Differences among membranes induced by different implants and cements: Experimental study

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ABSTRACT

Background: The objective of this study is to analyze and compare the characteristics of the membranes that form around cement spacers; as well as the one that develops around titanium and steel implants. **Materials and Methods:** 20 rabbits were divided into 2 groups of 10. In Group 1, an antibiotic-coated cement spacer was placed on the right femur, and a titanium elastic nail (TEN) on the left one. In Group 2, an antibiotic/steroid-coated cement spacer was placed on the right femur, and a steel pin on the left one. At 6 weeks, the membranes were removed and its macroscopic, imaging, biochemical and histological characteristics were evaluated. **Results:** Macroscopy: The membrane induced by the ATB-coated cement spacer was significantly wider, whereas the one induced by the steroid-coated cement spacer and the TEN was very thin and adherent. Microscopy: The membrane induced by the steroid-coated cement spacer showed less inflammation ($p = 0.0502$) and was similar to the one induced by the steel pin ($p = 0.322$). Steel pins showed greater epithelial proliferation ($p = 0.026$), which was scarce on the membrane induced by the steroid-coated cement spacer ($p = 0.071$). There was a mild tendency towards less active vascular proliferation ($p = 0.107$) in the group of the steroid-coated cement spacer vs. the one without steroids. There were no differences between the steel pin and the TEN ($p = 0.737$). X-rays and CT showed no significant differences ($p = 0.988$). In MRIs, most of the responses indicated lack of osseointegration in the steel pin group due to metallic artifacts. **Conclusions:** Different materials (titanium, steel and cement) with different agents added to them (antibiotics and steroids), alter the membranes both macroscopically and histologically. The steroid-coated cement spacer showed less inflammation and fibrosis, less vascular proliferation, and thinner and adherent membranes.
Key words: Cement spacer; peri-implant membrane; Masquelet membrane; antibiotic-coated cement.

Diferencias entre las membranas inducidas por diferentes implantes y cementos. Estudio experimental

RESUMEN

Introducción: El objetivo de este estudio fue analizar y comparar las características de las membranas que se forman alrededor de espaciadores de cemento y aquellas que rodean a implantes de titanio y acero. **Materiales y Métodos:** Veinte conejos en 2 grupos de 10: grupo 1, espaciador de cemento con antibióticos en fémur derecho y clavo de titanio (TEN) en fémur izquierdo; grupo 2, espaciador de cemento con antibióticos más corticoide en fémur derecho y clavija de acero en fémur izquierdo. A las 6 semanas se extrajeron las membranas. Se evaluaron sus características macroscópicas, bioquímicas, histológicas y en las imágenes. **Resultados:** *Macroscopia:* la membrana del cemento con antibióticos era significativamente más ancha y, en el cemento con corticoide y el TEN, era muy fina y adherente. *Microscopia:* menos inflamación en el cemento con corticoide ($p = 0,0502$), sin diferencias con las clavijas ($p = 0,322$). La proliferación epitelial era mayor en las clavijas ($p = 0,026$) y escasa en el cemento con corticoide ($p = 0,071$). Hubo una leve tendencia a la proliferación vascular ($p = 0,107$), de menor actividad, en el grupo con corticoide vs. sin corticoide. No hubo diferencias entre clavija y TEN ($p = 0,737$). No hubo diferencias significativas en las radiografías y la tomografía ($p = 0,988$). En la resonancia magnética, la mayoría de las respuestas en el grupo 2 indicaron sin osteointegración, debido a distorsión de la imagen (metal). **Conclusiones:** Diferentes materiales y los diferentes agregados alteran macroscópica e histológicamente las membranas. El cemento con corticoide presentó menor inflamación y fibrosis, menos proliferación vascular, y membranas más finas y adherentes.
Palabras clave: Espaciador de cemento; membrana peri-implante; membrana de Masquelet; cemento con antibióticos.

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INTRODUCTION

Membranes formed around implants and foreign bodies have been studied for years. They form around bullets, splinters or as a reaction to therapeutic interventions (pacemakers, arthroplasties, osteosynthesis); in the latter cases, the membrane that forms around the implant is, in general, damaging to purpose the implant.[1,2] There have been many efforts to control the thickness and vascularization of these membranes, as well as their formation. In general, the material of the implant, the topography of its surface, its porosity, dimensions, location and hydrophobicity, can affect the formation of the membrane.[1,3]

Surgeons' challenge in patients with bone loss lays on selecting the ideal reconstruction method for each patient. The two-stage reconstruction technique described by Masquelet, based on the formation of an induced membrane around the surgical cement and the subsequent addition of a bone graft, has gained great popularity for its simplicity and low cost, and for being technically simple and easy to reproduce.[4] Many methods have been tested to increase bone formation and decrease the time until consolidation with the Masquelet technique. Among the most popular are the variation in the time elapsed between the two stages, the supplementation with growth factors or cell therapies, and the use of different polymethylmethacrylates. In 2016, Masquelet noted that the best combination between a cement-induced membrane and the osteoconductive and osteoinductive material placed inside the membrane has not yet been established.

The current literature states that research should focus on the addition of substances that are beneficial on the constitution of the membrane to improve the incorporation of bone grafts and reduce the time between the first and second surgical stages.[5,6] On the other hand, surgeons' reasons to use the "preferred metal" in bone loss vary and, when choosing between different implant materials, they must balance the advantages and disadvantages of using steel or titanium.[7-9]

The purpose of this experimental study was to analyze and compare the characteristics of the membranes that form around antibiotic-coated cement spacers and antibiotic plus steroid-coated cement spacers, as well as the one that surrounds titanium and steel implants.

MATERIALS AND METHODS

An experimental study was carried out using 20 New Zealand rabbits, with an average weight of 2,500 kg, divided into two groups of 10 rabbits each. The procedure was approved by the Institutional Ethics Committee of our institution. Both femurs were intervened.

In group 1, an antibiotic-coated (gentamicin plus vancomycin) cement spacer was placed in the right femur and a titanium prosthesis (2.0-mm TEN nail) in the left femur. In group 2, an antibiotic- (gentamicin plus vancomycin) plus steroid-coated (hydrocortisone) cement spacer was placed in the right femur and a steel prosthesis (2.0-mm pin) in the left femur. The surgical procedure was carried out at the experimental surgery laboratory of an authorized university center.

Anesthetic induction was performed with IV ketamine (60 mg/kg), as well as diazepam 1/3 IM and 2/3 IR (5 mg/kg). All rabbits were given IM cephalomycin 20 mg/kg before and after surgery. As postoperative analgesia, IM ketorolac 1 mg/kg was administered.

A longitudinal lateral approach to the axis of the femur was performed, and dissection was made in planes until reaching the bone and raising the periosteum. In group 1, a 4-cm long titanium prosthesis (2.0-mm TEN nail fragment) was placed in the left femur and an equal-sized antibiotic-coated (gentamicin plus vancomycin) cement spacer was placed in the right femur (Figures 1 and 2). In group 2, a 4-cm long steel prosthesis (steel pin fragment) was placed in the left femur and an equal-sized antibiotic- (gentamicin plus vancomycin) and steroid (hydrocortisone) powder-coated cement spacer was placed in the right femur. The surgical wound was closed in planes. The SUBITON® G surgical cement (dosage of 40 g of powder and 20 mL of sterilized liquid plus gentamicin) was used, which was molded during the surgical procedure, forming cylindrical studs of 4 cm long, with an average weight of 0.5 g. During its preparation, 2 g of vancomycin powder was added for group 1, and 2 g of vancomycin plus hydrocortisone powder for group 2.

At 6 weeks, all rabbits were euthanized, and x-rays, MRIs and CT scans of both femurs were taken (Figures 3 and 4). The x-rays, MRIs and CT scans were evaluated by a radiologist specialized in the musculoskeletal system, who measured the formation of bone bridges and inflammation with the different materials placed in each rabbit. The MRIs were performed with a Philips® Achieva 1.5 T device and an 8-channel knee antenna was used. The CT scans were performed with a Siemens SOMATOM Sensation Multi-slice 64-channel scanner. The formation of bone bridges was determined by crosses (x = 1/3, xx = 2/3, xxx = 3/3).

When extracting the different materials, the thickness of the membrane, the adhesion of the membrane to the material and the bone formation around the material were evaluated. These three aspects were classified by the authors using a scale of 0 to 3 crosses, according to their magnitude, where 0 indicates absence of these parameters and 3 indicates highly prominent parameters. The membranes formed around the implants and the cement were then meticulously dissected so as not to damage them, and were preserved in 10% formalin. For histological evaluation, sections were obtained by sectioning a 2 x 2 cm sample, which was then included in paraffin. Multiple 6-mm thick cuts were made with a microtome and stained with hematoxylin-eosin to be observed under conventional light microscopy.

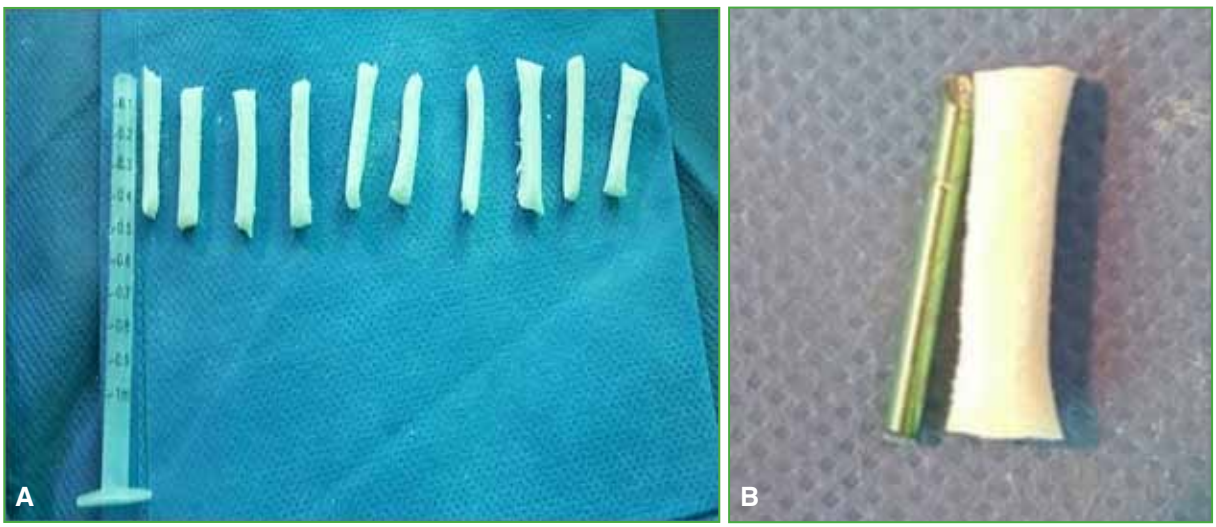

Figure 1. A. Preparation of cement spacers using tuberculin syringes. B. Titanium nail and cement spacer.

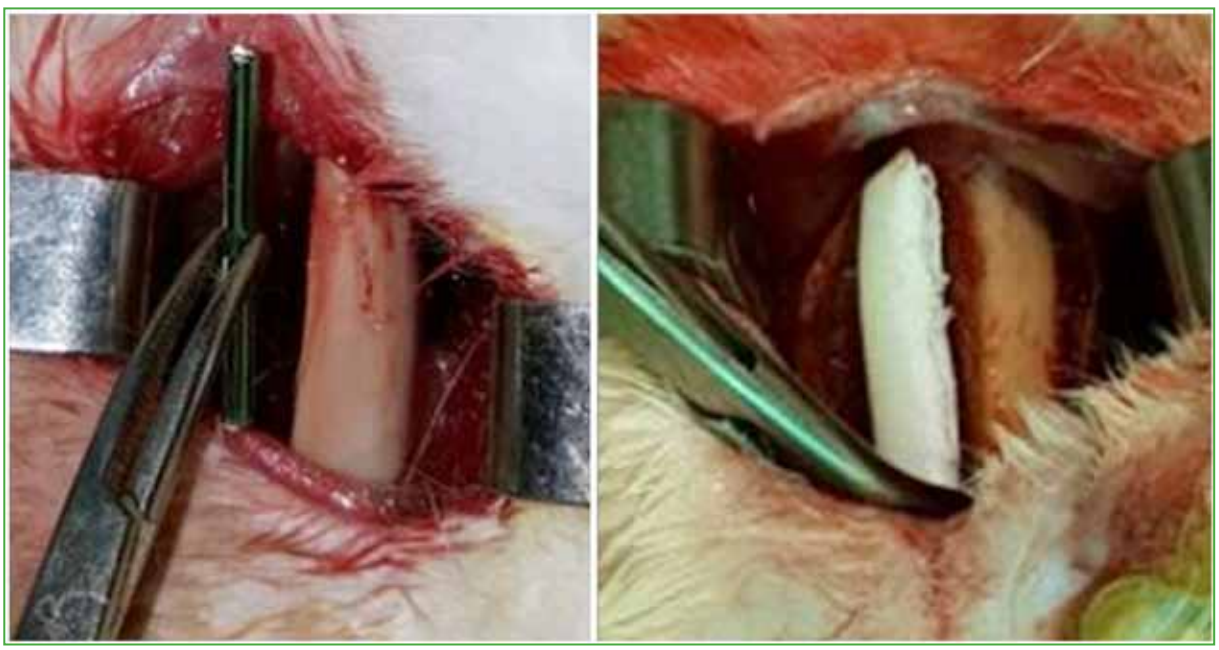

Figure 2. Surgical approach. Complete raising of the periosteum and placement of the different materials parallel to the rabbit's femur.

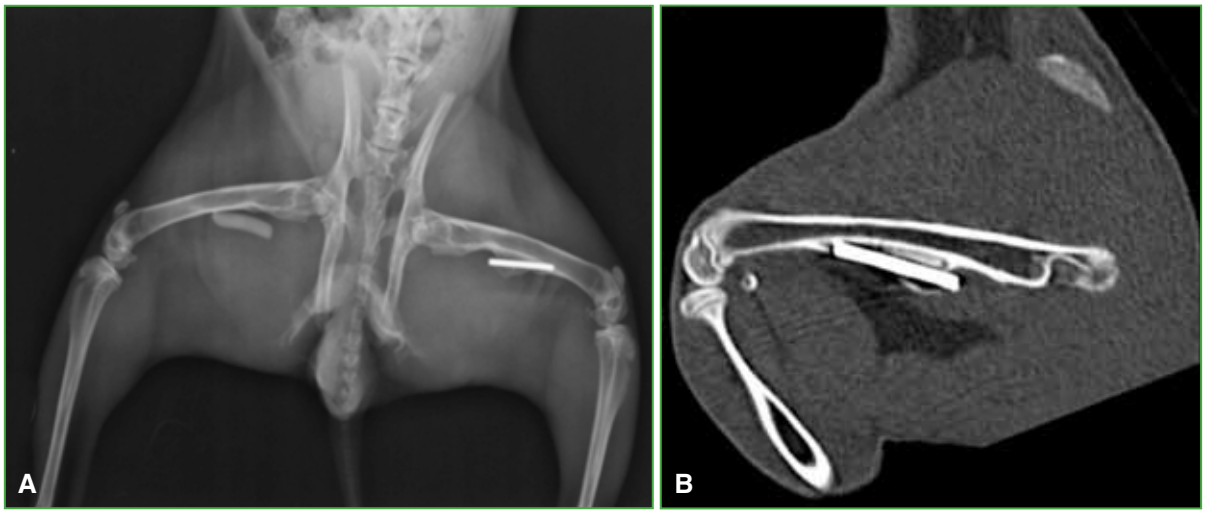

Figure 3. **A.** X-ray of both femurs. Antibiotic-coated cement spacer placed in the right femur and TEN nail placed in the left femur. **B.** CT scan. TEN nail.

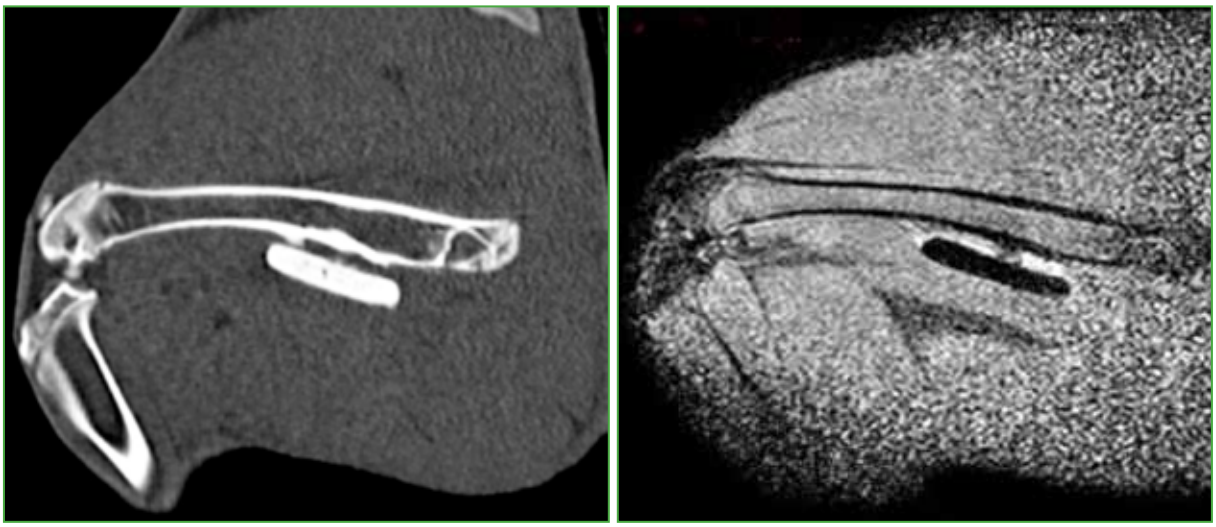

Figure 4. MRI. Antibiotic-coated cement spacer.

A qualitative and quantitative evaluation of the samples was performed, and the cellular and vascular proliferation, and the degree of fibrosis and inflammation were determined. All these variables were expressed by crosses according to their magnitude (- = absent, + = mild, ++ = moderate, +++ = intense). All samples were evaluated by the same pathologist.

RESULTS

After six weeks of placing the different materials, 17 rabbits were evaluated: 9 from group 1 and 8 from group 2 (3 died: 1 from group 1 and 2 from group 2).

When extracting the different materials, it was macroscopically observed that a membrane formed around the antibiotic-coated cement spacer was significantly wider than the rest, followed by the one surrounding the steel pins, which was very thin in the groups using steroid-coated cement spacers and titanium nails; on the contrary, the membrane showed greater adhesion in the group of titanium nails and steroid-coated cement spacers. Significant bone formation was only observed around titanium nails (Table and Figures 5-8).

Table. Masquelet's membrane macroscopic characteristics.

Membrane	Group 1, right thigh	Group 2, right thigh	Group 1, left thigh	Group 2, left thigh
Material	PMMA + antibiotics + steroids	PMMA + antibiotics	Titanium	Steel
Thickness	1.2	2.75	1	1.8
Adherence	2.4	1.75	3	2
Bone formation	0.2	0.5	1.5	0.4

PMMA = polimetilmetacrilato.

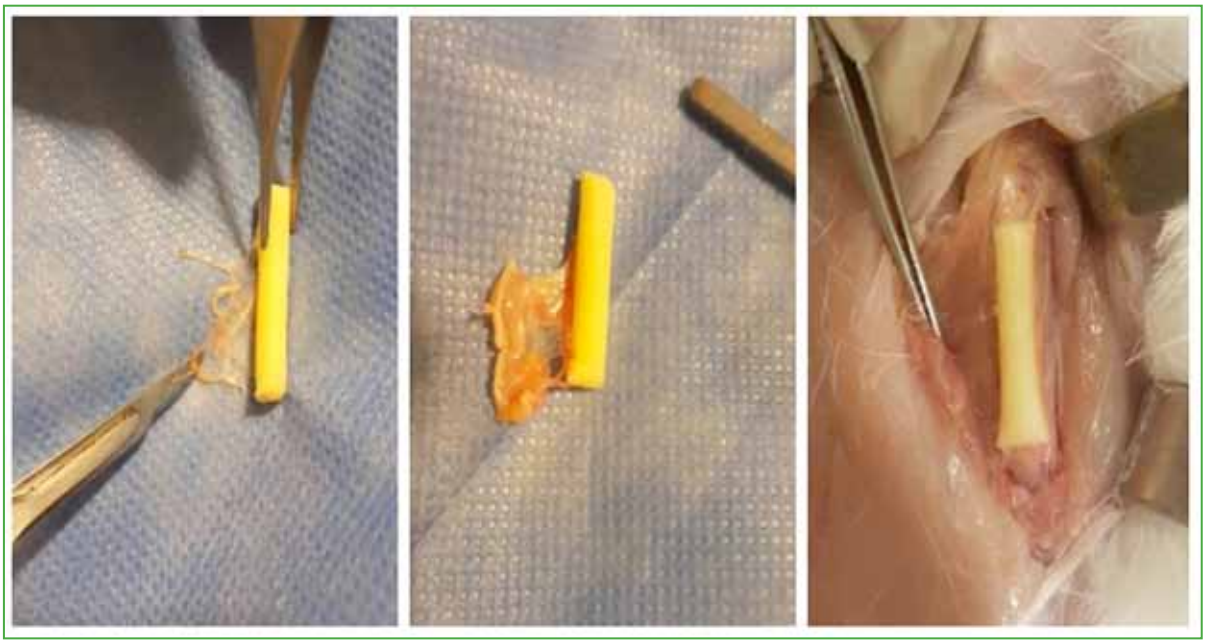

Figure 5. Masquelet's membrane in antibiotic-coated cement spacer.

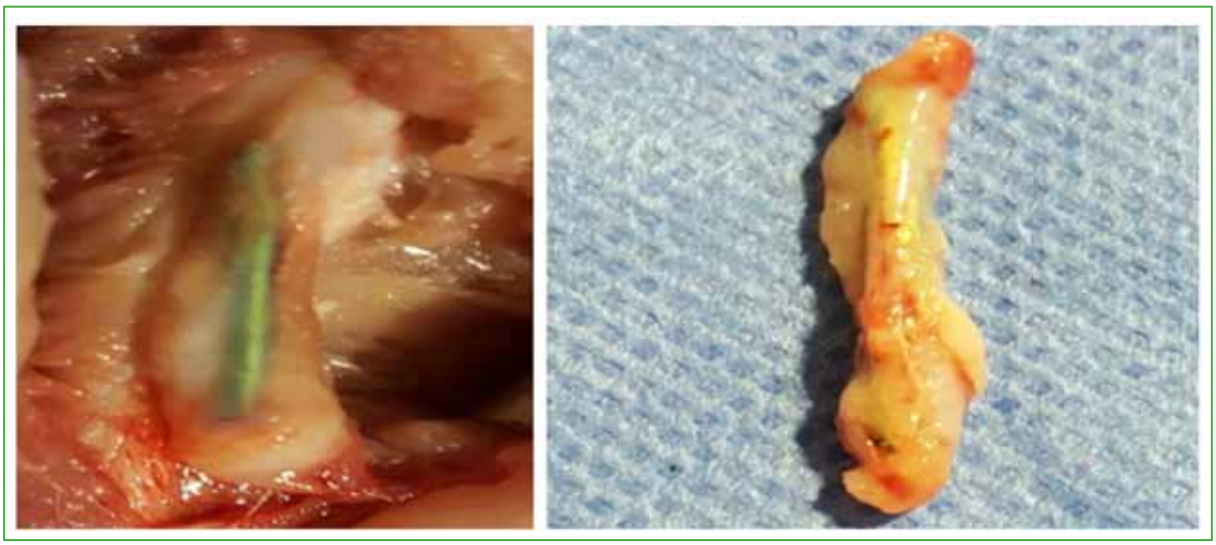

Figure 6. Masquelet's membrane in titanium nail.

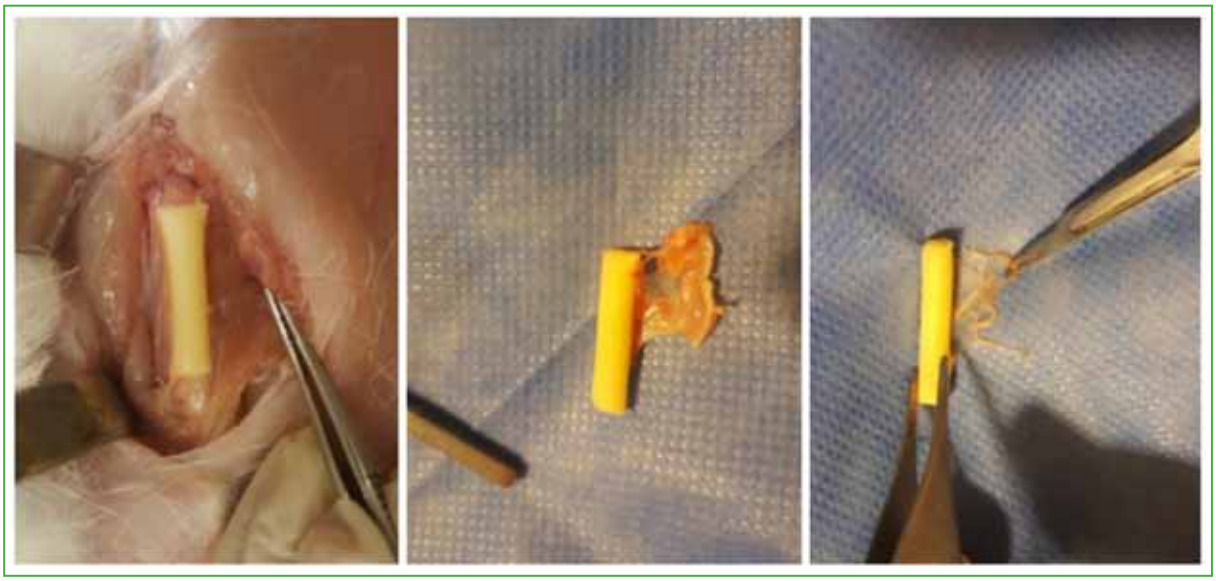

Figure 7. Masquelet's membrane in antibiotic- plus steroid-coated cement spacer.

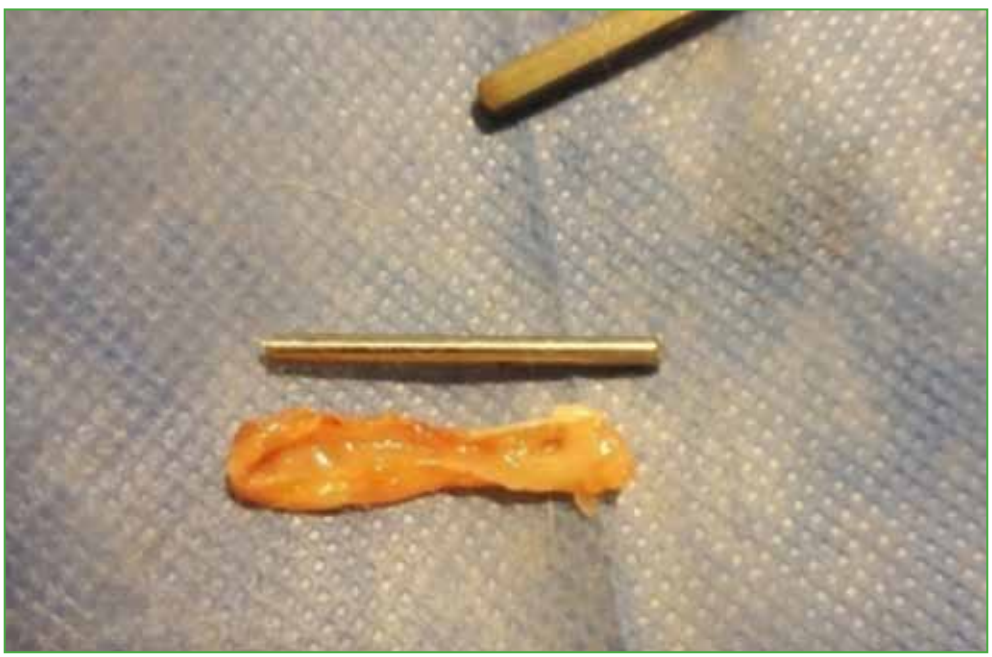

Figure 8. Masquelet's membrane in steel pin.

Regarding pathology, in general, the group with the antibiotic- plus steroid-coated cement spacer presented less inflammation ($p = 0.0502$) than the group with non-steroid-coated cement spacers, and there were no differences from the ones that received a pin ($p = 0.322$) vs. nail; $p = 0.365$). The latter (pin) presented higher epithelial proliferation activity ($p = 0.026$), while the activity was mild in animals with antibiotic-coated cement spacers and low in those with steroids ($p = 0.071$).

The presence of fibrosis and vascular proliferation did not differ among the groups (p = 0.85 and p = 0.369, respectively), but there was a mild tendency (p = 0.107) to lower vascular activity in the group treated with steroid-coated cement spacers. The groups in which a pin and a nail were placed did not differ regarding this indicator (p = 0.737) (Figure 9).

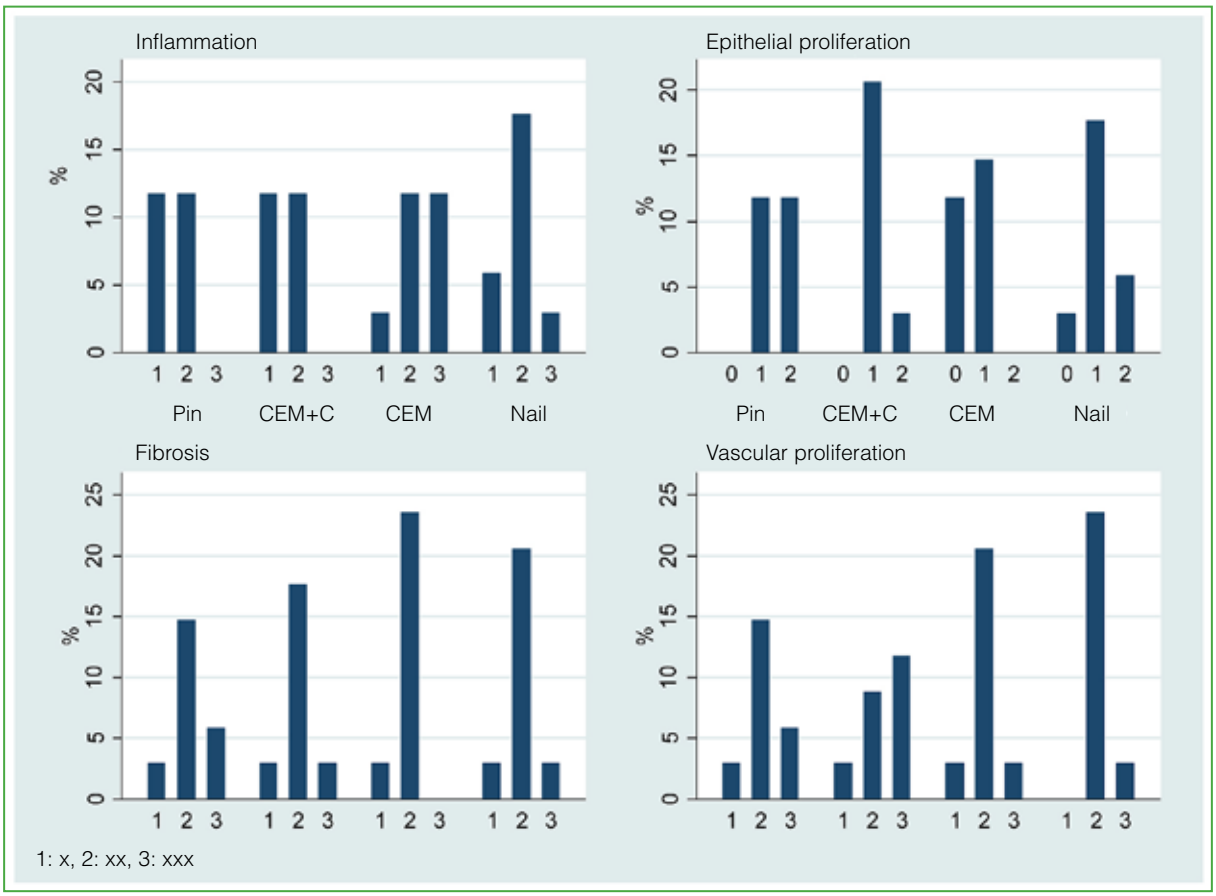

Figure 9. Response distribution: inflammation, epithelial proliferation, fibrosis and vascular proliferation for the two groups treated with a pin, a steroid-coated cement spacer and a nail.

There were no significant differences between the sides (right = steroid-coated cement spacer, left = pin) in x-rays and CT scans (p = 0.310, p = 0.988, respectively). Regarding MRIs, most of the responses indicated absence of bone integration or a rating scale of up to 1/3. 87% of the animals that received pins had no signs of bone integration due to image distortion or loss of signal produced by the material (metal), while that same percentage of animals treated with the steroid-coated cement spacer showed a bone integration of 1/3 and 2/3. This trend was significant (p = 0.003) to distinguish between the two groups (Figure 10).

Inflammation observed on the MRIs of the first group did not show significant differences (p = 0.592) between cement spacers and nails, which suggests that it not depend on the technique used, since the proportions of this sign are similar (3/8 vs. 2/8, respectively). Regarding the second group (steroid-coated cement spacer vs. steel pin), equal proportions were observed, without significant differences (p = 0.319).

Figure 10. Frequency distribution in the assessment scale for both treatments performed, in a paired manner, in the animals studied.

DISCUSSION

Segmental bone defects can be secondary to high-energy trauma, tumor resections, infections or revision surgeries.[10-12] The goal of the treatment is to eradicate the tumor or infection and the pain, and achieve consolidation while maintaining bone length and alignment with a functional articular range.[13-17] Different reconstruction options for segmental bone losses have been described, and the placement of an antibiotic-coated cement spacer (a technique known as *Masquelet's induced membrane*) is among the most commonly used.[12-18]

The limitations of this study were the absence of a bone defect, so materials were not subject to weight-bearing; not having performed the second stage of the technique (consisting of bone graft placement) and not having performed a Western-Blot of the samples (to determine growth factors and vascular endothelial growth factor). Our study showed that the membrane formed around the steroid-coated cement spacer was thin and adherent, with a macroscopic similarity to the one formed around the titanium nail, but showed no bone formation around it like the titanium nails did, and presented less vascularization than antibiotic-coated cement spacers. However, this difference was not statistically significant.

Cement-induced membranes are fibrous capsules that share some similarities with the periosteum.[19] The technique described by Masquelet combines the induction of a vascularized membrane by means of a cement spacer and the subsequent placement of a cancellous bone graft. The main role of the spacer is mechanical, since it fills dead spaces, prevents invasion of fibrous tissue, maintains bone length and alignment, and prepares the recipient bed for a future bone graft. In addition, it releases high local concentrations of antibiotics, with minimal systemic distribution. The secondary role is biological, since it induces the formation of a surrounding membrane. This membrane is similar to synovial lining and mainly composed of collagen type I and fibroblasts. Its inner surface is well vascularized. It can secrete bone morphogenetic protein 2 (BMP-2), vascular endothelial growth factor, central binding factor α1, interleukin 6, collagenase I, and other growth factors to stimulate bone defect reconstruction.[20-22] Removal of this inner layer of the cement-induced membrane results in vascular bed bleeding, but preserves the mechanical function of the rest of the fibrous capsule of this membrane, thus enriching the biological environment of the bed and increasing bone regeneration. In our study, we observed the same macroscopic and histological characteristics of a synovial membrane, with presence of fibroblasts and vascular and epithelial proliferation.

The morphological and molecular structural changes of the membrane induced by time suggested that the optimal time for autologous bone grafting is 6 to 8 weeks.[20-24]

Bone grafts must be covered by healthy tissues in order to be revascularized. According to Masquelet *et al.*,[6] the role of the induced membrane is to protect the graft from the environment to prevent resorption.

Pelissier *et al.*[25] found high concentrations of these osteogenic factors from week 4 after the first stage of the surgery. In our study, although we were unable to perform any quantitative assay of osteogenic factors due to lack of reagents, we did found histologically large vascular proliferation, which suggests the presence of vascular endothelial growth factor, with the exception of group 2 (steroid-coated cement spacer), in which less vascular proliferation was observed, as well as less epithelial proliferation and less inflammation in comparison to group 1 and the contralateral limb of the same group. The membrane works to prevent soft tissue protrusion at the site of the bone defect, provides a framework for osteoconduction, maintains adequate vascularization and creates a closed space where osteogenic cells and substances are preserved.

The polymethylmethacrylate used to fill bone defects is usually prepared with antibiotics, which increases local concentration within the defect, but the addition of antibiotics has an impact on the properties of the cement-induced membrane.[26] The proportion of antibiotics added when preparing the cement should not exceed 5% of its mass (e.g., 2 g of antibiotic every 40 g of cement), so that it does not weaken and increase the risk of cement fracture. We used vancomycin to prepare the cement, since this agent has certain characteristics, according to Murray's criteria, that make it possible to use in spacers (it is a thermostable, hypoallergenic and water-soluble drug with a suitable antibiotic spectrum and is available in powder).[27]

The choice of the implant material is important in terms of resistance to infection. Tissue adhesion to the implant is the most important factor in its resistance to infections. Without adhesion, mechanical irritation induces the formation of a capsule with a dead space within, and this capsule decreases the inflow of cells that promotes bacterial colonization and the growth and spread of bacteria.[7,8] In this aspect, titanium is biologically superior to steel due to its greater resistance to infection, its adherence to peripheral tissues and the decrease in dead spaces.[8] Greater tolerance observed with titanium implants in both animals and humans is well documented.[28-30]

CONCLUSIONS

The membranes that formed around osteosynthesis materials did not differ significantly from a pathological point of view. There were, however, differences between the membranes formed around steroid-coated cement spacers and antibiotic-coated cement spacers. In the first case, less inflammation and fibrosis were observed, but also less vascular and epithelial proliferation.

With regards to bone integration, no significant differences were observed between the groups (right = steroid-coated cement spacer, left = pin) ($p = 0.310$ and $p = 0.988$, respectively) on x-rays and CT scans. According to the MRIs of group 2, most of the responses lacked bone integration or had a rating scale of up to 1/3 in the animals treated with the pin due to image distortion or signal loss produced by the material (metal). On the other hand, in animals treated with the steroid-coated cement spacer, bone integration was 1/3 and 2/3, and had statistical significance ($p = 0.003$).

Regarding inflammation assessed by MRI, the first group showed no significant differences ($p = 0.592$) between the antibiotic-coated cement spacer and TEN nail. In the second group, equal proportions were observed regarding the presence or absence of inflammation in animals treated with steroid-coated cement spacers ($p = 0.319$).

Conflict of interest: Authors claim they do not have any conflict of interest.

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