores prevents soft tissue growth internally and allows interstitial fluid diffusion.[14] Studies show satisfactory results for bone reconstructions with titanium meshes.[9,17–19]

However, morphology factors such as pore size and space maintenance, are discussed to ensure greater predictability. Studies in literature have demonstrated space maintenance importance[9,20–23] and also that barriers need to be malleable enough to promote required geometry to ensure bone gain in height and thickness.[17] Furthermore, it has been suggested that larger diameter pore size allows new bone angiogenesis and better nutrient diffusion. But, smaller diameter pore size with completely occlusive meshes may limit the neovascularization process, but also restrict fibrous connective tissue invasion.[9,24] Authors have suggested that bone growth occurs in 50-μm diameter pore size meshes.[25]

Until now, the ideal mechanical barrier for GBR remains in studies, aiming analyze factors such as, occlusivity, stability, ideal pore size, peripheral sealing between barrier, and bone tissue, blood supply required and providing proliferation of osteoprogenitor cells.[26] However, some clinical and preclinical studies have demonstrated limited bone regeneration and soft tissue infiltration when occlusive membranes were not used in association with titanium mesh.[24,27,28]

Lim et al. used titanium mesh after implant installation and, despite high exposure rate, authors did not observe bone regeneration in many samples, suggesting the additional use of occlusive membrane.[28] The presence of soft tissue layer below titanium mesh and lack of mineralization soft tissue evidence, demonstrates possibility on using occlusive membrane.[24,29,30] Despite mechanical barrier needed to obtain GBR criteria, good results were demonstrated after use of different pore size titanium meshes,[8,9,16,30–33] which shows that results are inconclusive.

Thus, the present study aims to evaluate collagen membrane influence in GBR when associated with titanium meshes and, moreover, to evaluate differences in pore size and thickness of titanium meshes.

2 | MATERIALS AND METHODS

2.1 | Ethical aspects and financial support

Initially, this research project was sent to University of Ribeirão Preto ethics committee (CEP/UNAERP), which was duly approved and registered through code ComÉt: 15/2015. ARRIVE guidelines were consulted in reporting this study.[34]

2.2 | Samples characterization

Twenty-eight male Wistar rats (*Rattus norvegicus* albinus) with an average weight of 410.8 g were used in this study, all originated from UNAERP central laboratory. The animals were kept in appropriate plastic boxes with food and water ad libitum before and during experimental period, and remained in the UNAERP laboratory in a 12-hour cycle environment of light and temperature between 22°C and 24°C.

2.2.1 | Experimental groups

Animals were randomly allocated into four main experimental groups, according to titanium meshes used:

- Group P300: titanium meshes,[*] with 0.3-mm thickness and 3-mm pore size (n = 7);
- Group P175: titanium meshes,[†] with 0.3-mm thickness and 1.75-mm pore size (n = 7);
- Group P85: titanium meshes,[‡] with 0.04-mm thickness and 0.85-mm pore size (n = 7);
- Group P15: titanium meshes,[§] with 0.04-mm thickness and 0.15-pore size (n = 7).

Each animal of each experimental group had one femur test and one control as described below:

- Test (T): femur in which bovine bone graft associated with porcine collagen[¶] (BC) and collagen membrane[#] were used.
- Control (C): femur in which only BC was used.

To determine collagen membrane use, right and left femurs of each animal were randomized using the Microsoft Excel 14.0.7 program.[‖] Randomization was only revealed after opening surgical wound and complete visualization of femur.

2.3 | Surgical procedure

After weighing, animals received general anesthesia, obtained by the association of 0.08 mL/100 g of ketamine hydrochloride and 0.04 mL/100 g of xylazine hydrochloride, via intramuscular injection. Subsequently, tricotomy was performed with electric cutter and then local asepsis using a 2% chlorhexidine solution.

Initial incision was made parallel to long axis of the femur, in thigh outer portion, using #3 scalpel handle, mounted with #15 scalpel blade[**] (Fig. 1A). The incision area was established by femur bulkier portion, through palpation. Muscle tissues were secluded until complete periosteum exposure.

After complete visualization of femur, three perforations were drilled with a 1.3-mm bur[††] attached at a 20:1 angle[‡‡] in 500 rpm under irrigation. Perforations did not reach bone marrow to avoid risk of fracture, just to evidence graft area, and had 1 mm of distance between each other, which was measured with a periodontal probe (Figs. 1B and 1C). Then each block of bone graft BC (Fig. 1D) was equally divided into four samples, so that one BC was enough for four femurs. BC sample was then positioned over perforations, and titanium mesh was positioned and fixed with two gingival screws of 5 mm high and 1.6 mm in diameter.[§§] In groups P300 and P175, screws were positioned on opposite sides of femur (Figs. 1E and 1G). In groups P85 and P15, screws were positioned only at the top of femur (Figs. 1F and 1H). Following randomization, 10 mm × 15 mm collagen membrane was positioned above titanium mesh, only in femur test (Fig. 1I). Surgical area was closed using absorbable sutures.[¶¶]

After surgery, animals received a single intramuscular antibiotic dose of 24,000 IU/kg penicillin G-benzathine at a dose of 0.01 mL per 100 g of the body weight and dipyrone 500 mg in water. Twenty-four hours after surgical procedure, animals were anesthetized using the same anesthesia technique previously reported. Then, each animal was positioned for in vivo microcomputed tomography (μCT) analysis, thus determining baseline analysis.

Animals were kept in appropriate plastic boxes throughout 30-day experimental period. After this period, animals were anesthetized for in vivo μCT analysis, determining 30-day analysis. At the end, animals were euthanized with 150 mg/kg 2.5% sodium thiopentate intraperitoneal injection.[##]

2.4 | In vivo computerized microtomography

For images acquisition, SkyScan Model 1176 Microtomograph[***] was used. This device consists of a microfocus X-ray tube with high voltage source (65 kV, 382 μA), resolution of 18 μm, with copper aluminum filter, rotation step (0.7/360°) + 2× off-set camera and 1. Microtomographic analyses of three-dimensional microarchitecture of graft and neoformed bone were performed. Reconstruction software (NRecon v.1.6.9), analysis software (DataViewer version 1.4.1 and CTAn v.1.14.4), and 3D navigation software (CTVol v.2.2) were used for image processing.

In vivo μCT analysis was performed 24 hours (baseline) and 30 days after surgical procedure. Baseline analysis aimed to capture BC initial volume and served as reference for comparisons. All measurements were performed by a masked examiner. Titanium mesh and gingival screws were used as reference to determine region of interest (ROI). Therefore, ROI is the cross-section area selected below titanium

[*] Neodent - Grid Panel 20, diamond pore, Curitiba, PR, Brazil

[†] Neodent - Grid Panel 20, circular pore, Curitiba, PR, Brazil

[‡] Bionnovation Surgitime Titanium, Bauru, SP, Brazil

[§] Bionnovation Surgitime Titanium, Bauru, SP, Brazil

[¶] Bio-Oss Collagen Geistlich Pharma, São Paulo, SP, Brazil

[#] BioGide Geistlich Pharma, São Paulo, SP, Brazil

[‖] Microsoft, Santa Rosa, CL

[**] Swann-Morton, Sheffield, England

[††] Neodent Bone Graft Kit, São Paulo, SP, Brazil

[‡‡] Koncept-Kavo, Joinville, SC, Brazil

[§§] Neodent gingival screw, Curitiba, PR, Brazil

[¶¶] Vicryl Ethicon 5.0, Johnson Prod., São José dos Campos, Brazil

[##] Thiopentax, Cristália, Brazil

[***] Bruker-microCT SkyScan, Kontich, Belgium

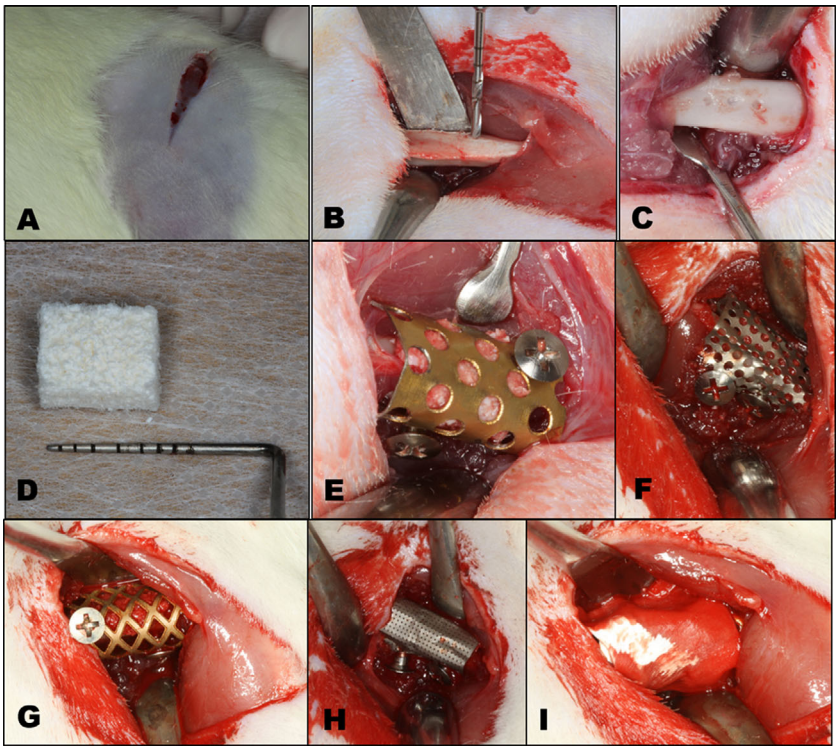

FIGURE 1 Illustration images of surgical procedure. **A**) initial incision; **B and C**) perforations in femur; **D**) Bio-Oss Collagen; **E**) Group P175 titanium mesh; **F**) Group P 300 titanium mesh; **G**) Group P85 titanium mesh; **H**) Group P15 titanium mesh; **I**) Collagen membrane above titanium mesh

mesh (graft or neoformed bone tissue). Adding all collective ROIs, volume of interest (VOI) was obtained, representing a selected 3D volume. All volumetric measurements were performed in a VOI and were determined according to the following definitions: tissue volume (TV): total VOI between titanium mesh and gingival screws; BV: binary total volume inside VOI done by the program to identify bone tissue; % of bone formation (BV/TV); and bone mineral density (BMD): measured in g/cm and refers to mineral amount in bone.

2.5 | Histological processing

After euthanasia, right and left femurs were fixed in 10% neutral formalin for 48 hours. Decalcification was performed by means of 10% EDTA solution (pH 7.0, EDTA), with changes from two to three times a week, for an average period of 60 days, until complete decalcification. Then, samples were washed in running water for 24 hours. In each sample, screw and titanium mesh were removed without damaging surrounding tissues. Dehydration and diaphanization process was started, through successive baths in increasing alcohols and xylol series. After paraffin inclusion, serial sections 5-μm thick were obtained from the most central point of the graft area, using electronic microtome.* Harris hematoxylin and Mason trichrome technique was used for staining.

2.6 | Histological analyses

Sections were analyzed under light microscopy for evaluation of bone quality. Images were captured by a Leica DC 300F camcorder† coupled to a Leica MZFL III stereomicroscope.‡ In a descriptive way, cellular pattern and remaining biomaterial were evaluated.

2.6.1 | Characterization of bone type

Bone type definition was determined by image analysis, captured with 2.5× magnification objective. For new bone quantification, a computerized grid measuring 2 cm × 2 cm and containing six columns and six lines was used. The presence of bone formation was determined by intersection points (%) between horizontal and vertical lines. These measures were made at three points: at both peripheral points and at the most central point. The final percentage was determined as average.

2.6.2 | Measurement of BC/femur interface

Using a 10× magnification objective image, newly formed bone/femur interface was measured in central region of the grafted area, which was considered representative. For this, Image J program§ was used.

* Microm HM 335E, SA, Germany

† Leica Microsystems, Nussloch, Germany

‡ Leica Microsystems, Nussloch, Germany

§ National Institutes of Health, Bethesda, MD

TABLE 1 μCT results

Groups		Tissue volume (TV, mm^3)	Bone volume (BV, mm^3)	BV/TV (%)	Bone mineral density (BMD)
P300	Test	125.53 ± 24.85	27.22 ± 5.28	21.95 ± 3.91	0.10 ± 0.03[a]
	Control	135.91 ± 27.77	35.95 ± 7.84	26.77 ± 4.57	0.10 ± 0.03[b]
P175	Test	112.3 ± 28.32	32.58 ± 13.12	30.45 ± 14.20	0.10 ± 0.02[c]
	Control	121.39 ± 15.22	33.70 ± 16.46	27.54 ± 11.26	0.11 ± 0.2[d]
P85	Test	120.10 ± 38.32	21.48 ± 5.85	18.27 ± 3.89	0.05 ± 0.01
	Control	124.81 ± 29.16	26.41 ± 9.92	22.26 ± 9.64	0.04 ± 0.01
P15	Test	84.06 ± 34.19	22.8 ± 9.02	27.24 ± 5.09	0.06 ± 0.02
	Control	87.95 ± 21.94	25.87 ± 8.15	30.85 ± 12.33	0.05 ± 0.01

Tissue volume (mm^3), bone volume (BV) (mm^3), and relationship between BV and tissue volume (%).

No statistically significant differences were observed. Bone mineral density (g/cm^3). Significant differences between meshes pore size >1 mm and meshes pore size <1 mm (ANOVA, Tukey, P <0.05). Level of significance was considered 95% (ANOVA, Tukey).

[a]Statistical differences between P300-T X P85-T, P85-C e P15-C.

[b]Statistical differences between P300-C X P85-T, P85-C e P15-C.

[c]Statistical differences between P175-T X P85-T, P85-C e P15-C.

[d]Statistical differences between P175-C X P85-T, P85-C, P15-T, P15-C.

T = Test, C = Control.

2.7 | Analysis of results

Statistical analysis was performed with GraphPad Prism version 7 statistical program.* Microtomographic data and histological measurements of bone type and BC/femur interface were compared between groups through ANOVA two criteria test, with Tukey post test. A significance level of 5% was used for all statistical analyses.

3 | RESULTS

Surgical procedure occurred with some intercurrences. Group P175 and P85 lost two animals in each group. P15 group lost one animal, totaling 23 animals in the experiment. Excluded animals were euthanized because of fractures or because they became weakened during healing period. In this way, experimental groups presented sample number as: group P300, n = 7; Group P175, n = 5; Group P85, n = 5; Group P15, n = 6.

3.1 | Volumetric measurements

Numerical values were distributed in Table 1 for each three-dimensional parameter evaluated. Initially, a lower tissue graft volume was observed in the P300 and P175 groups. After the 30-day period, tissue volume was similar in all groups. In all volumetric parameters after 30 days, no statistically significant differences were observed.

In relationship to BMD after 30 days, denser tissue was observed in the titanium meshes with pore size >1 mm ($P < 0.05$), with mean and SD of 0.1 ± 0.03 and 0.11 ± 0.02 in control femur, and 0.01± 0.03 and 0.10 ± 0.02 in test femur, for groups P300 and P175, respectively (Table 1). Tridimensional reconstructions demonstrated titanium meshes, screws, and bone graft set at baseline and 30 days.

3.2 | Histological analysis

3.2.1 | Histological description

Bone neoformation pattern was similar in all groups. Presence of bone formation was observed, mainly in peripheral graft area region, besides spaces with absence of bone formation, corresponding to disorganized connective tissue and blood cells. In addition, presence of isolated osteoclasts, osteocytes in gaps, empty gaps corresponding to remnant graft, osteoblasts aligned on new bone surface and graft can be observed (Fig. 2).

3.2.2 | Bone type analysis

Percentage of non-mineralized areas were 37 ± 18.8, 40.5 ± 15.9, 45.1 ± 10.9, 33.3 ± 7.1 for control femur; 42.9 ± 9.5, 31.7 ± 10, 45 ± 21.12, 45.3 ± 26.8 for test femur, at P300, P175, P85, and P15, respectively. No statistically significant differences were observed in intergroup comparisons.

3.2.3 | BC/femur interface

In grafted central region, new bone formation was in close contact with femur in 73.5 ± 26.5, 78.6 ± 22.1, 80.1 ± 11.7, 88.3 ± 9.7 for P300, P175, P85, and P15, respectively. No statistically significant differences were observed in intergroup comparisons.

* GraphPad Software, San Diego, CA

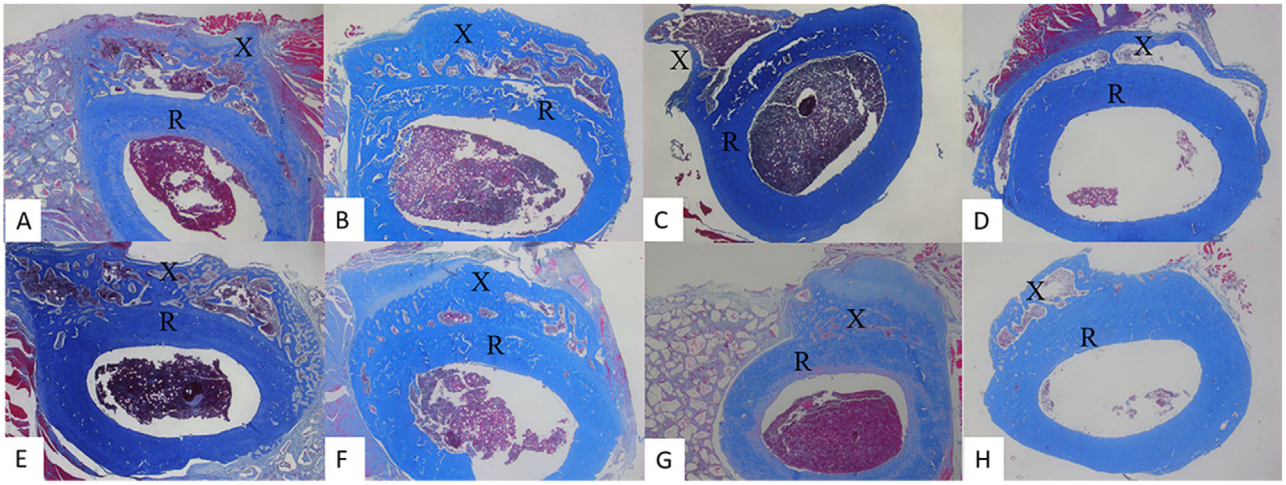

FIGURE 2 Histologic samples. **A**) Bone formation in Group P300, femur test; **B**) Bone formation in Group P175, femur test; **C**) Bone formation in Group P85, femur test; **D**) Bone formation in Group P15, femur test; **E**) Bone formation in Group P300, femur control; **F**) Bone formation in Group P175, femur control; **G**) Bone formation in Group P85, femur control; **H**) Bone formation in Group P15, femur control. For all images, original magnification of 2.5×. X = grafted area; R = femur; stain = Harris hematoxylin and Mason trichrome

4 | DISCUSSION

In this present study, four types of titanium mesh were associated with bone graft and collagen membrane to evaluate additional use of occlusive membrane influence in titanium mesh reconstructions and evaluate the influence of mesh thickness and pore size in new bone formation. Pore size ranged from 15 to 300 μm, and thickness from 4 to 30 μm. The model used in the study was a rat femur, due to greater disposition in adapt titanium meshes. In addition, an exophytic bone formation model, to gain bone tissue out of skeletal framework, was used as opposed to bone formation surrounded by defect walls or alveoli. Thus, new bone tissue was formed over the outer surface of femur, without creating a defect.

The collagen membrane used in the present study is absorbable, composed of type I and III porcine collagen, in a double layer. The inner surface, facing bone tissue, consists of disorganized collagen fibers, which allow osteoblasts proliferation. The outer surface facing soft tissue is dense and prevents fibroblasts proliferation.[35] An in vivo study in rat femur demonstrated that membrane absorption process is initiated after 4 weeks and completed after 6 weeks.[15]

It was suggested that occlusive barrier uses in association with titanium meshes could minimize soft tissue formation in bone defect, aiming to obtain a biomaterial or combination of biomaterials that would allow bone tissue formation of better quality.[8,9,16,30,33] Shin et al.[26] evaluated the effect of using collagen membrane in conjunction with 50-μm titanium mesh and allogeneic bone graft in rabbit calvaria GBR. After 8-week histomorphometric analysis, authors observed new bone formation of 10.81% ± 5.38% and 15.16% ± 6.76% in groups without and with collagen membrane, respectively, and did not find statistically significant differences.[26] Our results showed similarities with the authors. In test femurs, percentage of bone formation was observed, ranging from 18.3% to 30.5%; in control femurs, variation was observed from 22.3% to 30.9%, according to mesh pore size. Likewise, no statistical differences were observed in intra- and intergroup comparisons. In spite of different analyses and numerical percentage of newly formed bone, our results are compatible with previous results, suggesting that in bone graft presence, use of collagen membrane does not interfere with final bone volume.

On the other hand, Lim et al.[36] compared use of titanium mesh alone and in association with collagen membrane, without bone graft. They observed that below titanium mesh, there was dense fibrous tissue when membrane was not used. Also, authors observed mesh exposure.[36] These results are not in agreement with Shin et al. and our results, probably due to experimental model difference and presence of bone graft, which was recommended in previous studies after demonstration of decrease in new bone formation.[26]

Bone graft used in this study is composed by a combination of inorganic bovine bone particles and a purified collagen matrix at 9:1 (BC). When compared with β-tricalcium phosphate for GBR with collagen membrane in rat calvaria, BC demonstrated a small amount of newly formed bone besides presence of structures similar to bone tissue, but without osteocytes, suggesting to be graft remnants. They also observed that, after 10 weeks, BC did not develop complete closure of defect.[37] Araújo et al. observed similar results and identified 40% filling with new bone after 4 weeks in dogs alveoli grafted with BC.[38]

After histological analysis, our results showed that percentage of non-mineralized areas ranged from 33.3% to 45.1%. According to Bonucci,[39] the main difference between

compact and spongy bone depends on its porosity. The percentage of voids related to osteon channels, osteocyte canaliculi, and Volkmann channels will determine this porosity. In compact bones, voids ranged from 5% to 30%. If presence of voids is >30%, bone may be characterized as spongy, with density ranging from 0.1 to 0.9 g/cm^3. Results of BMD and percentage of non-mineralized areas suggest that new bone type was spongy for all groups, regardless of collagen membrane and pore size. In addition, gaps of empty osteocytes were also observed, suggesting remaining graft after 30 days, according to Kato et al.[37]

When evaluating BC/femur interface, it was observed that 73.5% to 88.3% of this interface was complete, and there was no statistically significant difference between groups. This suggests that, even in exophytic areas, BC may be integrated into femoral bone tissue. Also, 33% of samples with pore size >1 mm and 20% of samples with pore size <1 mm presented isolated formation of bone tissue in concentric lamellae (osteon). It was suggested that, to obtain osteon formation, it would be ideal to use meshes with pore size of at least 150 μm.[25] In our results, even in low proportion, it was possible to observe osteons formation in intermediate stages, in all pore size, with or without collagen membrane.

Through in vivo μCT results, it was observed that there was no volumetric difference in newly formed bone using different pore size. Numerically, P15 mesh showed a higher percentage of BV (30.9 ± 12.33), despite its thickness, when compared with other groups (P300 = 26.8 ± 4.6, P175 = 27.5 ± 11.3, P85 = 22.3 ± 9.6). Rakhmatia et al.[9] also did not observe significant difference in volumetric parameters due to pore size after 8 weeks in rats. In this case, titanium mesh was used without bone graft in a 7 mm defect in rat calvaria. Also, they observed that mesh thickness between 100 and 200 μm would be ideal for larger bone reconstructions, avoiding collapse within the defect.

In relationship to BMD, it was observed that meshes with larger pore size presented higher density, and the difference was statistically significant. Studies suggested that titanium meshes with larger pore size would have a lower mineral density, different from demonstrated by our results.[9] Numerically, Rakhmatia et al[9] observed density between 8 and 10 g/cm^3 in meshes with pore size between 20 and 100 μm in rats. Our values ranged from 0.04 to 0.11 g/cm^3, and in P300 and P175 meshes density was 0.10 and 0.11 g/cm^3 (control), respectively, and 0.11 g/cm^3 in both test femurs. However, considering bone graft characteristics, reduced BMD and absence of complete filling of graft area may be related to newly formed BC pattern. Rakhmatia et al.[9] also showed that, BV meshes showed no differences when comparing healing periods of 4 and 8 weeks; they suggest that after healing period, BV will be similar in meshes evaluated, justifying a 30-day healing period.

The present study demonstrated limitations in relationship to thinner meshes (P15 and P85) stabilization, which may have suffered interference during animal movement. Even so, volumetric analyses showed results compatible with those obtained in groups P175 and P300, which had thickness of 30 μm. It has been suggested in the literature that meshes with thickness <50 μm showed a tendency to collapse.[9,18,32] Our results showed no interference of 30-μm thickness. We suggest that, when using thinner meshes for large vertical bone reconstructions, screw tents should be used to avoid collapse and reduction of bone gain.[40] Different rates of bone formation and BMD observed in the literature studies and our results suggest that mesh pore size may not be the only factor interfering with new bone formation. Factors such as physical characteristics of material, thickness, chemical composition, biocompatibility,[41] besides pore shape, manufacturer, vascularization, and use of bone graft, serve as a framework.

5 | CONCLUSIONS

Despite the experimental study limitations and supported by the findings of this investigation, data demonstrated that combined usage of collagen membrane in GBR procedures with titanium mesh did not show improvements in new bone quality in a rat femur model. However, titanium mesh pore size specifications have influence in bone quality.

ACKNOWLEDGMENTS

This study was financed in part by the Coordenação de Nível Superior—Brasil (CAPES)—finance code 001 and National Council for Scientific and Technological Development (CNPq) Research Productivity Fellowship – process number 307917/2017-7 to Dr. Taba Jr. This study received biomaterials donation from the following companies: Neodent (Curitiba, PR Brazil), Geistlisch Pharma (São Paulo, SP, Brazil), and Bionnovation Biomedical (Bauru, SP, Brazil). Study sponsors had no involvement in study design, analysis, interpretation data, and manuscript writing. The authors report no conflicts of interest related to this study.

AUTHOR CONTRIBUTIONS

Cristine D'Almeida Borges contributed in conception or design of the work, data collection, data analysis and interpretation, and drafting the article; Paulo Esteves Pinto Faria, conception or design of the work, data collection, data analysis and interpretation, and drafting the article; Paula Gabriela Faciola Pessôa de Oliveira, data collection and critical revision of the article; Mariana Sales de Melo Soares, data collection and critical revision of the article; Milla Sprone Tavares Ricoldi, data analysis and interpretation, and critical revision of the article; Monalisa Sena Costa, data collection and

critical revision of the article; Arthur Belém Novaes Júnior, critical revision of the article and final approval of the version to be published; Paulo Tambasco de Oliveira, data analysis and interpretation, and critical revision of the article; and Mário Taba Júnior, conception or design of the work, drafting the article, critical revision of the article, and final approval of the version to be published.

REFERENCES

1. Adell R, Eriksson B, Lekholm U, Branemark PI, Jemt T. Long-term follow-up study of osseointegrated implants in the treatment of totally edentulous jaws. *Int J Oral Maxillofac Implants*. 1990;5:347-359.
2. Branemark PI, Adell R, Albrektsson T, Lekholm U, Lundkvist S, Rockler B. Osseointegrated titanium fixtures in the treatment of edentulousness. *Biomaterials*. 1983;4:25-28.
3. Adell R. Clinical results of osseointegrated implants supporting fixed prostheses in edentulous jaws. *J Prosthet Dent*. 1983;50:251-254.
4. Rasia-dal Polo M, Poli PP, Rancitelli D, Beretta M, Maiorana C. Alveolar ridge reconstruction with titanium meshes: a systematic review of the literature. *Med Oral Patol Oral Cir Bucal*. 2014;19:e639-646.
5. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg*. 1988;81:672-676.
6. Hammerle CH, Karring T. Guided bone regeneration at oral implant sites. *Periodontol 2000*. 1998;17:151-175.
7. Hurley LA, Stinchfield FE, Bassett AL, Lyon WH. The role of soft tissues in osteogenesis. An experimental study of canine spine fusions. *J Bone Joint Surg Am*. 1959;41-A:1243-1254.
8. Artzi Z, Dayan D, Alpern Y, Nemcovsky CE. Vertical ridge augmentation using xenogenic material supported by a configured titanium mesh: clinicohistopathologic and histochemical study. *Int J Oral Maxillofac Implants*. 2003;18:440-446.
9. Rakhmatia YD, Ayukawa Y, Furuhashi A, Koyano K. Microcomputed tomographic and histomorphometric analyses of novel titanium mesh membranes for guided bone regeneration: a study in rat calvarial defects. *Int J Oral Maxillofac Implants*. 2014;29:826-835.
10. Hammerle CH, Jung RE. Bone augmentation by means of barrier membranes. *Periodontol 2000*. 2003;33:36-53.
11. Zitzmann NU, Scharer P, Marinello CP, Schupbach P, Berglundh T. Alveolar ridge augmentation with Bio-Oss: a histologic study in humans. *Int J Periodontics Restorative Dent*. 2001;21:288-295.
12. Zitzmann NU, Scharer P, Marinello CP. Long-term results of implants treated with guided bone regeneration: a 5-year prospective study. *Int J Oral Maxillofac Implants*. 2001;16:355-366.
13. Hammerle CH, Lang NP. Single stage surgery combining transmucosal implant placement with guided bone regeneration and bioresorbable materials. *Clin Oral Implants Res*. 2001;12:9-18.
14. Strietzel FP, Khongkhunthian P, Khattiya R, Patchanee P, Reichart PA. Healing pattern of bone defects covered by different membrane types—a histologic study in the porcine mandible. *J Biomed Mater Res B Appl Biomater*. 2006;78:35-46.
15. Bunyaratavej P, Wang HL. Collagen membranes: a review. *J Periodontol*. 2001;72:215-229.
16. Miyamoto I, Funaki K, Yamauchi K, Kodama T, Takahashi T. Alveolar ridge reconstruction with titanium mesh and autogenous particulate bone graft: computed tomography-based evaluations of augmented bone quality and quantity. *Clin Implant Dent Relat Res*. 2012;14:304-311.
17. Rakhmatia YD, Ayukawa Y, Furuhashi A, Koyano K. Current barrier membranes: titanium mesh and other membranes for guided bone regeneration in dental applications. *J Prosthodont Res*. 2013;57:3-14.
18. Eisig SB, Ho V, Kraut R, Lalor P. Alveolar ridge augmentation using titanium micromesh: an experimental study in dogs. *J Oral Maxillofac Surg*. 2003;61:347-353.
19. Gutta R, Baker RA, Bartolucci AA, Louis PJ. Barrier membranes used for ridge augmentation: is there an optimal pore size. *J Oral Maxillofac Surg*. 2009;67:1218-1225.
20. Simion M, Jovanovic SA, Tinti C, Benfenati SP. Long-term evaluation of osseointegrated implants inserted at the time or after vertical ridge augmentation. A retrospective study on 123 implants with 1-5 year follow-up. *Clin Oral Implants Res*. 2001;12:35-45.
21. Brunel G, Brocard D, Duffort JF, et al. Bioabsorbable materials for guided bone regeneration prior to implant placement and 7-year follow-up: report of 14 cases. *J Periodontol*. 2001;72:257-264.
22. von Arx T, Kurt B. Implant placement and simultaneous periimplant bone grafting using a micro titanium mesh for graft stabilization. *Int J Periodontics Restorative Dent*. 1998;18:117-127.
23. Maiorana C, Santoro F, Rabagliati M, Salina S. Evaluation of the use of iliac cancellous bone and anorganic bovine bone in the reconstruction of the atrophic maxilla with titanium mesh: a clinical and histologic investigation. *Int J Oral Maxillofac Implants*. 2001;16:427-432.
24. Her S, Kang T, Fien MJ. Titanium mesh as an alternative to a membrane for ridge augmentation. *J Oral Maxillofac Surg*. 2012;70:803-810.
25. Welsh RP, Pilliar RM, Macnab I. Surgical implants. The role of surface porosity in fixation to bone and acrylic. *J Bone Joint Surg Am*. 1971;53:963-977.
26. Shin SI, Herr Y, Kwon YH, Chung JH. Effect of a collagen membrane combined with a porous titanium membrane on exophytic new bone formation in a rabbit calvarial model. *J Periodontol*. 2013;84:110-116.
27. Park SH, Lee KW, Oh TJ, Misch CE, Shotwell J, Wang HL. Effect of absorbable membranes on sandwich bone augmentation. *Clin Oral Implants Res*. 2008;19:32-41.
28. Lim HC, Kim MS, Yang C, et al. The effectiveness of a customized titanium mesh for ridge preservation with immediate implantation in dogs. *Clin Implant Dent Relat Res*. 2015;17(suppl 2):e652-660.
29. Louis PJ, Gutta R, Said-Al-Naief N, Bartolucci AA. Reconstruction of the maxilla and mandible with particulate bone graft and titanium mesh for implant placement. *J Oral Maxillofac Surg*. 2008;66:235-245.
30. Proussaefs P, Lozada J. Use of titanium mesh for staged localized alveolar ridge augmentation: clinical and histologic-histomorphometric evaluation. *J Oral Implantol*. 2006;32:237-247.
31. Dupoirieux L, Pourquier D, Picot MC, Neves M. Comparative study of three different membranes for guided bone regeneration of rat cranial defects. *Int J Oral Maxillofac Surg*. 2001;30:58-62.
32. Zellin G, Linde A. Effects of different osteopromotive membrane porosities on experimental bone neogenesis in rats. *Biomaterials*. 1996;17:695-702.
33. Lundgren AK, Sennerby L, Lundgren D. Guided jaw-bone regeneration using an experimental rabbit model. *Int J Oral Maxillofac Surg*. 1998;27:135-140.

34. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *J Pharmacol Pharmacother*. 2010;1:94-99.
35. Zitzmann NU, Naef R, Scharer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. *Int J Oral Maxillofac Implants*. 1997;12:844-852.
36. Lim HC, Lee JS, Choi SH, Jung UW. The effect of overlaying titanium mesh with collagen membrane for ridge preservation. *J Periodontal Implant Sci*. 2015;45:128-135.
37. Kato E, Lemler J, Sakurai K, Yamada M. Biodegradation property of beta-tricalcium phosphate-collagen composite in accordance with bone formation: a comparative study with Bio-Oss Collagen(R) in a rat critical-size defect model. *Clin Implant Dent Relat Res*. 2014;16:202-211.
38. Araujo MG, Liljenberg B, Lindhe J. Dynamics of Bio-Oss Collagen incorporation in fresh extraction wounds: an experimental study in the dog. *Clin Oral Implants Res*. 2010;21:55-64.
39. Bonucci E. Basic composition and structure of bone. In: An YH, Draughn RA, ed. *Mechanical Testing of Bone and the Bone-Implant Interface*. Boca Raton, FL: CRC Press; 2000:3-22.
40. Jegham H, Masmoudi R, Ouertani H, Blouza I, Turki S, Khattech MB. Ridge augmentation with titanium mesh: a case report. *J Stomatol Oral Maxillofac Surg*. 2017;118:181-186.
41. Galgut P, Pitrola R, Waite I, Doyle C, Smith R. Histological evaluation of biodegradable and non-degradable membranes placed transcutaneously in rats. *J Clin Periodontol*. 1991;18:581-586.

How to cite this article: Borges CD, Faria PEP, Pessôa de Oliveira PGF, et al. Influence of collagen membrane on bone quality in titanium mesh reconstructions—Study in rats. *J Periodontol*. 2020;91:1673–1681. https://doi.org/10.1002/JPER.19-0399