



Influence of collagen membrane on bone quality in titanium meshes reconstructions - study in rats

Running title - Influence of collagen membrane on bone quality

Summary sentence - No influence of collagen membrane on guided bone regeneration with titanium meshes.

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Abstract

Background: New bone formation and tissue remodeling are the major challenges in today's implantology. 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 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 (T): bovine bone graft associated with porcine collagen and collagen membrane was used; Control (C): 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 $> 1\text{mm}$ demonstrated higher mineral bone density in comparison to meshes with pore size $< 1\text{mm}$ ($p < 0.05$), regardless the combined usage of collagen membrane. All groups showed a spongy bone formation after 30 days.

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

Introduction

Dental implants for tooth replacement have been supported by studies¹⁻³, but in some cases, residual bone volume is often insufficient for optimal rehabilitation. Thus, restoration of bone volume has become necessary to achieve success in rehabilitation with implants⁴. 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 process⁵⁻⁷. 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 osteoconductors⁸.

Studies comparing the best type of barrier to be used have been published in literature since the last 60 years^{4, 6, 9-12}. Occlusive membranes in association with bone graft material demonstrated a gain of bone tissue in several studies¹¹⁻¹⁴. Absorbable membranes, for example, maintain a temporary barrier between 6 to 8 weeks¹⁵, 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 maintenance⁹, which is essential in vertical bone reconstructions¹⁰.

Titanium mesh barriers have been shown that bone volume to be created can be planned prior to the surgical procedure and mesh can be molded to maintain volume during healing period without graft compression by the flap¹⁶. The presence of pores prevents soft tissue growth internally and allows interstitial fluid diffusion¹⁴. Studies shows 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 been demonstrated space maintenance importance^{9, 20-23} and also that barriers needs to be malleable enough to promote required geometry to ensure bone gain in height and thickness¹⁷. Furthermore, it has been suggested that larger diameter pore size allows new bone angiogenesis and better nutrients diffusion. On the other hand, smaller diameter pore size completely occlusive meshes may limit neovascularization process, but also restrict fibrous connective tissue invasion^{9, 24}. Authors have suggested that bone growth occurs in 50 μm diameter pore size meshes²⁵.

Up until present time, 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 cells²⁶. However, some clinical and preclinical studies have demonstrated limited bone regeneration and soft tissue infiltration when occlusive membranes were not used in association to 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 membrane²⁸. 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 need to obtain GBR criteria, good results were demonstrated through 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. Moreover, evaluate differences in pore size and thickness of titanium meshes.

Material and methods

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 study³⁴.

2. Samples characterization

Twenty-eight male Wistar rats (*Rattus norvegicus albinus*) with an average weight of 410.8 grams 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 UNAERP's laboratory in a 12 hours cycle environment of light and temperature between 22 and 24°C.

2.1. Experimental groups

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

- 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).

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

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

^{**} Bionnovation® Surgitime Titanium, Bauru, SP, Brazil

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.

3. Surgical Procedure

After weighing, animals received general anesthesia, obtained by the association of 0.08 ml / 100g of Ketamine Hydrochloride and 0.04 ml / 100g 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^{†††} (Figure 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 for delimit graft area, and had 1 mm of distance

^{††} Bionnovation® Surgitime Titanium, Bauru, SP, Brazil

^{†††} Bio-Oss Collagen Geistlich® Pharma, São Paulo, SP, Brasil

^{§§} BioGide Geistlich® Pharma, São Paulo, SP, Brasil

^{***} Microsoft® Corporation, Santa Rosa, California

^{†††} Swann-Morton, Sheffield, England

^{†††} Neodent® Bone Graft Kit, São Paulo, SP, Brasil

^{§§§} Koncept-Kavo®, Joinville, SC, Brazil

between each other, which was measured with a periodontal probe (Figure 1B and C). Then each block of bone graft BC (Figure 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 femur opposite sides (Figure 1E and G). In groups P85 and P15, screws were positioned only at the top of femur (Figure 1F and H). Following randomization, 10 mm x 15 mm collagen membrane was positioned above titanium mesh, only in femur test (Figure 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* computerized microtomograph 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* computerized microtomography (μ -CT) analysis, determining 30 days analysis. At the end, animals were euthanized with intraperitoneal intraperitoneal^{††††} 150 mg / kg 2.5% sodium thiopentate intraperitoneal injection.

^{****} Neodent® gingival screw, Curitiba, PR, Brazil

^{††††} Vicryl Ethicon 5.0, Johnson Prod., São José dos Campos, Brazil

^{††††} Thiopentax, Cristália, Brazil

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 μ A), resolution of 18 μ m, with copper aluminum filter, rotation step (0,7 / 360°) + 2x off-set camera and 1. Microtomographic analyzes of three - dimensional microarchitecture of graft and neoformed bone were performed. For image processing was used 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.

In vivo computerized microtomography analysis was performed 24 hours (baseline) and 30 days after surgical procedure. Baseline analysis aimed to capture BC initial volume and serve as reference for comparisons. All measurements were performed by a blind examiner. Titanium mesh and gingival screws was used as reference to determine region of interest (ROI). Therefore, ROI is the cross-section area selected below titanium mesh (graft or neoformed bone tissue). Summing 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 following definitions: tissue volume (TV): total volume of interest between titanium mesh and gingival screws; bone volume (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^3 and refers to mineral amount in bone.

^{§§§§} Bruker-microCT SkyScan, Kontich, Belgium

5. Histological processing

After euthanasia, right and left femurs were fixed in 10% neutral formalin for 48 hours. Decalcification was carried out by means of 10% ethylenediaminetetraacetic acid 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.

5.1. Histological analyzes

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.

5.1.1. Characterization of bone type

Bone type definition was determined by image analysis, captured with 2.5X magnification objective. For new bone quantification, a computerized grid measuring 2 cm X 2 cm and containing six columns and six lines was used. The presence of bone formation was

**** Microm HM 335E, SA®, Germany

†††† Leica Microsystems GmbH, Nussloch, Germany

††††† Leica Microsystems GmbH, Nussloch, Germany

determined by intersection points (%) between horizontal and vertical lines. These measures were made at three points: at both peripheral and at the most central point. The final percentage was determined as average.

5.1.2. Measurement of BC/femur interface

Using a 10X 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.

6. 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 analyzes.

Results

Surgical procedure occurred with some interurrences. 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.

^{§§§§§} National Institutes of Health, Bethesda, USA
^{*****} GraphPad Software Inc., San Diego, CA, USA

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 relation to BMD after 30 days, denser tissue was observed in the titanium meshes with pore size > 1 mm ($p < 0.05$), with mean and standard deviation 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.

2. Histological analysis

2.1. Histological description

Bone neoformation pattern was similar in all groups. It was observed presence of bone formation, 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 (Figure 2).

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.

2.3. BC/femur interface

In grafted central region, new bone formation were 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.

Discussion

In this present study, four types of titanium mesh were associated with bone graft and collagen membrane in order to evaluate additional use of occlusive membrane influence in titanium mesh's 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 and 30 μm . The model used in the study was a rat femur, due to greater disposition in adapt titanium meshes. In addition, 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 proliferation³⁵. In vivo study in rat femur demonstrated that membrane absorption process is initiated after 4 weeks and completed after 6 weeks¹⁵.

It was suggested that occlusive barrier uses in association with titanium meshes could minimize soft tissue formation in bone defect, aiming acquire 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 $10.81\% \pm 5.38\%$ and $15.16\% \pm 6.76\%$ in groups without and with collagen membrane, respectively, and did not find statistically significant differences²⁶. 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 inter-group comparisons. In spite of different analyzes and numerical percentage of new bone formed, our results are compatible with previous results, suggesting that in bone graft presence, use of collagen membrane does not interfere 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 exposure³⁶. 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 formation²⁶.

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 to β -tricalcium phosphate for GBR with collagen membrane in rat calvaria, BC demonstrated small amount of new bone formed besides presence of structures similar to bone tissue, but without osteocytes, suggesting to be grafts 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 in 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 range from 5 to 30%. If presence of voids is greater than 30%, bone may be characterized as spongy, with density ranging from 0.1 to 0.9 g / cm³. Results of BMD and percentage of non-mineralized areas suggest that new bone type was spongy for all groups, regardless 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, in order 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* μ -CT results, it was observed that there was no volumetric difference in new bone formed using different pore size. Numerically, P15 mesh showed a higher percentage of bone volume (30.9 ± 12.33), despite its thickness, when compared to 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 μ m would be ideal for larger bone reconstructions, avoiding collapse within the defect.

In relation to BMD, it was observed that meshes with larger pore size presented higher density, and difference was statistically significant. Studies suggested that titanium meshes with larger pore size would have a lower mineral density, different from demonstrated by our results⁹. Numerically, Rakhmatia et al.⁹ observed density between 8 and 10 g/cm^3 in meshes with pore size between 20 and 100 μ m 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 femur. 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 add that, regarding bone volume meshes showed no differences when comparing healing period of 4 and 8 weeks. They suggest that after healing period, bone volume will be similar in meshes evaluated, justifying 30 days healing period of this study.

This present study demonstrated limitations in relation to thinner meshes (P15 and P85) stabilization, which may have suffered interference during animal movement. Even so, volumetric analyzes showed results compatible with those obtained in groups P175 and P300, which had thickness of 30 μ m. It has been suggested in the literature that meshes with thickness less than 50 μ m 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 in order to avoid collapse and reduction of bone gain⁴⁰. 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 new bone formation. Factors such as physical characteristics of material, thickness, chemical composition, biocompatibility⁴¹, besides pore shape, manufacturer, vascularization and use of bone graft to serve as a framework.

Conclusions

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

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Figure Legends

Figure 1 - Illustration images of surgical procedure. A – initial incision; B, 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.

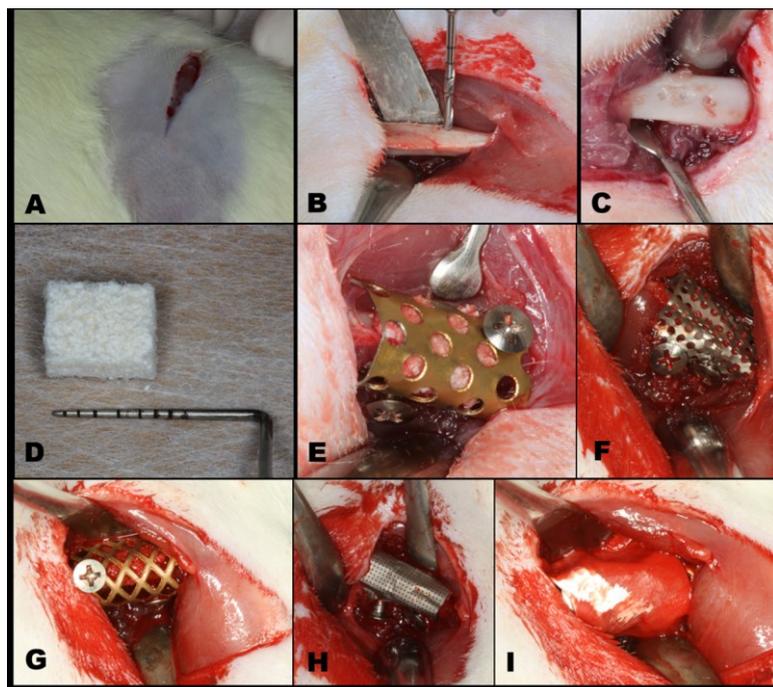
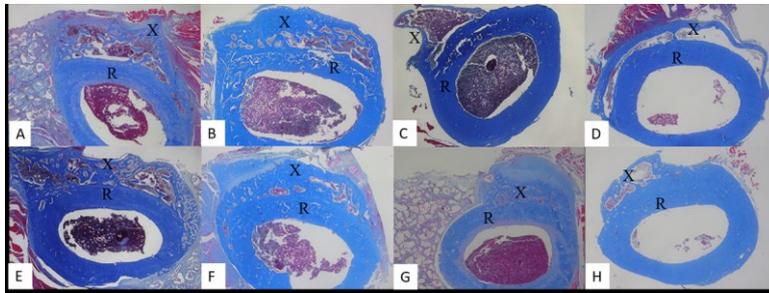


Figure 2 – Histologic samples. **A** – Bone formation in Group P300, femur test; **B** - Bone formation in Group P175, femur test; **C** - Bone formation in Group P85, femur test; **D** - Bone formation in Group P15, femur test; **E** - Bone formation in Group P300, femur control; **F** - Bone formation 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 2.5X. X: grafted area; R: femur.



Tables

GROUPS		TISSUE VOLUME (TV, mm ³)	BONE VOLUME (BV, mm ³)	BV/TV (%)	BONE MONERAL DENSITY (BMD)
P300	TEST	125.53 ± 24.85	27.22 ± 5.28	21.95 ± 3.91	0.10 ± 0.03 f
	CONTROL	135.91 ± 27.77	35.95 ± 7.84	26.77 ± 4.57	0.10 ± 0.03 e
P175	TEST	112.3 ± 28.32	32.58 ± 13.12	30.45 ± 14.20	0.10 ± 0.02 a *
	CONTROL	121.39 ± 15.22	33.70 ± 16.46	27.54 ± 11.26	0.11 ± 0.2 b y
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

Table 1 – μ -CT results. Tissue volume (mm³), Bone volume (mm³) and relation between bone volume and tissue volume (%). No statistically significant differences were observed. Bone mineral density (g/cm³). Significant differences between meshes pore size > 1mm and meshes pore size < 1 mm (Anova, Tukey, p <0.05). Level of significance was considered 95% (Anova, Tukey). (f) Statistical differences between P300-T X P85-T, P85-C e P15-C; (e) Statistical differences between P300-C X P85-T, P85-C e P15-C; (a) Statistical differences between P175-T X P85-T, P85-C e P15-C; (b) Statistical differences between P175-C X P85-T, P85-C, P15-T, P15-C. T: Test, C: Control.