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RESEARCH ARTICLE

Collagen membrane from bovine pericardium for treatment of long bone defect

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Abstract

The treatment of bone regeneration failures has been constantly improved with the study of new biomaterials. Techgraft® is a collagen membrane derived from bovine pericardium, which has been shown to have biocompatibility and effectiveness in tissue repair. However, its use in orthopedics has not yet been evaluated. Therefore, the purpose of this study was to characterize a bovine pericardium collagen membrane and evaluate the effects of its use in the regeneration of a bone defect in rat tibia. Scanning electron microscopy, atomic force microscopy, weight lost and water uptake tests, and mechanical test were performed. Afterwards, the membrane was tested in an experimental study, using 12 male Sprague Dawley rats. A bone defect was surgically made in tibiae of animals, which were assigned to two groups ($n = 6$): bone defect treated with collagen membrane (TG) and bone defect without treatment (CONT). Then, tibiae were submitted to micro-CT. The membranes preserved their natural collagen characteristics, presenting great strength, high water absorption, hydrophilicity, and almost complete dissolution in 30 days. In the experimental study, the membrane enhanced the growth of bone tissue in contact with its surface. A higher bone volume and trabeculae number and less trabecular space was observed in bone defects of the membrane group compared to the control group at 21 days. In conclusion, the Techgraft membrane seems to have favorable characteristics for treatment of long bone repair.

KEYWORDS
biomaterial, bone repair, collagen, pericardium

1 | INTRODUCTION

Bone healing is a natural event but, in some situations, the process is impaired. For such cases, diverse procedures and materials have been studied to improve bone repair. A target of interest in biomaterials, collagen has been studied for being a natural constituent of the body, and it has been shown to support cell growth with low immune response.[1] Collagen membranes are used in bone repair as a physical barrier that promotes bone formation. However, some disadvantages of collagen membranes are their difficult production, poor mechanical properties, and fast degradation.[2]

The Techgraft® is a biocompatible and absorbable collagen membrane produced from bovine pericardium that has shown benefits for the repair of calvaria[3] and heart valves.[4] Its method of production emerged from studies by Goiss et al.[5] and the membrane might be an alternative for orthopedic applications. However, the characteristics of a biomaterial affect the possibility of its use in tissue repair,[6] and thus the evaluation of the effects of Techgraft on bone healing in vivo

is needed. As bone undergoes microstructural changes during repair, the assessment of this process enables interpreting the healing progress and the treatment efficacy.

Thus, the aim of this study was to (1) assess the characteristics of Techgraft membranes and (2) evaluate the in vivo microstructural outcome of bone healing using Techgraft.

2 | MATERIALS AND METHODS

2.1 | Membranes characterization

Techgraft bovine pericardium collagen membranes were acquired from Baumer® Industry, Brazil. The membranes were prepared following the protocol described by Goissis et al.[5] The pericardium samples were treated in alkaline solution (3 ml/g) with 6% dimethyl sulfoxide, salts (3.0 M chloride and 0.9 M sulfate), alkali metals (1.2 M K+ and 2.0 M Na+), and an alkaline earth metal (0.91 M Ca++). The resulting material was equilibrated in solution (6 ml/g) containing 0.13 M chloride, 0.17 M sulfate, 0.13 M K+, 0.7 M Na+, and 0.7 M Ca++. The salts were removed by consecutive washing in 3% boric acid, 0.3% ethylenediaminetetraacetic acid pH 11, and deionized water. The membranes were lyophilized and supplied in aseptic packaging. A total of 98 membranes were used in this study, in two different dimensions: 62 membranes in dimensions of 10 × 10 mm, and 36 in dimensions of 30 × 5 mm. They were distributed according to analysis: Scanning electron microscopy (SEM) ($n = 1$), atomic force microscopy (AFM) ($n = 1$), water uptake ($n = 30$), weight loss ($n = 30$), mechanical test ($n = 30$), and experimental study ($n = 6$). The membranes characterization was performed in I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho.

2.1.1 | Scanning electron microscopy

Scanning electron microscopy (JSM-6010 LV, JEOL, Japan) was used to analyze qualitatively the morphology of intact membrane and at each time-point after weight loss test. Samples were mounted on stubs and sputter-coated with a layer of gold for observation at 1000x magnification. The membranes were observed over their entire area to verify homogeneity before taking the images. All images were taken at the mid-point of the specimen for consistency.

2.1.2 | Atomic force microscopy

Atomic force microscopy (Dimension Icon, Bruker) was used to evaluate the topography of membranes. The intact membrane was assessed in areas of 1 × 1 μm in two different points of the surface of the same specimen. Two points were chosen at random to observe homogeneity. The morphology of collagen fibrils was qualitatively evaluated, the average surface roughness was quantitatively evaluated. The mean of fibril transverse length was obtained by the measures of three different fibrils using the NanoScope Analysis Software (Bruker).

2.1.3 | Water uptake

Collagen membranes of 10 × 10 mm ($n = 30$) were immersed in 1 ml of phosphate-buffered saline (PBS, pH 7.0) in an oven set at 37°C for 1, 3, 7, 14, and 30 days. Six membranes were maintained in PBS for each time-point: 1 day ($n = 6$), 3 days ($n = 6$), 7 days ($n = 6$), 14 days ($n = 6$), and 30 days ($n = 6$). At day 1, the same membranes were evaluated at 1, 2, 4, 6, 8, and 24 h. All specimens were weighed in prior to place them in the saline (i_w). At the end of each time-point, the membranes were removed from the PBS, blotted with filter paper to remove liquid excess and reweighed immediately (f_w). The swelling (S) was calculated using the following formula:

$$S(\%) = (f_w - i_w)/i_w \times 100,$$

where f_w = final weight (mg) and i_w = initial weight (mg).

For the measures, a balance with 0.001 g precision was used (Denver), and the weight was determined in mg.

The liquid pH was measured using a pH meter at the end of each time-point.

2.1.4 | Weight loss

Other 30 samples were submitted to weight loss analysis. Membranes (10 × 10 mm) pre-weighted (iw) were immersed in 1 ml of PBS (pH 7.0) at 37°C for 1, 3, 7, 14, or 30 days ($n = 6$ for each time-point). At the end of time-points the membranes were removed from the PBS solution, blotted with filter paper and dried at room temperature for 6 h, and then reweighed (fw). The weight loss (W) was calculated using the following formula:

$$W(\%) = (f_w - i_w)/i_w \times 100,$$

where f_w = final weight (mg) and i_w = initial weight (mg).

2.1.5 | Mechanical test

The mechanical properties of collagen membranes were studied using an Instron 5543 equipped with a 5 kN load cell, loading rate of 1 mm/min and Bluehill® Universal Materials Testing Software. The membranes, with a dimension of 30 × 5 mm, were fixed in plastic frames resulting in specimens with a planar dimension of 5 mm (membrane width) × 10 mm of gauge length corresponding to the gap between the parallel strips of the plastic frame (Figure 1). The plastic frame was cut along the discontinuous lines before testing. Figure 2 represents a membrane submitted to the tensile test.

The tensile test was performed in intact membranes ($n = 6$) and at two of the time-points of the water uptake (1 and 3 days) and weight loss tests (1 and 3 days), $n = 6$ for each time-point. Membranes were previous mounted in the plastic frames and so immersed in PBS during 1 or 3 days. At the end of time-points of water uptake protocol, the membranes were tested immediately after removed from liquid. For the weight loss, the membranes were removed from liquid, dried at room temperature for 6 h and then tested. The thickness of each membrane was measured using a digital caliper (Mitutoyo®) and this value was used for determining the cross-sectional area of the membranes. By the test, tensile strength (MPa), fracture strain (%) and Young's Modulus (MPa) were obtained.

2.2 | Experimental study

The experimental study was approved by the local Ethics Committee for Animal Experimentation of University of São Paulo under process number 188/2017. Twelve Sprague Dawley rats 9 weeks old were used. They were maintained under standard laboratory conditions with temperature at 22 ± 2°C, 55 ± 5% humidity, 12-h light and dark cycles, and free access to water and food. The animals were assigned to two groups ($n = 6$): control group, which was not treated (CONT) and treatment group, which received the Techgraft membrane (TG). After 1 week of adaptation, the animals of both groups were submitted to a surgical procedure to create a circular bone defect.[7]

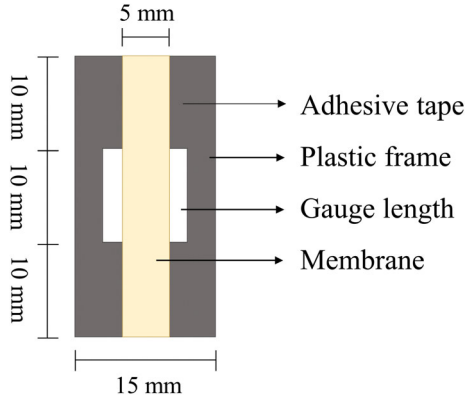

FIGURE 1 Schematic figure to illustrate the membrane (30 × 5 mm) fixed in the plastic frame

2.2.1 | Surgical procedure

The animals were anesthetized with an intramuscular injection of xylazine (10 mg/kg) and ketamine (70–80 mg/kg) and positioned with an external rotation of the hip and triple flexion. A skin and muscle incision was made 10 mm under the knee junction, exposing the tibia. With a 2.9-mm external diameter trephine (2.7-mm internal diameter) coupled to an electric motor, set to 3000 rpm (Micro Motor 210/105 L, Strong®, Korea) a unicortical defect was produced in the medial face of the proximal region of the tibia, under physiological solution irrigation. The bone defect of the TG group animals was covered with a collagen membrane, cut into a size of 5 × 10 mm, fixed below the musculature (Figure 3). In the CONT group animals the defect did not receive treatment.

Subsequently, muscle and skin were sutured. The animals received analgesic for 3 days post-surgery. After experimental times, the animals were euthanized with an overdose of anesthetic, and right tibiae were dissected and stored in 70% ethanol to be analyzed by micro-CT.

2.2.2 | Microcomputed tomography

The micro-CT characterization was performed using a SkyScan 1272 scanner (Bruker Micro-CT, Belgium). Samples were scanned using the

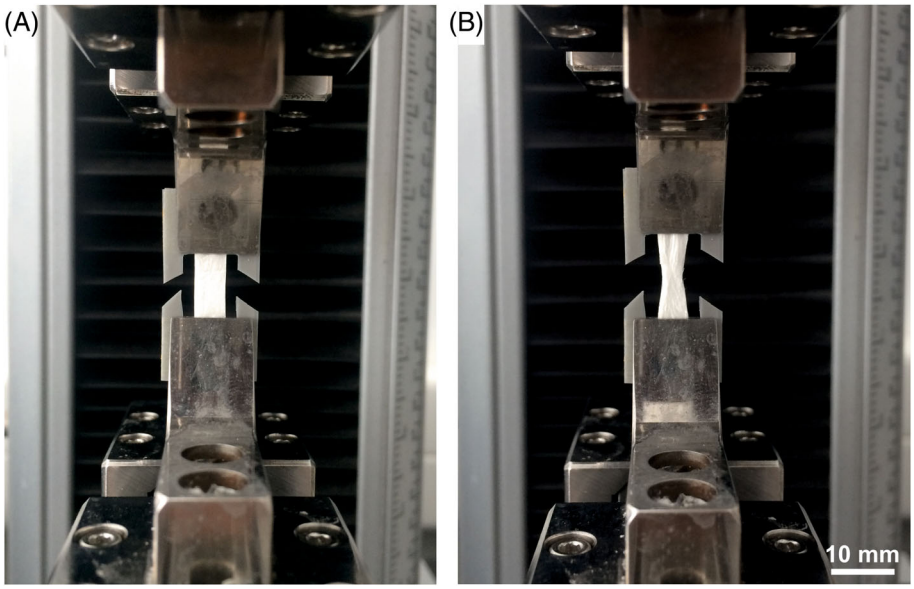

FIGURE 2 (A) Membrane positioned in the mechanical test machine. (B) Membrane being submitted to the tensile test

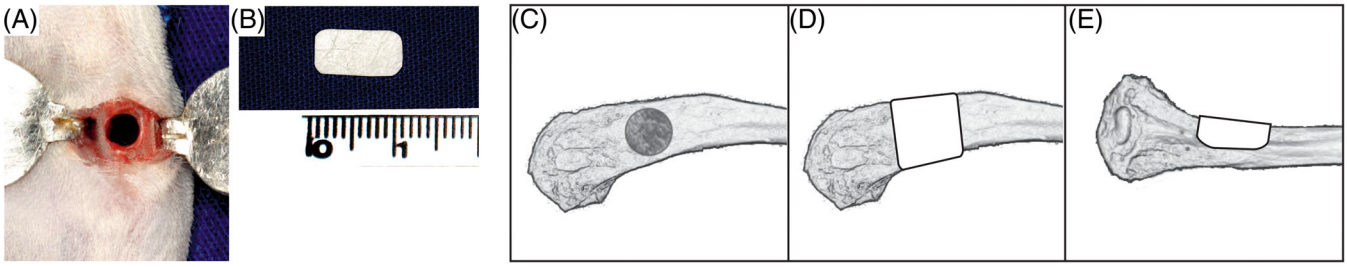

FIGURE 3 (A) Bone defect in medial face of tibia. (B) Techgraft membrane of 10 × 5 mm used to cover bone defects in the TG group. (C) Schematic figure of bone defect in the medial face of tibia. (D) Schematic figure of membrane covering bone defect on the medial face of tibia. (E) Schematic figure showing membrane in contact with bone in part of posterior face of tibia to be fixed below musculature

x-ray set at 60 kV and current of 166 μA. An aluminum filter of 0.25 mm thickness, 30 μm pixel size, and 0.4° rotation step over a rotation range of 360° was applied. The reconstructions were performed using the NRecon software (v1.6.10.2). The region of interest was selected by drawing a circle of 3.0 mm diameter corresponding to the total bone defect site and an internal circle of 1.65 mm diameter in the center of the defect, using always the same volume of interest. The parameters evaluated with the CT Analyzer software (v1.15.4.0) were: BV/TV (%), Tb.Th (mm), Tb.Sp (mm), and Tb.N (1/mm). Bone mineral density (BMD) was measured automatically in each sample, calculated using gray values previously calibrated by specific phantoms.

2.3 | Statistical analysis

Statistical analysis was carried out using the SPSS program (IBM). The Shapiro–Wilk test was used to verify data distribution and the *t*-test was applied to compare groups. One-way analysis of variance was used to evaluate mechanical properties of membranes in different conditions. *P* values <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Membrane characterization

3.1.1 | Scanning electron microscopy

Scanning electron microscopy images of the dry membrane showed a surface with few undulations and a fibrous aspect. No relevant differences were observed over the same specimen. After 1 day of immersion in PBS, qualitative analysis showed an appearance suggestive of swollen fibers on the membranes. At 3 days of immersion, suggestive more swollen fibers were observed. The fibrous appearance was no longer striking, but it was still visible. At 7 days, the fibrous aspect was not more observed. The fibers were thinner and dispersing. At 14 and 30 days a gelatinous aspect was observed. It is no longer possible to identify collagen fibers, an amorphous surface with an irregular shape was observed. Figure 4 shows the membrane aspect at the different time-points.

3.1.2 | Atomic force microscopy

The collagen fibrils that maintain the structure after the membrane manufacturing process were observed by AFM. The collagen fibrils of 103.52 nm of transverse length presented in parallel groups with a striated appearance (Figure 5). The average roughness of membranes was 8.12 nm.

3.1.3 | Water uptake

In this analysis, the membranes were immersed in PBS, then after the time-points, they were quickly blotted with filter paper, and weighed immediately. A weight gain reflects the membrane ability to absorb water. Membranes showed high water uptake in the first hour, which showed as progressive weight gain with time until the seventh day. In the first hour, the weight increase was 386%. After 8 h, it reached 422%, rising to a total of 458% at the end of the first day. The increase was 486% at 7 days. The weight gain was reduced at 14 days (194%) and 30 days (145%).

The pH started neutral without great change in the 1 day (pH = 7.26) showing an increase at 7 days (pH = 8.01). Probably, due to the disintegration of material and dissolution of molecules in the liquid. After 14 days, the pH tended to neutral and at 30 days the pH normalized to 7.43 (Figure 6).

3.1.4 | Weight loss

In this analysis, the membranes were weighed after drying. A weight loss is related to membrane disintegration. The weight loss started at 7 days (−6.6%). At 14 days, the dry membranes deformed and their weight decrease more (−34.1%). At 30 days, the dry membranes were almost disintegrated, showing a weight loss of −94.3%.

3.1.5 | Mechanical test

All intact membranes fractured in the medial region, indicating that there was no fragile or defective region. Some samples were lost

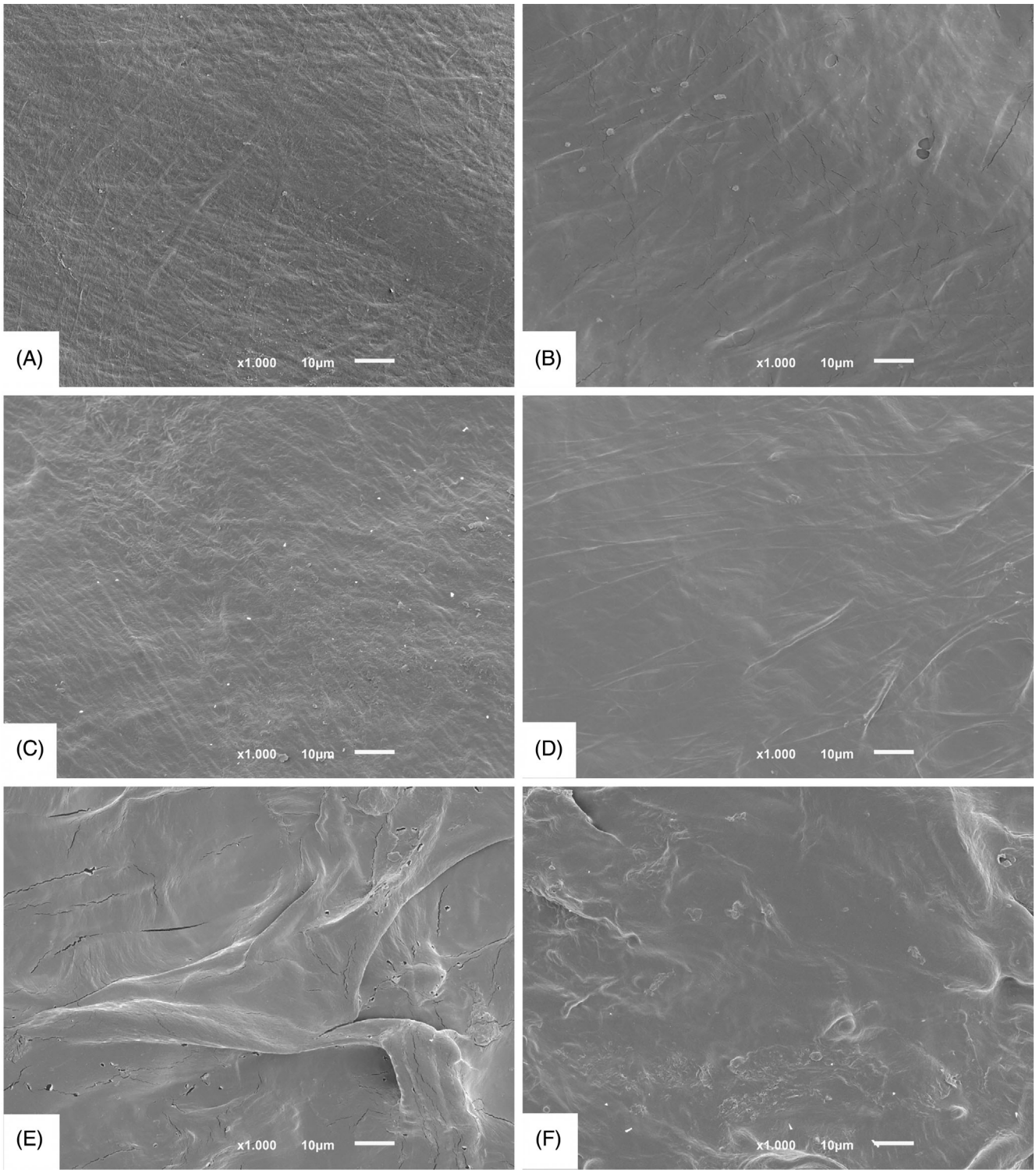

FIGURE 4 Photomicrographs of the membrane surface in the dry condition (A), and after 1, 3, 7, 14, and 30 day of weight loss test (B–F). Magnification of 1000x

during the experiment due to instability of the membrane or its detachment from the plastic frame with the following final number of samples: intact condition ($n = 6$); 1 day ($n = 5$) and 3 days ($n = 3$) in dry condition; 1 day ($n = 6$) and 3 days ($n = 5$) in wet condition. The mean tensile strength observed in intact membranes was 40.62 (6.93) MPa, a fracture strain of 29.09 (5.00) % and Young's Modulus of 513.51 (123.90) MPa. Membranes had higher tensile strength in dry than in wet condition ($p < 0.001$), but no difference were found between intact membranes compared to 1 day dry ($p = 0.804$) and 3 days ($p = 0.071$). In the wet condition, 1 day compared to 3 days

showed no difference for tensile strength ($p = 0.245$), fracture strain ($p = 0.892$), and Young's Modulus ($p = 0.964$). Similarly, between 1 and 3 days in the dry condition, no differences were found for tensile strength ($p = 0.392$), fracture strain ($p = 0.528$), and Young's Modulus ($p = 0.975$). Wet membranes (1 and 3 days) showed lower Young's Modulus compared to intact ($p < 0.001$) and dry membranes ($p < 0.001$). Fracture strain showed no difference between dry and wet membranes ($p > 0.05$). Membranes showed decreased strength along dry and wet condition time-points (Table 1). Figure 7. represent the stress–strain curve of the samples according to the tensile test.

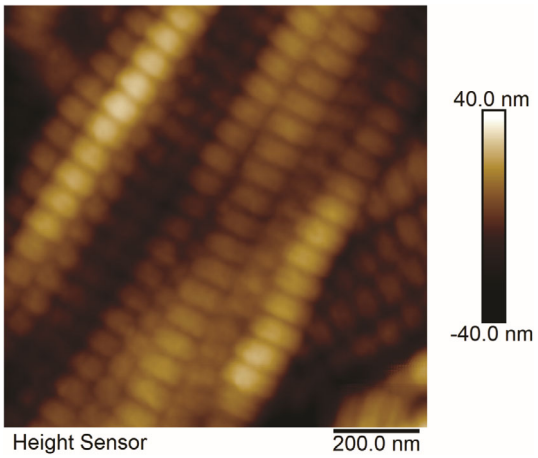

FIGURE 5 Photomicrograph of membrane topography by AFM

3.2 | Experimental study

3.2.1 | Micro-CT

Bone healing was visible in both groups. Cross-sectional views showed a higher volume of newly formed bone in the TG group, with more trabecular bone adjacent to the membrane, in the top levels of defect. In the control group (CONT), the new bone was thinner than in the treated group (TG).

The percentage of bone volume (BV/TV%) at 21 days in the CONT, with a mean of 45.13 (6.44) %, was lower than in the TG, with a mean of 55.70 (3.33) %, showing significant difference ($p = 0.005$). The TG group showed higher mean of Tb.N when compared to CONT ($p = 0.001$) and lower Tb.Sp ($p = 0.018$) at 21 days. A mean of 1.54 (0.09) 1/mm trabeculae number was found in the CONT group and

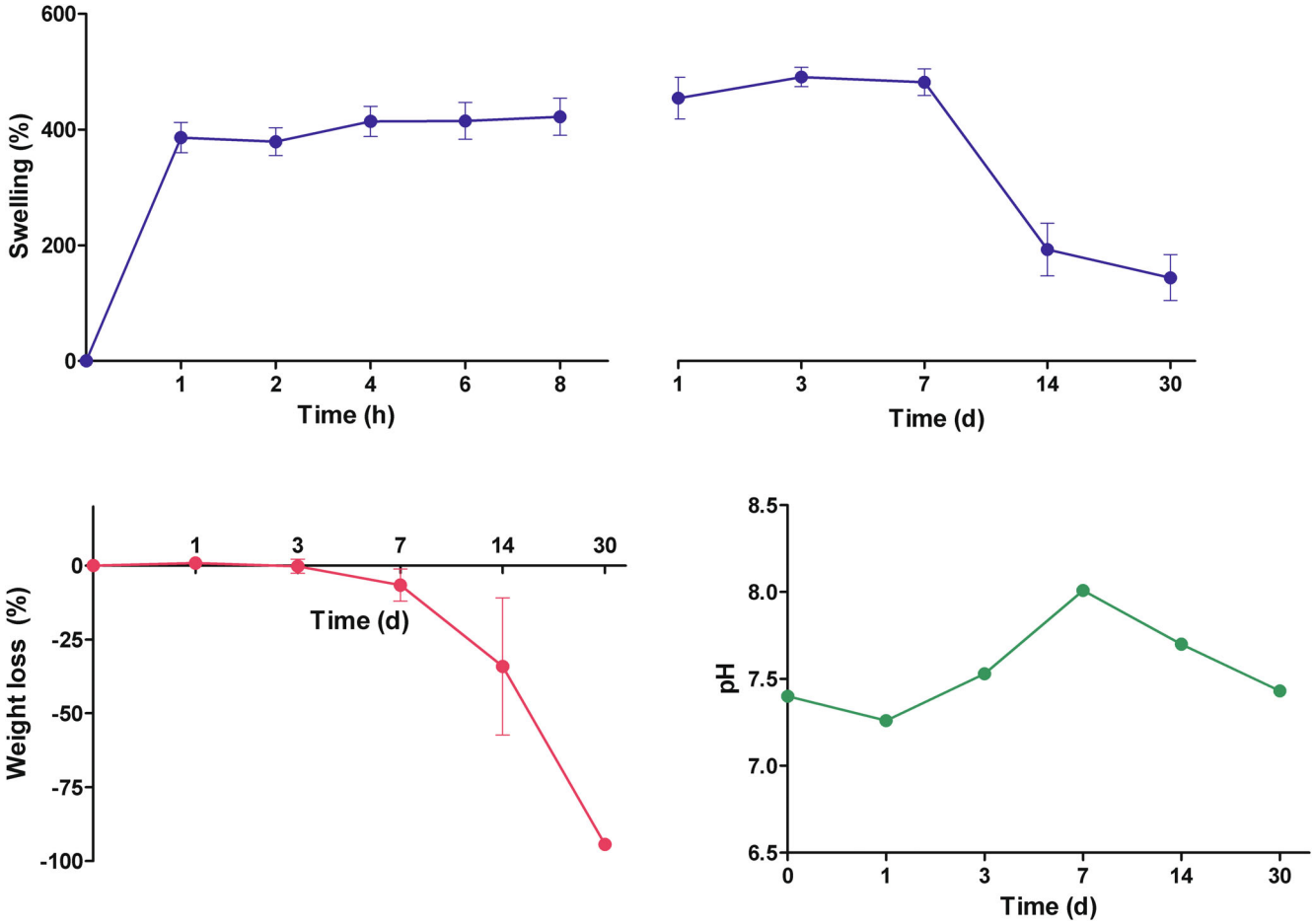

FIGURE 6 Membrane swelling in the first 8 h up to 30 days; membrane weight loss and liquid pH in the 1 day up to 30 days

TABLE 1 Mechanical properties of collagen membranes during the tensile test presented in mean (standard deviation)

		Dry test		Wet test	
Condition	**Intact**	**1 day**	**3 days**	**1 day**	**3 days**
Tensile strength (MPa)	40.62 (6.93)	36.93 (4.37)	29.59 (8.16)	9.27 (5.45)	2.12 (1.83)
Fracture strain (%)	29.09 (5.00)	44.12 (4.86)	34.35 (19.49)	44.03 (7.13)	39.38 (7.17)
Young modulus (MPa)	513.51 (123.90)	423.86 (84.64)	457.34 (79.76)	41.25 (14.24)	10.78 (8.79)

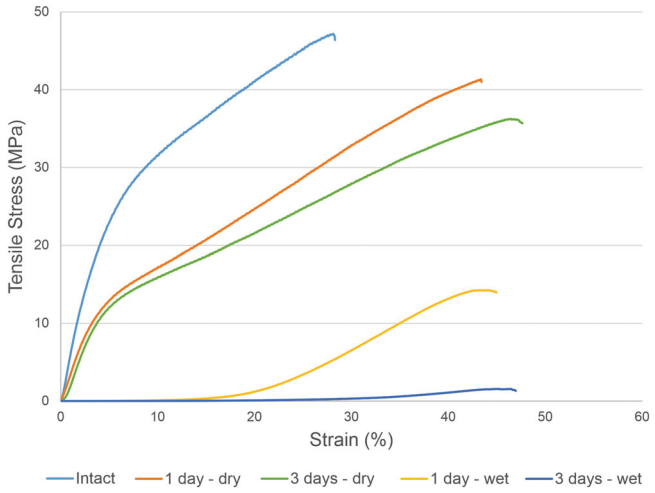

FIGURE 7 Mechanical tensile test of membranes. One representative stress–strain curve was presented for each condition

2.12 (0.29) 1/mm in the TG group. The higher number of trabeculae in TG group seen in images corroborates the quantitative difference. Furthermore, the higher trabeculae separation in the CONT group of 0.34 (0.07) mm indicates less new bone formation compared with the TG group of 0.24 (0.02) mm. There was no difference between groups for Tb.Th ($p = 0.316$), with mean of 0.29 (0.04) mm in the CONT group and 0.27 (0.04) mm in the TG group. For BMD, the TG group showed a mean of 0.37 (0.09) g/cm^2 in the CONT and 0.40 (0.02) g/cm^2 in the TG, with no significative difference between groups ($p = 0.554$). Figure 8 shows tibial section and quantitative results of micro-CT.

4 | DISCUSSION

The purpose of this study was to explore the characteristics of the Techgraft membrane and evaluate its use in the treatment of long bone defects. The membrane use showed positive effects causing higher new bone volume than the untreated group.

An important aspect of biomaterials is a manufacturing process that produces a material with a desired set of functions. To be used as a biomaterial, collagen from animal sources is chemically treated, including esterification, cross-linking, acylation, and blocking of the guanidine groups of arginine residues. The alkaline treatment through

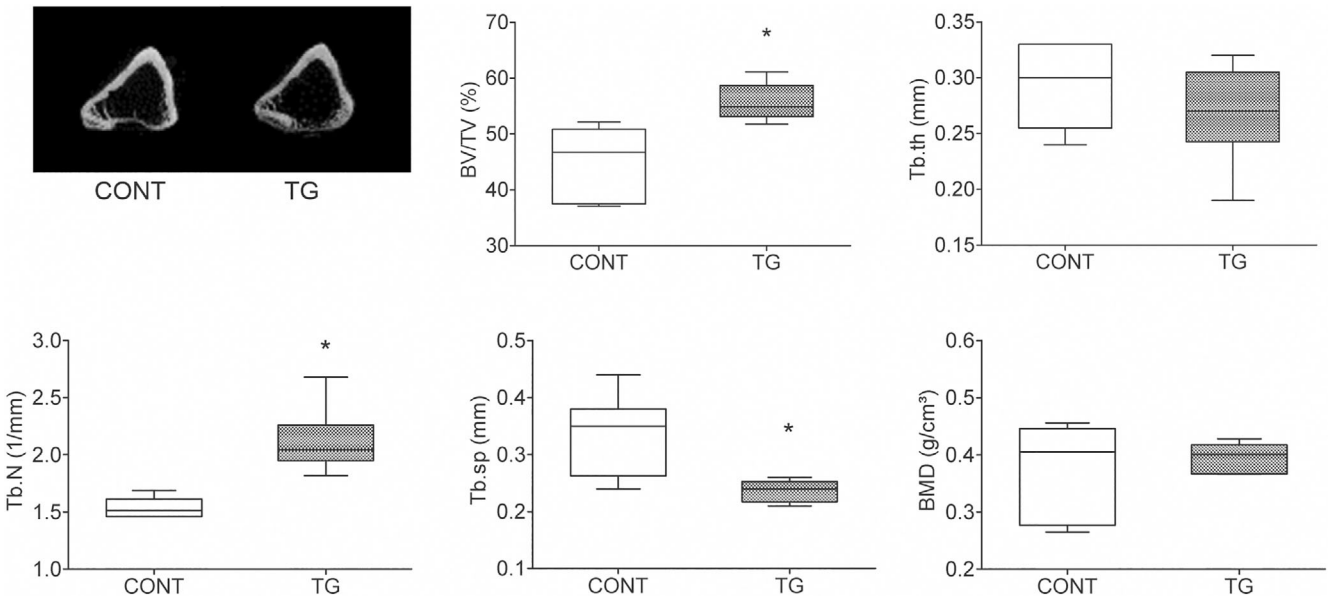

FIGURE 8 Tibiae cross-sectional views. In the treated group (TG), a deposition of bone in the top levels of the defect was observed, while in the control group (CONT) the deposition started in the bottom levels. The graph shows the percentage of bone volume per tissue volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and bone mineral density (BMD) in the CONT and TG groups at 21 days. *$p < 0.05$

hydrolysis of carboxamide groups enhances piezoelectric properties of collagen by a relatively simple and low-cost technique.[8] In addition, collagen matrix preparation by hydrolysis of side-chain amides of asparagine and glutamine provides biomimetic mineralization.[9] In deamidation by selective hydrolysis of asparagine and glutamine carboxamide side-chains, the hydrolyzation time could alter fibrils formation.[10] The hydrolysis for production of the Techgraft membrane did not alter collagen fibrils, as the preservation of the collagen natural structure was confirmed. In SEM and AFM analyses, a native collagen structure could be observed suggesting an adequate production technique that produced a biomaterial with structure functional to be used in the promotion of bone repair. Molecules of collagen are organized in long fibrils, an inherent characteristic of collagen type I. Collagen molecules have a well-known sequence of amino acids making the triple helix aggregated in tropocollagen structures organized in fibrils, fibers, and collagen bundles. In the AFM images, the hierarchical organization of collagen molecules was clearly seen, with striated fibrils grouped in fibers that are twisted together and with overlapped tropocollagen units forming lacunae, which give the striated appearance of collagen fibrils.[11] The collagen structure organization observed could contribute to cell adhesion in membrane surface.

Biomaterials are confectioned to promote tissue regeneration. The main purpose to choose the ideal biomaterial is the knowledge of the repair process in which it will be applied and its finality in this process. In case of Techgraft its application in bone repair requires hydrophilicity, mechanical strength, malleability, and bioactivity. In the water uptake test, the membrane absorbed the liquid in the first hour and the equilibrium was maintained. Swelling is an important characteristic of biomaterials due to the high presence of water in the extracellular matrix, allowing water-soluble factors to enter the membrane.[12] Also, a hydrophilic surface favors cell attachment.[13] Thus, an ideal biomaterial membrane should have affinity to a liquid environment without affecting the capacity of maintaining a barrier and without causing morphology damage. Our collagen membrane showed higher water uptake during the first 24 h. This can show the strong hydrophilicity from Techgraft. We observed that at the last time-point (30 days) the membrane maintained its usual aspect in liquid immersion, but when removed from the liquid, its structure was altered, indicating that it has water affinity and it maintains its structure when in liquid. Water uptake performance found in Techgraft is important functional characteristics of a biomaterial to be used in bone repair.

The weight loss test associated with the water uptake test provides notions about the ability of the material to absorb the water, its behavior in liquid medium and disintegration. At 14 days, reduction in weight gain was observed in water uptake and weight loss analysis. This finding is probably assigned to dissolution of biomaterial in the liquid medium. So, this fact does not mean that membranes had a loss of water absorption capacity, but a mass loss. This could be confirmed by the increasing pH along time-points. The pH went from neutral to basic over time, which could indicate dissolution of collagen molecules in the water.

The biodegradation process enables tissue remodeling, replacing biomaterial for natural cells. Biodegradation of collagen is a positive characteristic of the membrane because it avoids an additional surgery to remove the membrane, as is required in when nonabsorbable membranes are used.[14] The fast degradation rate of collagen membranes is considered a problem, because it could be reabsorbed before the end of bone healing. In our study, an almost complete dissolution was observed after 30 days. However, more than just a barrier, the main advantages of the membrane are the initial signaling and properties that support cell adhesion and differentiation to accelerate the bone healing process.[15] Therefore, the time that the membrane remained stable in vivo appear to have been sufficient to promote satisfactory bone repair.

The membranes act as a scaffold for the migration of osteogenic cells and protect against connective tissue infiltration.[16] There are no established standard mechanical properties. The variability found in the literature reflects different analyses and different levels of collagen evaluations.[11] Membranes may differ in geometry, dimensions, and composition. In addition, the sample alignment, the load cell, and the grips used influence the results. Therefore, the membranes need to be strong enough according to their goals in the repair process. Membranes need to be flexible enough to be handling and molded to the bone surface, controlled elasticity to maintain dimensional stability and mechanical integrity, and strong enough to allow manipulation and adjustment.[17] Techgraft showed stability in liquid for up to 30 days and could be tested by tensile testing up to 3 days after immersion in liquid. The results showed that membranes had tensile strength similar of intact membranes even after 3 weeks of immersion in PBS, showing that liquid medium did not affect drastically mechanical properties of the membranes in the initial phase of bone repair. The product instructions suggest that membranes should be hydrated in saline before being placed in the surgical site. However, excessive swelling could alter mechanical properties and supporting function of the biomaterial, associated with its stiffness. In the present study, even after wet, the membranes were able to resist some tensile strength, contributing to the idea that the membranes would probably not disintegrate quickly when placed in bone defect, acting in a satisfactory time to promote bone repair.

The ability to withstand tensile strength was reduced over time. No significative difference was observed when the dried membranes were compared to the intact ones for tensile strength. Membranes showed a decreasing elastic limit with time, but high fracture strain was found at all-time points. In the present work, the membranes showed lower tensile strength in the wet condition. Van der Rijt et al.[18] found, similarly, at the fibrillar collagen level, a lower strength in a wet environment than at room temperature. Moisture decreased the rigidity of the membranes. Confirming the present study, Raz et al.[19] found that membranes showed lower resistance to tensile loads in wet condition. The authors[19] compared three different collagen membranes: Bio-Gide, made of porcine dermis types I and III collagen; Remaix, composed of purified porcine collagen fibers intermingled with porcine elastin fibers; and Ossix Plus, made of porcine tendon type I collagen. All of them showed higher tensile

strength values in the dry condition: Remaix dry 10.4 (2.66) MPa and wet 5.25 (1.35) MPa, Bio-Gide dry 4.6 (0.94) MPa and wet 1.68 (0.54) MPa, and Ossix Plus dry 5.13 (2.48) MPa and wet 1.2 (0.14) MPa.[19]

Ha et al.[20] found tensile strengths of 27.6 MPa in silk membrane, 3.5 MPa in collagen membrane and 4.3 MPa in polytetrafluoroethylene membrane. In the study of Silva et al.,[21] GenDerm, a commercial collagen membrane, showed mechanical strength with a tension value of 19 MPa, while the experimental collagen-rich membrane, from the porcine intestinal submucosa, showed 6.2 MPa. The results of the present study showed acceptable tensile strength as found in the literature. However, the comparison with other studies is not reliable due to the different parameters defined and methodologies used. The mechanical test was performed to define properties of the membrane and to know its behavior. Since, to date, there are no exact standards, comparisons with other commercial membranes or other compositions can help to elucidate the tensile strength of Techgraft. For other experiments, it would be useful to compare Techgraft to a nonabsorbable membrane.

The main disadvantage in the use of collagen membranes is the low mechanical strength. In some studies, specific treatments improve mechanical properties of collagen membranes.[17,22] The Techgraft shows are strong enough to support handling to be applied to the bone defect and remain stable to support bone repair in the period studied without extra specifics treatments. Indeed, the mechanical test shows no structural defects in the membranes, or weakness points.

Biological and structural specifications need to precede clinical use. Thus, laboratory characterization and experimental study guide us to elucidate the clinical response of the membrane. Membranes created a favorable environment for bone formation, indicated by the presence of newly formed bone close to the membrane in the TG. Collagen fibers of the pericardium are entwined,[23] which could cause a favorable trabeculae distribution of newly formed bone, and consequently lead to a high trabeculae number. Many biological reactions occur on surfaces or interfaces of biomaterials, so topography is an important feature to be evaluated. Roughness could be contributed to bone formation in membrane surface, since it is an important characteristic in the promotion of cell adhesion in biomaterials.[24] In contrast, trabeculae separation was higher in the CONT, suggesting a slower bone repair in the absence of treatment. Turri et al.[25] showed that new bone formation in groups treated with collagen membrane was higher under the membrane, in the top region of the defect at 6 days, corroborating our findings. Turri et al.[25] also observed that at 28 days the bone area was larger in the membrane-TGs in the center and top of the defect compared to control.

The goal of the experimental section was to compare treatments. A CONT was adopted as reference, because bone repair involves a systemic response, so the contralateral bone would not be the best comparison between treatments. In addition, the bone would not be able to complete the bone repair in the experimental time studied. Thus, a CONT submitted to the same surgical procedure provided a better comparison. It was possible minimize adverse effects using a homogeneous group of rats controlled by age and weight.

Membrane treatment can also improve bone healing in osteoporotic conditions. In a previous study, collagen membranes from bovine pericardium increased bone volume in calvaria bone defects in both normal and osteoporotic rats.[26] In the present study, the collagen membrane from bovine pericardium improved bone healing in long bone defect as well.

The use of membranes is well established in periodontal regeneration,[27] for which different biomaterials are proposed for membranes and tissue augmentation. For example, collagen membranes used in association with β-tricalcium phosphate alloplast is a method described for periodontal regeneration.[28] In another study, bone repair was improved when strontium and hydroxyapatite augmentation was combined with collagen membranes.[29] The release of growth factors by membranes is also studied in bone defect healing. Collagen membranes containing osteogenic protein-1 (OP-1) improved bone healing in rat mandible, increasing new bone formation significantly, compared with membrane alone and the CONT.[30] Thus, the favorable characteristics of Techgraft, concerning strength, swelling, and capacity to support bone growth, support its use combined with synergistic treatments in new strategies for bone therapies.

The development of new treatments requires extensive preclinical experimentation. Therefore, the membrane characterization followed by its in vivo application was essential to understand the effects of membrane use on bone. The use of membranes from bovine pericardium in long bone healing seems to have positive effects, similar to that found on flat bones.[3,26,31] The membrane led to optimal bone repair, accelerating the initial stage of the process and increasing bone volume. Considering our results, collagen membranes could be a promising alternative for bone repair. Further analyses in the cellular and molecular levels need to be carrying out to better understand the effects of Techgraft membrane in bone repair, and the future studies combining Techgraft and other grafts are encouraged.

5 | CONCLUSIONS

Collagen membranes showed good structural and mechanical characteristics for use in bone repair therapies. Although the membrane had a high dissolution rate, it maintained its stability in liquid environment and the time it remained in vivo was enough to initiate bone repair. Membranes had positive effects in bone defect treatment, improving microstructural properties of newly formed bone and seem to be an advantageous treatment alternative.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Yamanaka JS, upon reasonable request.

REFERENCES

1. Abou Neel EA, Bozec L, Knowles JC, et al. Collagen – emerging collagen based therapies hit the patient. *Adv Drug Deliv Rev*. 2013;65(4):429-456.
2. Burg KJL, Porter S, Kellam JF. Biomaterial developments for bone tissue engineering. *Biomaterials*. 2000;21:2347-2359.
3. Miguel FB, Barbosa A Jr, de Paula FL, Barreto IC, Goissis G, Rosa FP. Regeneration of critical bone defects with anionic collagen matrix as scaffolds. *J Mater Sci Mater Med*. 2013;24(11):2567-2575.
4. Costa JNL, Pomerantzeff PMA, Braile DM, Ramirez VA, Goissis G, Stolf NAG. Comparação entre o pericárdio bovino decelularizado e o pericárdio bovino convencional utilizado na confecção de biopróteses valvares cardíacas. *Rev Bras Cir Cardiovasc*. 2005;20(1):14-22.
5. Goissis G, Plepis AMG, Rocha JLA. Process for the extraction of collagen from tissues with aqueous organic solvent in alkaline media. *Braz Pat Ind Prop*. 1994;1:1-24.
6. Billström GH, Blom AW, Larsson S, Beswick AD. Application of scaffolds for bone regeneration strategies: current trends and future directions. *Injury*. 2013;44(Suppl. 1):S28-S33.
7. Carlos BL, Yamanaka JS, Yanagihara GR, et al. Effects of latex membrane on guided regeneration of long bones. *J Biomater Sci Polym Ed*. 2019;30(14):1291-1307.
8. Rocha LB, Goissis G, Rossi MA. Biocompatibility of anionic collagen matrix as scaffold for bone healing. *Biomaterials*. 2002;23(2):449-456.
9. Goissis G, Da Silva Maginador SV, da Conceição Amaro Martins V. Biomimetic mineralization of charged collagen matrices: in vitro and in vivo study. *Artif Organs*. 2003;27(5):437-443.
10. Bet MR, Goissis G, Lacerda CA. Characterization of polyanionic collagen prepared by selective hydrolysis of asparagine and glutamine carboxyamide side chains. *Biomacromolecules*. 2001;2(4):1074-1049.
11. Sherman VR, Yang W, Meyers MA. The materials science of collagen. *J Mech Behav Biomed Mater*. 2015;52:22-50.
12. Correlo VM, Pinho ED, Pashkuleva I, Bhattacharya M, Neves NM, Reis RL. Water absorption and degradation characteristics of chitosan-based polyesters and hydroxyapatite composites. *Macromol Biosci*. 2007;7(3):354-363.
13. Wei J, Igarashi T, Okumori N, et al. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts. *Biomed Mater*. 2009;4(4):045002.
14. Pellegrini G, Pagni G, Rasperini G. Surgical approaches based on biological objectives: GTR versus GBR techniques. *Int J Dent*. 2013;1:1-13.
15. Elgali I, Turri A, Xia W, et al. Guided bone regeneration using resorbable membrane and different bone substitutes: early histological and molecular events. *Acta Biomater*. 2016;29:409-423.
16. Elgali I, Omar O, Dahlin C, Thomsen P. Guided bone regeneration: materials and biological mechanisms revisited. *Eur J Oral Sci*. 2017;125(5):315-137.
17. Wang J, Qu Y, Chen C, et al. Fabrication of collagen membranes with different intrafibrillar mineralization degree as a potential use for GBR. *Mater Sci Eng C*. 2019;104(109959):1-8.
18. Van der Rijt J, Van der Werf K, Bennink M, Dijkstra P, Feijen J. Micromechanical testing of individual collagen fibrils. *Macromol Biosci*. 2006;6(9):697-702.
19. Raz P, Brosh T, Ronen G, Tal H. Tensile properties of three selected collagen membranes. *Biomed Res Int*. 2019;2019:1-8.
20. Ha YY, Park YW, Kweon HY, Jo YY, Kim SG. Comparison of the physical properties and in vivo bioactivities of silkworm-cocoon-derived silk membrane, collagen membrane, and polytetrafluoroethylene membrane for guided bone regeneration. *Macromol Res*. 2014;22(9):1018-1023.
21. Silva MJ, Gonçalves CP, Galvão KM, D'Alpino PHP, Nascimento FD. Synthesis and characterizations of a collagen-rich biomembrane with potential for tissue-guided regeneration. *Eur J Dent*. 2019;13(3):295-302.
22. Chu C, Deng J, Xiang L, et al. Evaluation of epigallocatechin-3-gallate (EGCG) cross-linked collagen membranes and concerns on osteoblasts. *Mater Sci Eng C*. 2016;67:386-394.
23. Buchaim RL, Goissis G, Andreo JC, et al. Biocompatibility of anionic collagen matrices and its influence on the orientation of cellular growth. *Ciência Odontológica Bras*. 2007;10(3):12-20.
24. Sprangers S, Everts V. Molecular pathways of cell-mediated degradation of fibrillar collagen. *Matrix Biol*. 2017;1:1-11.
25. Turri A, Elgali I, Vazirisani F, et al. Guided bone regeneration is promoted by the molecular events in the membrane compartment. *Biomaterials*. 2016;84:167-183.
26. Hirata HH, Munhoz MAS, Plepis AMG, et al. Feasibility study of collagen membranes derived from bovine pericardium and intestinal serosa for the repair of cranial defects in ovariectomised rats. *Injury*. 2015;46(7):1215-1222.
27. Corinaldesi G, Lizio G, Badiali G, Morselli-Labate AM, Marchetti C. Treatment of Intrabony defects after impacted mandibular third molar removal with bioabsorbable and non-Resorbable membranes. *J Periodontol*. 2011;82(10):1404-1413.
28. Fahmy RA, Kotry GS, Ramadan OR. Periodontal regeneration of dehisence defects using a modified perforated collagen membrane. A comparative experimental study. Futur. *Dent J*. 2018;4(2):225-230.
29. Cardemi C, Elgali I, Xia W, et al. Strontium-doped calcium phosphate and hydroxyapatite granules promote different inflammatory and bone remodelling responses in normal and ovariectomised rats. *PLoS One*. 2013;8(12):1-17.
30. Ozaki M, Takayama T, Yamamoto T, et al. A collagen membrane containing osteogenic protein-1 facilitates bone regeneration in a rat mandibular bone defect. *Arch Oral Biol*. 2017;84:19-28.
31. Cunha MR, Santos AR Jr, Genari SC. Cultura de osteoblastos sobre membranas de colágeno polianiônico: avaliação preliminar do potencial de indução da formação de tecido ósseo visando reparação tecidual. *Bol Med Veterinária - UNIPINHAL*. 2005;1(1):73-85.

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