SOCIEDADE BRASILEIRA DE ORTOPEDIA E TRAUMATOLOGIA

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Original article

In vivo evaluation of porous hydrogel pins to fill osteochondral defects in rabbits☆

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ARTICLE INFO

Article history:
Received 14 January 2016
Accepted 29 March 2016
Available online 29 December 2016

Keywords:
Articular cargilage
Hydrogels/chemistry
Rabbits

ABSTRACT

Objective: This experimental study aimed to evaluate the biological performance of poly (L-co-D, L-lactic acid)-co-trimetilene carbonate/poly (vinyl alcohol) (PLDLA-co TMC/PVA), hydrogel scaffolds, as an implant in the filling (and not in the repair) of osteochondral defects in New Zealand rabbits, assessing the influence of the material in tissue protection *in vivo*.
Methods: Twelve rabbits were divided into groups of nine and 16 weeks. In each animal, an osteochondral defect was created in both medial femoral condyles. In one knee, a hydrogel scaffold was implanted (pin group) and in the other, the defect was maintained (control group). A histological analysis of the material was performed after euthanasia.
Results: The condyles of the pin group showed no inflammatory reaction and were surrounded by a fibrous capsule. The control group presented higher bone growth in the areas of the defect, but with disorganized articular cartilage, evident fibrosis, bone exposure, atrophy, and proliferation of synovial membrane.
Conclusion: The hydrogel pins are promising in *filling* osteochondral defects, generally do not cause inflammatory reactions, and are not effective in the *repair* of osteochondral defects.

☆ Study conducted at the Pontifícia Universidade Católica de São Paulo, Laboratório de Biomateriais, Faculdade de Ciências Médicas e da Saúde de Sorocaba, Sorocaba, SP, Brazil.

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http://dx.doi.org/10.1016/j.rboe.2016.03.009

Avaliação do desempenho *in vivo* de pinos porosos de hidrogel para preenchimento de defeito osteocondral em coelhos

Palavras-chave:
Cartilagem articular
Hidrogéis/química
Coelhos

RESUMO

Objetivo: Trabalho experimental para avaliar o desempenho biológico de arcabouços de hidrogel poli (L-co-D, L ácido lático)-co-trimetileno carbonato/poli (álcool vinílico) (PLDLA-co-TMC/PVA) como implante no preenchimento, e não no reparo, de defeito osteocondral em coelhos Nova Zelândia e verificar a influência do material na proteção tecidual *in vivo*.
Métodos: Foram usados 12 coelhos divididos em grupos de nove e 16 semanas. Em cada animal foi criado um defeito osteocondral em ambos os côndilos femorais mediais, em um foi implantado um arcabouço de hidrogel (grupo pino) e no outro foi mantido o defeito (grupo controle). Após o sacrifício dos animais, foi feita análise histológica do material.
Resultados: Os côndilos do grupo pino não evidenciaram reação inflamatória e estavam rodeados por cápsula fibrosa. Já no grupo controle, uma maior proliferação óssea foi observada nas áreas do defeito, porém com cartilagem articular desorganizada, fibrose evidente, atrofia com exposição óssea e proliferação de membrana sinovial.
Conclusão: Os pinos de hidrogel são promissores na função de *preenchimento* de defeitos osteocondrais, não ocasionam, de modo geral, reação inflamatória e não são eficazes no *reparo* de defeitos osteocondrais.

Introduction

Osteoarthritis is one of the diseases that most commonly affect humans.[1] Its prevalence increases with age, being common after 60 years.[2] The articular cartilage and the subchondral bone form a lubrication, stabilization, and uniform load distribution system, absorbing shocks and allowing movement with low friction for several decades.[2–4] Thus, cartilage protects subchondral bone from high stress and reduces normal contact pressure.[2,5] Degraded cartilage evolves to joint pain, stiffness, and decreased movement. Due to low chondral regeneration capacity, osteoarthritis is one of the most important problems in orthopedics. With increasing human longevity and the practice of sports in recent decades, osteochondral injuries have been increasingly observed.[2]

Normal joint cartilage features a solid phase consisting mostly of collagen and proteoglycans (15–32%) and a fluid phase composed predominantly of water (68–85%).[2] Hyaline cartilage consists of 10% cells (chondrocytes) and a dense extracellular matrix, composed of 60–80% water, 10–20% type II collagen fibers, and 10–15% proteoglycans. The mechanical properties of the articular cartilage allow it to transmit loads of approximately eight times the body weight, due to exudation and movement of the fluid through the pores of cartilage, conferring a friction coefficient of 0.008 μ (mi).[4,6]

The subchondral bone is a thin layer of dense, hard bone in contact with the articular cartilage, while trabecular bone is composed of an abundant matrix (collagen fibers and minerals) that serves to transmit loads.[6]

Being avascular, the cartilage depends on the vascularization of the bone marrow for the migration of mesenchymal cells responsible for the healing process.[7,8] Furthermore, superficial lesions of the articular cartilage without subchondral bone involvement have little intrinsic repair capacity.[9]

Both in primary and secondary osteoarthritis, cartilage is the tissue that undergoes the greatest damage. Morphological changes to the cartilage include loss of its homogeneous nature, fragmentation, fibrillation, fissures, and ulcerations. With disease progression, occasionally no cartilage remains and areas of the subchondral bone become exposed.[10]

Three stages can be considered in the process of tissue regeneration: necrosis, inflammation, and repair.[11,12] Nonetheless, healing of lesions restricted to the hyaline cartilage may not occur this way.[13] Superficial lesions of the articular cartilage that do not reach the subchondral bone tend not to heal.[9] In these lesions, there is a degeneration of the cartilage from the surface area; thin portions of collagen fibers with scaly appearance are observed. With lesion progression, vertical cracks with an uneven and dull appearance can be observed in the articular cartilage.[4,14]

Chondral lesions trigger an inflammatory process and the hematoma quickly organizes itself into fibrin clots, white blood cells, and bone marrow elements.

Undifferentiated bone marrow and vascular endothelium cells are converted into primitive fibroblasts, which, with input of capillaries and fibrin clots, turn into vascular fibroblastic repair tissue.[11] Depending on the mechanical and biological stimuli, this fibrocartilage tissue will form a cartilaginous tissue.[13] Newly formed bone migrates from the base of the defect to the articular surface in the area in contact with subchondral bone. Fibrocartilaginous tissue fills the transition zone and interrupts the formation of bone tissue.[4,11,14]

Osteochondral defect repair tissue has a different composition than normal cartilage.[15] Chondrocytes synthesize proteoglycans of lower molecular weight. As collagen type II fibers have a smaller diameter and more irregular arrangement, this configuration favors water permeability. This newly formed tissue presents a lower elastic modulus when compared with normal cartilage tissue.[4,16,17]

Surgical treatment of chondral and osteochondral lesions is a major challenge. The formation of cartilaginous or fibrocartilaginous tissue can be stimulated, repairing or replacing such injuries with a tissue that presents similar characteristics.[4,13]

There are no replacement tissues whose mechanical properties are similar to that of the original tissue.[18] Currently, there is ongoing research on tissue engineering using synthetic three-dimensional systems with porous scaffolds.[19,20]

Polymers such as poly-p-dioxanone (PPD), polylactic acid (PLA), polyglycolic acid (PGA) and its copolymers PLLA and PLGA, and poly-α-hydroxy acids, as well as their decay, bioreabsorption, and biocompatibility characteristics, have been thoroughly studied.[4,21–24]

A non-porous and non-absorbable hydrogel (PLDLA-co-TMC/PVA) was used in the present study to fill the osteochondral defect rather than to make a repair that allowed the growth of new tissue.

The *in vivo* biological response to PLDLA-co-TMC/PVA hydrogel pins in the filling of osteochondral defects is unknown.

This study aimed to evaluate the biological performance of PLDLA-co-TMC/PVA hydrogel scaffolds as an implant in the *filling*, rather than in the *repair* of osteochondral defects in New Zealand rabbits, and to assess the influence of the material in *in vivo* tissue protection.

Material and methods

This study was approved by the Research Ethics Committee of this institution (CEUA/FCMS/PUCSP, Number 2013/10).

Preparation of polymer scaffolds (hydrogel pins)

Hydrogel pins are composed of a semi-interpenetrating polymer based on polyvinyl alcohol (PVA) and poly-L-co-D, L-lactic acid-co-trimethylene carbonate (PLDLA-TMC). The hydrogel was made with a 10% m/v PVA solution in water and 10% of the PLDLA-TMC compound relative to the mass of the PVA used. The solution was then poured into polytetrafluoroethylene (PTFE) scaffolds with a 4.1 mm diameter, and was submitted to a freeze–thaw process for two days. After this process, pins were removed from the scaffolds, cut at a length of 13 mm, sterilized with UV radiation, and implanted (Fig. 1).

In vivo study of the implanted pins and controls

Twelve New Zealand rabbits of both sexes were used, aged between 120 and 150 days and weighing between 3.5 kg and 4.5 kg.

After a preoperative eight-hour fasting, the animals were submitted to general anesthesia with intramuscular ketamine hydrochloride (30 mg/kg) associated with xylazine chloride (5 mg/kg). A trichotomy of the operated area was made, and rabbits were placed on a proper operating table in the supine position. Sterilization was conducted with alcoholic chlorhexidine 0.5%, applied with sterile gauze. Surgeon used sterile surgical gloves and the surgical materials were sterilized by autoclaving at 180 °C for 2 h.

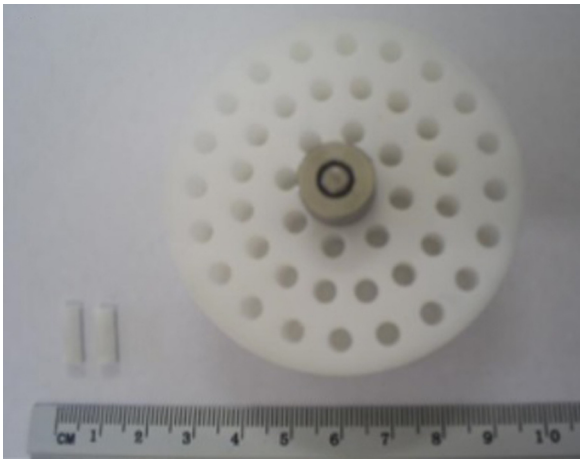

Fig. 1 – PLDLA-co-TMC/PVA hydrogel pins and scaffold used for making the devices.

A medial parapatellar incision was used, with dissection by planes (subcutaneous and capsulotomy for patellar medial). Then, a lateral patellar dislocation was made to expose the femoral condyles. With the knee flexed at 90°, a cylindrical defect was created in the articular cartilage and in the subchondral bone of the medial femoral condyle with the use of a 3.3 mm/38.5 mm trephine drill; a 1 cm deep osteochondral cylinder was removed from both knees[7,13] (Figs. 2 and 3).

Fig. 2 – Hydrogel and trephine implant.

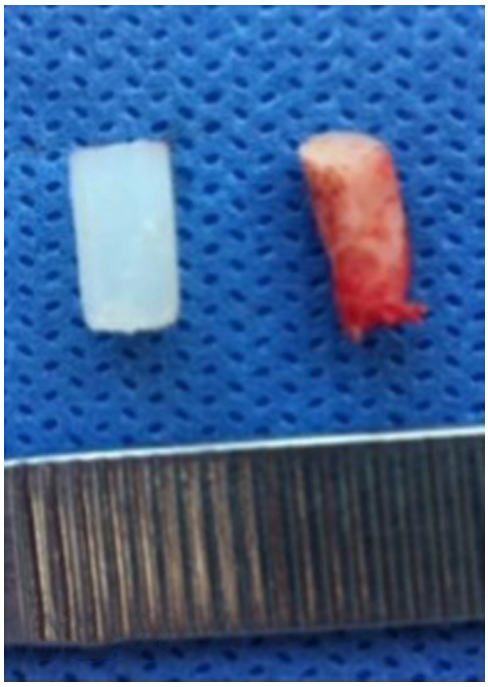

Fig. 3 – Implant and osteochondral cylinder.

The chondral defect was created in both medial and femoral condyles; on one side, the hydrogel pin was implanted (implant side) while on the other (control side), the defect was kept (Fig. 4).

Sutures were made in planes after washing with 0.9% saline solution; no bandages or immobilization devices were used. All animals were kept in individual cages with food and water *ad libitum*.

Removal of the material – euthanasia and tissue collection

Seven animals were euthanized after nine weeks, and five, after 16 weeks.

Halothane inhalation was used. After death was confirmed, the animal was placed in the supine position. Then, the medial femoral condyles of both knees were resected through the medial patellar access route.

Macroscopic evaluation

The condyles were individually identified, macroscopically observed, and photographed (Fig. 5). According to the modified Outerbridge classification, no tissue growth was observed on the pin (both at 9 and 16 weeks), and the pin group could not be included in the classification. In the control group, with nine or 16 weeks, alterations characteristic of Outerbridge II were observed.

Material processing – histological analysis

The femoral condyles were placed in glass containers with Bouin's solution (consisting of picric acid, acetic acid, and formaldehyde) for 24 h for fixation, which maintains tissue integrity after death.

The material was decalcified in EDTA 4.13% (the decalcification solution consisted of tetrasodium EDTA, sodium tartrate, potassium sodium tartrate, hydrochloric acid, and distilled water) for 21 days.

For histological processing, longitudinal parallel (craniocaudal) incisions were made and sequentially identified for each sample. This technique allowed a precise evaluation of the entire area of the condyle, including the initially injured area and the entire surface around the lesion.

Samples, properly identified, were dehydrated in a series of alcohol solutions, sequentially cleared in xylene I, II, and III,

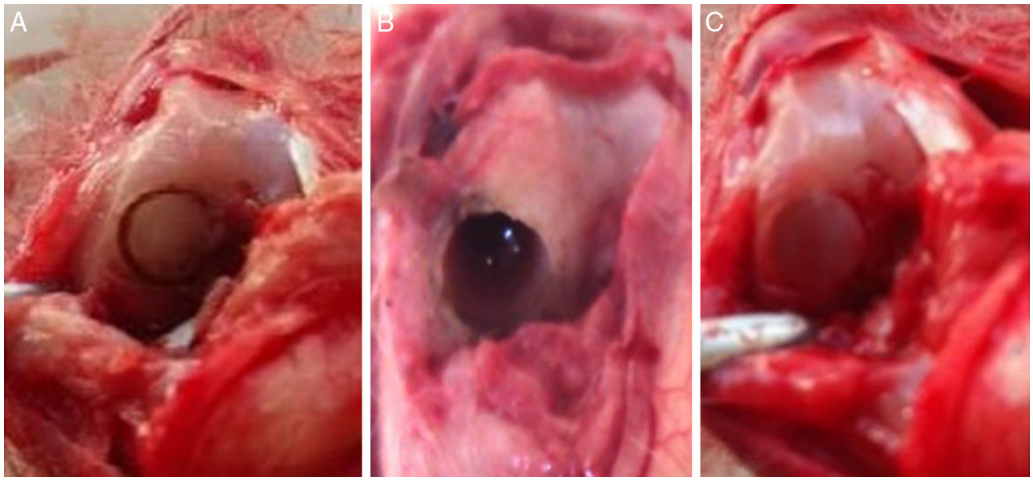

Fig. 4 – (A) Osteochondral defect *in situ*; (B) defect after removal of the osteochondral cylinder; (C) defect filled with the implanted hydrogel pin.

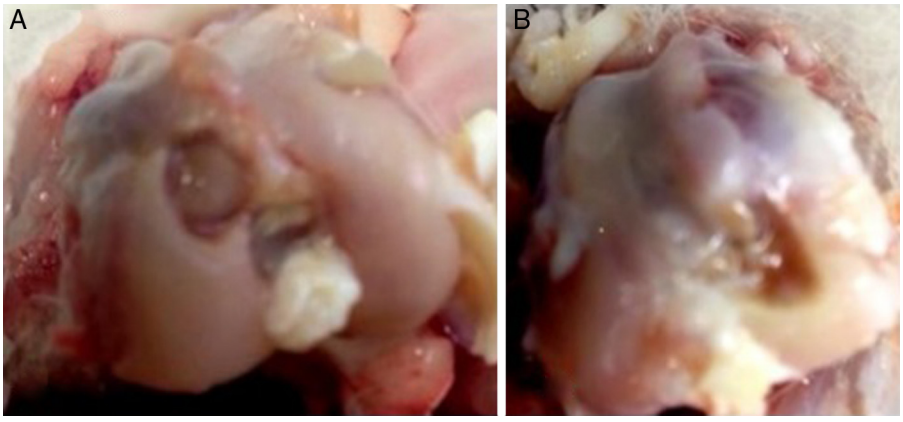

Fig. 5 – (A) With the hydrogel pin; (B) without the pin (control group).

and embedded in paraffin at 70 °C. After cutting 3 μm slices, samples were stained with hematoxylin–eosin and analyzed by conventional light microscopy.

Microscopic evaluation

On histologic examination of the sections of the distal femur, attention was directed particularly to the following elements:

1. type of tissue covering the articular surface: hyaline cartilage, fibrocartilage, bone tissue, or fibrous tissue;
2. state of the cartilaginous surface: smooth, with a depression, or irregular with the presence of cracks or fragments;
3. subchondral bone pattern;
4. presence or absence of necrosis and proper inflammatory response;
5. presence or absence of hyperplastic alterations on the cartilage or bone;
6. assessment of synovial membranes, when possible.

Results

The evaluation of results considered the amount of fibrosis, bone neoformation, and presence of cartilaginous tissue on the condylar surface.

In the pin group, euthanized nine weeks after the implant was placed, mild fibrosis was found in four animals, and marked fibrosis in two; no animal presented moderate fibrosis.

In the control group, euthanized nine weeks after the creation of the osteochondral defect, mild fibrosis was found in two animals, moderate in two, and severe in two.

In the pin group, euthanized after nine weeks after the implant was placed, one animal showed bone neoformation; in five, this neoformation was not observed. In the control group, bone neoformation was present in four animals, and absent in one.

Regarding the presence of cartilage in the joint surface, it was absent in four rabbits in the control group and present in one case. In one of the rabbits, an area that could not be defined as organized cartilaginous tissue was observed. In the pin group, presence of cartilage tissue was observed in four rabbits, while in two, it was not observed (Table 1 and Figs. 6 and 7).

One of the animals included in the group euthanized at 16 weeks presented surgical site infection and was excluded from the study.

Four rabbits from the pin group had mild fibrosis and one had no fibrosis.

Bone neoformation occurred in four rabbits in the control group; fragmentation of the material was observed

Table 1 – Group of rabbits at nine weeks.

Euthanasia at nine weeks	Groups	Fibrosis	Bone neoformation	Cartilaginous surface
Rabbit 1-9	Control	Marked	Absent	Absent
Rabbit 1-9	Pin	Discreet	Absent	Present
Rabbit 2-9	Control	Moderate	Present	Disorganized
Rabbit 2-9	Pin	Marked	Present	Absent
Rabbit 3-9	Control	Discreet	Present	Absent
Rabbit 3-9	Pin	Discreet	Absent	Absent
Rabbit 4-9	Control	Discreet	Present	Absent
Rabbit 4-9	Pin	Discreet	Absent	Present
Rabbit 5-9	Control	Marked	Present	Present
Rabbit 5-9	Pin	Discreet	Absent	Present
Rabbit 6-9	Control	Moderate	Absent	Absent
Rabbit 6-9	Pin	Marked	Absent	Present

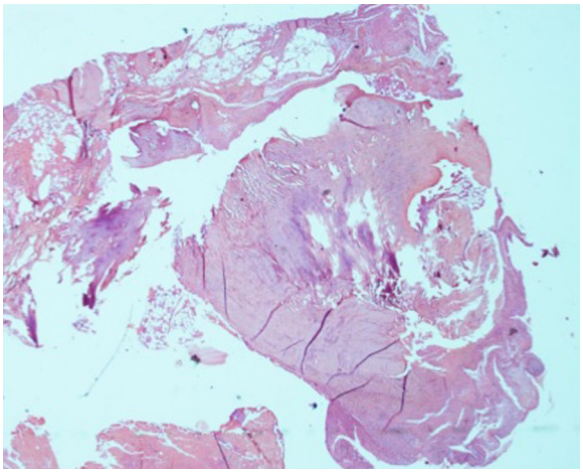

Fig. 6 – Histological slide of the pin group at nine weeks showing no inflammatory reaction and mild fibrosis.

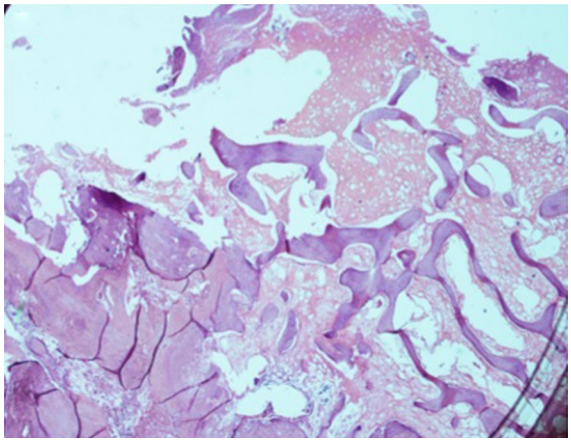

Fig. 7 – Histological slide of the control group at nine weeks showing bone neoformation, marked fibrosis, and tissue disorganization.

in one rabbit, which prevented the correct analysis. Bone neoformation was not observed in the pin group.

In the control group at 16 weeks, cartilage degeneration was observed in one case, while in the case with fragmentation of the material, it was not possible to analyze the presence or absence of cartilage. In the control group, cartilage on the joint surface was observed in two rabbits, and it was not present in one rabbit. In the pin group, five rabbits presented cartilage, vs. one in which it was absent (Table 2 and Figs. 8 and 9).

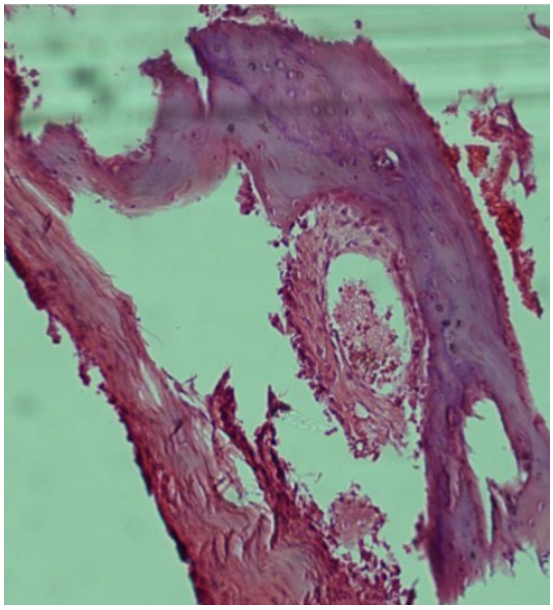

Fig. 8 – Histological slide of the pin group at 16 weeks showing mild fibrosis without bone formation.

Discussion

Of the 12 rabbits studied, only one from the group euthanized at nine weeks was excluded due to local infection. Thus, 11 animals were included in this study.

The histological analysis of the PLDLA-co-TMC/PVA scaffold implants after nine weeks showed that the pin does not induce an inflammatory reaction, but also does not stimulate bone neoformation. There is a stimulus of fibrous proliferation at the edges of the lesion from the articular cartilage, with formation of mainly fibrocartilage, as an attempt to heal the injury.

In the control group after nine weeks, more fibrosis and more bone formation were observed, either from articular cartilage from the edges of the lesion or from the trabeculae of compact bone surrounded by osteoprogenitor cells. However, there was no evidence of chondral lesion repair.

Table 2 – Group of rabbits at 16 weeks.

Euthanasia at 16 weeks	Groups	Fibrosis	Bone neoformation	Cartilaginous surface
Rabbit 1-16	Control	Discreet	Present	Present
Rabbit 1-16	Pin	Discreet	Absent	Absent
Rabbit 2-16	Control	Discreet	Present	Present
Rabbit 2-16	Pin	Discreet	Absent	Present
Rabbit 3-16	Control	Discreet	Present	Degenerated
Rabbit 3-16	Pin	Discreet	Absent	Present
Rabbit 4-16	Control		Fragmented	Infeasible analysis
Rabbit 4-16	Pin	Discreet	Absent	Present
Rabbit 5-16	Control	Moderate	Present	Absent
Rabbit 5-16	Pin	Discreet	Absent	Present

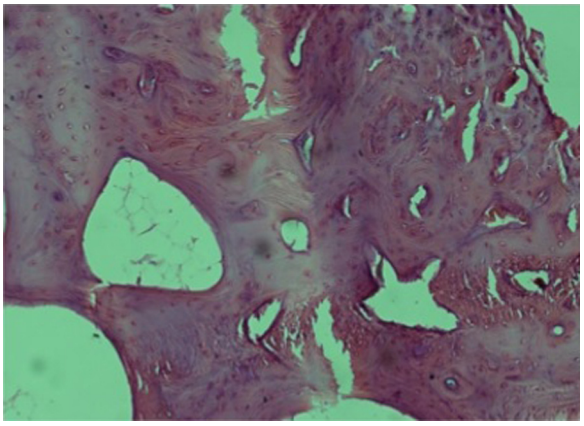

Fig. 9 – Histological slide of the control group at 16 weeks showing bone formation and fibrosis.

After 16 weeks, the condyles with hydrogel implants showed no inflammatory reaction and were surrounded by a fibrous capsule. The surrounding trabecular bone presented a preserved aspect and closure of the joint surface was observed, with the presence of hyaline cartilage or atrophic cartilage of fibro-hyaline appearance.

In the control group at 16 weeks, bone proliferation was observed in the defect areas. However, the articular cartilage was disorganized, with obvious fibrosis; atrophy with exposed bone and proliferation of the synovial membrane were observed.

Regarding bone neoformation, to the authors' surprise, one animal from the nine-week pin group presented growth of bone tissue with fibrous pattern. The other rabbits from this group showed no bone growth; in turn, bone formation was observed in the entire control group.

As expected, bone filling in osteochondral defect was observed in the control group euthanized at 16 weeks, most likely due to the increased time between surgery and euthanasia. Conversely, no animals in the pin group presented bone growth in the defect. This suggests that the presence of the implanted pin filled the entire space created by the defect.

Conclusion

Hydrogel pins were superior regarding the protection of the cartilaginous joint surface as devices for filling osteochondral defects. Nonetheless, they showed no reparative effect. In contrast, cartilage degradation in both the defect and the surrounding area were observed in the control group.

Conflicts of interest

The authors declare no conflicts of interest.

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