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Stimulation of Reparative Processes in Cartilage Defects in Rabbits Using AD (DIEE) Type Biophytomodulators

Cristian CRECAN[1)], Daniela OROS[1)], Ancu DINCĂ[2)], Pompei BOLFĂ[1)], Liviu OANA[1)], Lucia BEL[1)], Cosmin PEŞTEAN[1)], Ciprian OBER[1)]

[1] Faculty of Veterinary Medicine, USAMV Cluj-Napoca, cristi_crecan@yahoo.com
[2)] The Biosynthetic Research and Study Centre "Dinca Ancu" Bucharest

Abstract. The healing of cartilage lesions is a long process due to the lack of vascularization and the particular nutrition in this type of tissue. This study aims to histologically evaluate the healing preocess of lesions inflicted in the stifle joint cartilage of rabbits, then treated with AD (DIEE) type biophytomodulators. The study was performed on 33 rabbits of German Giant breed, aged 8 months. The animals were divided into 4 groups: a group of animals treated with AD (DIEE) type biophytomodulators (BF), an untreated control group (M) and two control groups treated with hyaluronic acid (H, HS), standard therapy for such pathology. There cartilage samples were collected at 14, 30 and 90 days post-operatively, and were then histologically stained (haematoxylin and eosin) and examined under light microscopy. The results obtained showed the presence of fibrous tissue at the lesion sites and the development of more advanced healing processes in the BF group compared to the untreated control group.

Keywords: cartilage, healing, rabbit, biophytomodulator.

INTRODUCTION

Articular cartilage represents the functional key of synovial joints. It varies in thickness, cell density and matrix composition, both between different joints and in the same joint.

All synovial joints contain cartilage composed of the same components that fulfills the same role. The qualities that make it remarkable and unique are its great pressure resistance, high resistance to wear and the ability to distribute pressure both on the surface and on a greater area of subchondral bone. There is no synthetic material that can reproduce these properties for such a long period of time (Bruckner et al., 1988).

Cartilage is composed of chondrocytes embedded in an organized structure, extracellular matrix consisting of collagen, proteoglycans, other proteins, ions and water. There are various interactions between extracellular matrix and chondrocytes that enable normal functioning of cartilage and maintain tissue integrity (Byers and Brown, 2006).

Posttraumatic repair and regeneration of mature articular cartilage are minimal, the cartilage not being able to repair through restitutio ad integrum; once appeared, structural alterations are impossible to fully repair by means of cartilaginous tissue (Buckwalter, 1995).

Not being inert, the cartilage is able, to some extent, to repair and regenerate, so that joint loading will not lead to arthrosis (Duncan et al., 1987). Certain treatments are able to at least partially restore the integrity of articular cartilage.Numerous studies have been conducted to better understand both the processes leading to cartilage degradation, alteration of composition and the processes related to cartilage repair and regeneration, with restoration of its function.

MATERIALS AND METHODS

The biological material used in this experiment consisted of a total of 33 German Giant breed rabbits, 8 months old, clinically healthy, from a single source, so they received the same housing conditions and care. The materials used were: sterile surgical instruments (scalpels, scissors, hemostatic forceps, suture needles) surgical drapes, absorbable and nonabsorbable sutures, enrofloxacin 5% (5% Enroxil®, Krka), disposable needles and syringes, saline, hyaluronic acid 10 mg/ml (Hyalgan®, FIDIA pharmaceuticals), specific reagents for histological processing.

Articular chondral lesions have been induced in the stifle joint of the animals, in the area of trochleea and lateral femoral condyle (Fig. 1). Cartilage defects were identical, with a length of 4.4 mm and 0.2 mm depth (Fig. 2). Before surgery animals underwent general anesthesia, using ketamine (Ketaminol 10 ®, MSD Animal Health) 35 mg/kg and xylazine (Xylazine Bio 2% ®, Bioveta) 4 mg/kg. The skin incisions were made to the side of the stifle joint and then articular capsule and lateral patellofemural ligament were exposed.

The capsule was longitudinally incised; the expression of synovial fluid occurred and chondral surface was evidenced. To highlight the place of choice for performing the defects, the patella was dislocated medially. Chondral defects were inflicted using a dental drill of 0.2/4.4 mm, adapted to a dental 3500 rpm micromotor. Irrigation with saline was used to avoid overheating and remove cartilage fragments (Fig. 3). Surgical reconstruction was performed in two layers: joint capsule was continuously sutured using 3.0 polyglycolic acid (Fig.4.) and the skin was sutured using interrupted sutures with 2.0 surgical silk (Fig.5.).

Fig.1. The site for the chondral defect

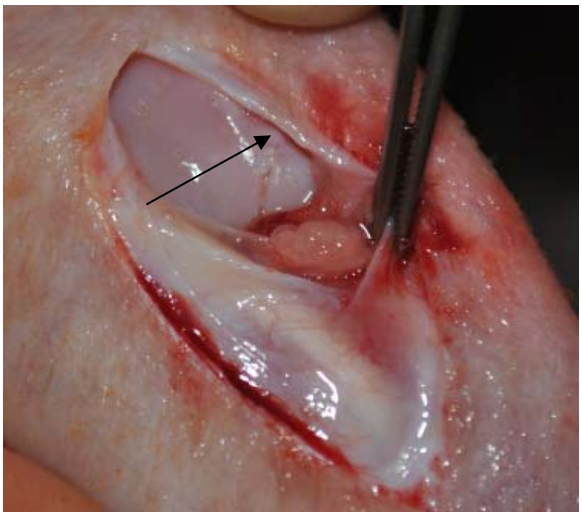

Fig.2. The chondral defect

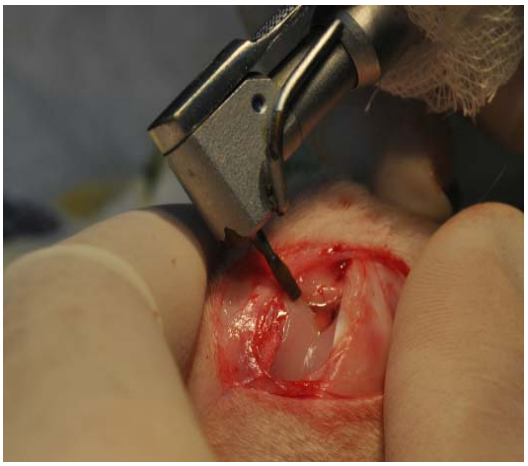

Fig.3. Removal of the cartilage

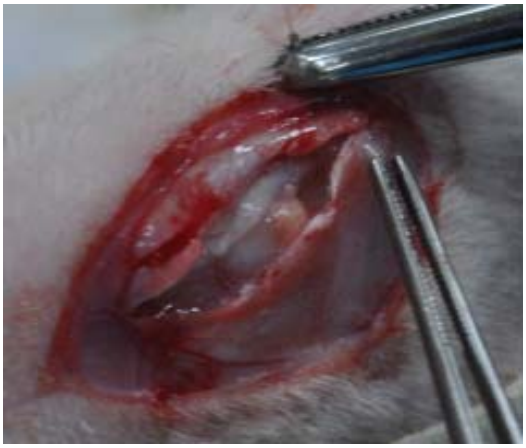

Fig.4. Suture of the joint capsule

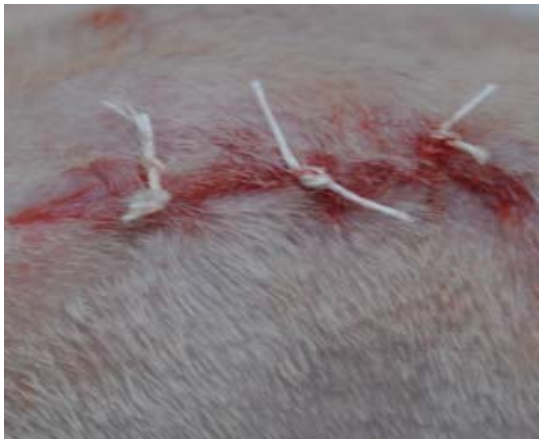

Fig.5. Skin suture

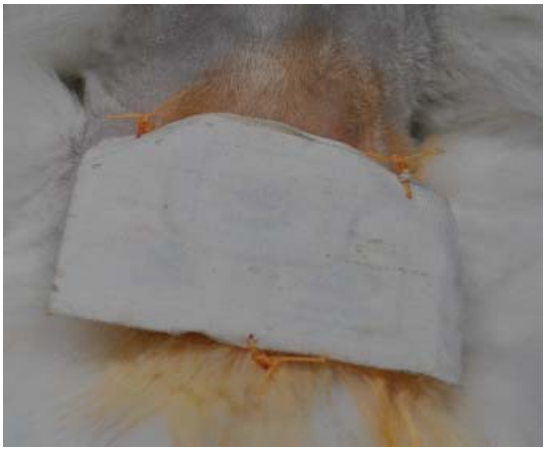

Fig.6. Applying of the AD-DIEE biophytomodulators

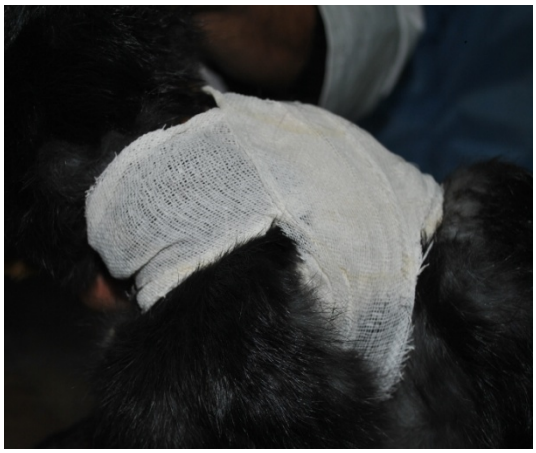

Fig.7. Securing the AD-DIEE biophytomodulators

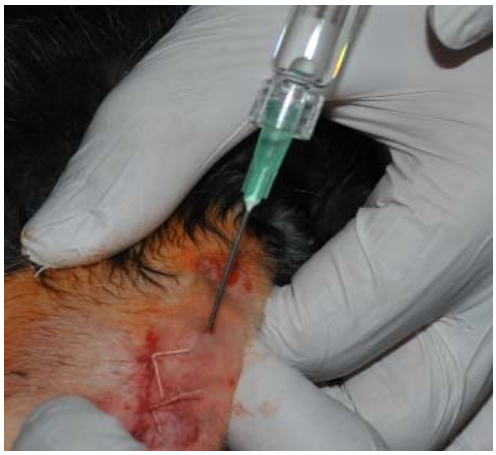

Fig.8. Injecting hyaluronic acid

The rabbits were randomly divided into four groups: group M (control), group BF (treated with biophytomodulators), group H (control treated with hyaluronic acid), group HS (control treated with hyaluronic acid in blood environment).

Three AD-DIEE type biophytomodulators were applied to the animals in the BF group. The devices were bound together with adhesive tape and applied to the skin in the dorsal cervical region using three interrupted sutures with nonabsorbable material (Fig. 6); a bandage was then applied in order to protect the devices. Protective dressing was applied to all individuals in the study to induce the same stress.

Animals from group HS have been injected with 5 mg hyaluronic acid (intra-articular) immediately after surgery (Fig. 8.). Animals in group H have been injected with 5 mg hyaluronic acid (intra-articular) five days after surgery.

All individuals received enrofloxacin 25 mg/animal 5 days post-operatively; also, they were weighed and clinically evaluated daily post-operatively. All animals were housed, cared for and fed identically.

In order to evaluate the results, the cartilage defect area was collected 14 days post-operatively. The animals were euthanized according to the laws in force. In group H, the sampling was done five days later, so the period passed after treatment was identical.

Harvested parts were fixed for 24 hours in 10% formalin, then decalcified with 20% trichloroacetic acid, washed for 24 h with tap water, dehydrated with alcohol, cleared with butyric alcohol (n-butanol) and included in paraffin at 57 °C. Five mm thick sections, stained by haematoxylin-eosin method were examined under the optical microscope (Olympus ® BX41 equipped with DP 25 video camera and CELL B - Olympus imaging software).

RESULTS AND DISCUSSION

Group M:

In the control group there is a very well developed connective matrix, larger than in the other groups, consisting of mucoid connective tissue. There are also small groups of chondrocytes and areas of cartilaginous metaplasia (Fig. 9). The reparative processes are in an early stage with a large number of fibroblasts and delicate collagen fibers (Fig. 10).

Group BF:

Primary mesenchymal cells have migrated in the defect area. There is chondral disorganization with zonal shortage of chondrocytes and at the edge of the lesion there are chondrocyte nests; the defect is filled with fibrous tissue. Areas of cartilaginous mataplasia were also noticed. Reparative processes are in somewhat more advanced stage than in the untreated control group, tissue architecture is better consolidated. There is a more advanced cartilage reconstruction compared to the control group. The regenerative processes are similar to those in animals treated with hyaluronic acid (Fig. 11, 12).

Group H and HS:

Following histological examination, a well developed matrix of chondrocytes was seen, with less fibrous tissue and areas with neovascularization; mucoid connective tissue and chondral metaplasia are similar to those seen in the BF group (Fig.13, 14).

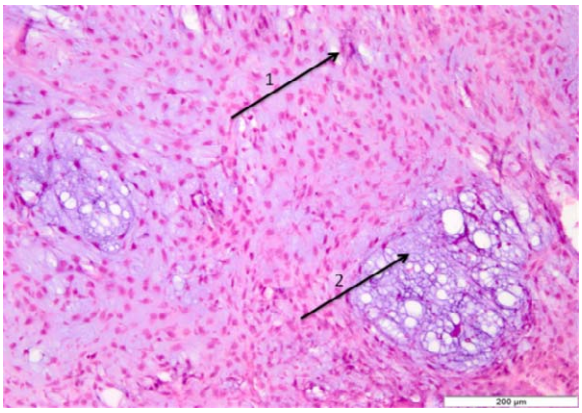

Fig. 9. Group M 14 d. postop.: 1. Mesenchymal cells, 2. Mucoid connective tissue

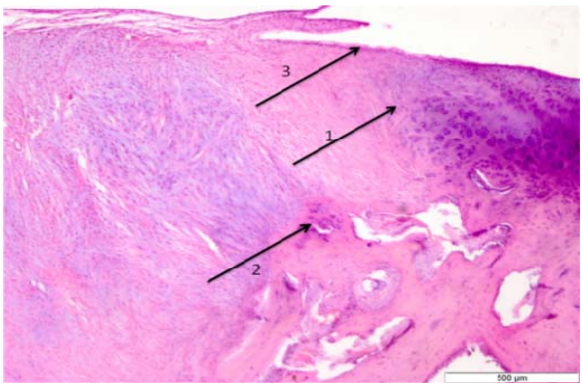

Fig.10. Group M 14 d. postop.: 1. Chondral metaplasia, 2. Chondrocyte nest, 3. Fibrous tissue

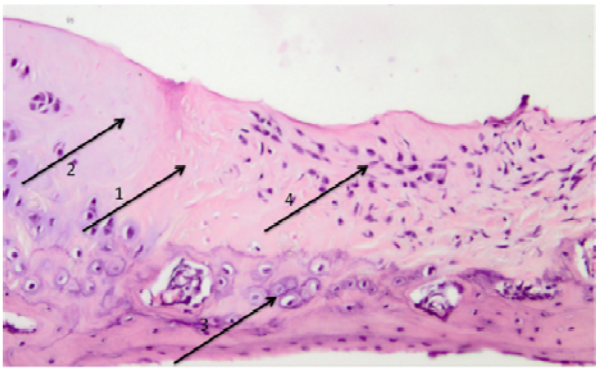

Fig.11. Group BF 14 d. postop.: 1. Fibrous matrix, 2. Dispersed cells, 3. Chondrocyte nests, 4. Primary mesenchymal cells

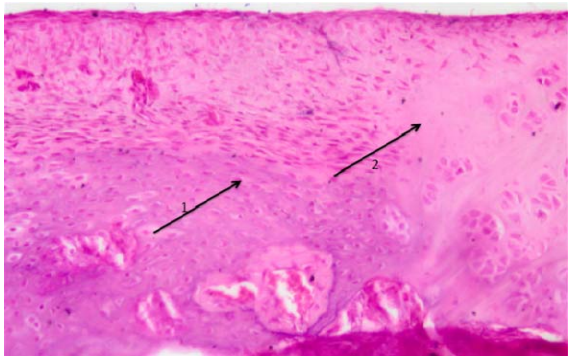

Fig.12. Group BF 14 d. postop.: 1. Newly formed cartilage, following metaplasia, 2. Mucoid connective tissue

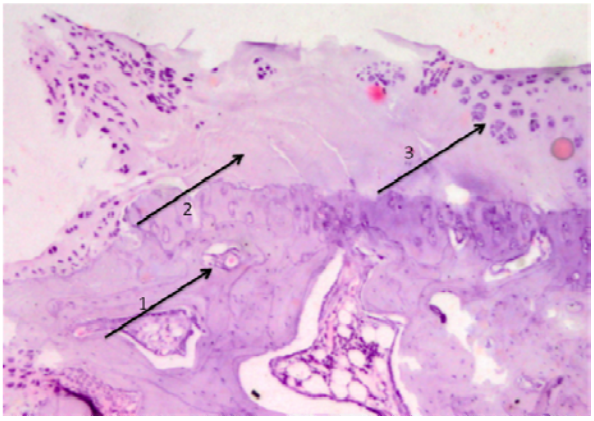

Fig.13. Group H 14 d. postop.: 1. Neovascularization, 2. Matrix of chondrocytes, 3. Chondrocyte nest

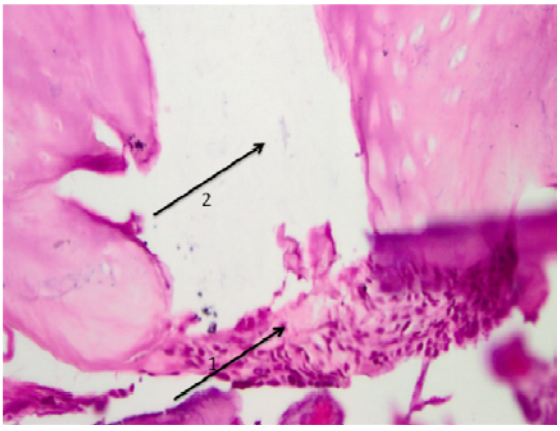

Fig. 14. Group H 14 d. postop.: 1. Delicate fibrous tissue at the edge of the defect, 2. Chondral discontinuity

CONCLUSIONS

The experimental model used for this study is suitable for the investigations concerning the pattern of healing of articular cartilage.

Reparative processes in the BF group evolved at a higher speed compared to the untreated control group (M), proving that AD-DIEE type biophytomodulators have a positive effect upon chondral healing.

Histological examination of samples taken from the H and HS groups showed reparative processes in a stage similar to those found in the BF group and more advanced compared to what was seen in the M group.

Results obtained in this study are encouraging for further research on the effect of AD-DIEE type biophytomodulators in joint therapy.

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