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AD BIOPHYTOMODULATORS PROMOTE HEALING SIMILARLY TO HYALURONIC ACID IN INDUCED CHONDRAL LESSIONS

BIOFITOMODULATORII AD PROMOVEAZĂ VINDECAREA, SIMILAR TERAPIEI CU ACID HIALURONIC, ÎN CAZUL DEFECTELOR CONDRALE INDUSE

C.M. CRECAN[1)], Simona Luminița OPREAN[2) *)], Mirela Alexandra TRIPON[1)], C. PEȘTEAN[1)], L. OANA[1)], C. OBER[1)], F. TĂBĂRAN[1)], B. SEVASTRE[1)], Daniela Claudia OROS[3)], A. DINCĂ[4)], A. BODNARIUC[1)], I. MORAR[1)]

ABSTRACT | REZUMAT

Biomaterials such as hyaluronic acid play an important role in the treatment of degenerative osteoarticular disease, due to its multiple pharmacological effects, non-toxicity and non-immunogenicity. Even though the role of the hyaluronic acid is incontestable, its administration via arthrocentesis does not come without a risk for multiple complications. In this case, biophytomodulators that are capable of re-establishing the energetic field of the organism, thus enhancing self-healing processes, can offer great benefits and are non-invasive. The purpose of this study was to compare the healing process in minimal chondral lesions using three different treatments on 36 rabbits: AD biophytomodulators (BF), hyaluronic acid 5 days post-surgery (H), hyaluronic acid right after surgery (HS) and no treatment (M). In order to assess the healing process, we evaluated each animal in terms of the lameness score and histologically at 15, 30 and 60 days post-surgery. In terms of lameness, group BF showed better results than lot M but inferior results compared to groups H and HS. Histologically, lot BF showed signs of chondrogenesis earlier than the other groups and no fissures were present, whereas fissures were found in all the other groups. In conclusion, AD biophytomodulators seem to promote cartilage healing in a similar pattern to that observed with hyaluronic acid.

Key words: AD biophytomodulators, hyaluronic acid, cartilage repair

Biomaterialele, așa cum este și acidul hialuronic joacă un rol important în terapia afecțiunilor osteoarticulare degenerative, datorită multiplelor activități farmacologice, a nontoxicității și nonimunogenicității. Deși rolul acidului hialuronic în astfel de afecțiuni este incontestabil, administrarea sa prin intermediul artrocentezei vine cu numeroase riscuri. În acest caz, biofitomodulatorii care au capacitatea de a reechilibra energetic organismul, stimulează auto-vindecarea și se aplică non-invaziv. Scopul acestui studiu a fost de a compara caracteristicile procesului de vindecare la nivel condral în urma aplicării a trei tratamente diferite: cu biofitomodulatori AD (BF), cu acid hialuronic la 5 zile post operator (H), cu acid hialuronic imediat după intervenția chirurgicală (HS) și un lot martor netratat (M). Pentru a evalua procesul de vindecare am monitorizat gradul de șchiopătură și am prelevat probe pentru examenul histologic în zilele 15, 30 și 60 post operator. În ceea ce privește gradul de șchiopătură am obținut rezultat mai bune la lotul BF comparativ cu lotul M însă rezultatele au fost inferioare loturilor H și HS. Histologic, la lotul BF au aparut fenomene de condrogeneză mai timpuriu și nu au existat fisuri condrale, comparativ cu celelalte loturi.

În concluzie, biofitomodulatorii AD par să promoveze procesele de reparare a cartilajului similar cu acidul hialuronic.

Cuvinte cheie: biofitomodulatori AD, acid hialuronic, vindecare cartilaj

Hyaluronic acid is widely used in both human and animal medicine due to its multiple pharmacological effects such as anti-inflammatory (12), regenerative (17), immunomodulatory (6), anticancerous (16), anti-diabetic (14), and anti-aging (15). In the treatment of joint disease, hyaluronic acid has multiple benefits including maintaining the viscosity of the synovial fluid, chondroprotection (1), synthesis of proteoglycan and glycosaminoglycan, anti-inflammatory, analgesic. However, most *in-vivo* studies have shown that after intra articular injection, hyaluronic acid seems to exhibit a mostly chondroprotective action (2). Biophytomodulators (BF) are devices created and patented by physicist Ancu Dincă – RO119756-2004 – and are capable of restabilizing the energetical field of an orga-

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
2) Iuliu Hațieganu University of Medicine and Pharmacy, Faculty of Pharmacy, Cluj-Napoca, Romania
3) Private Practitioner, Cluj-Napoca, Romania
4) Centre of Biosynergic Study and Research „Dincă Ancu" Bucharest
*) *Corresponding author: lopreans@umfcluj.ro*

nism, modified by various pathological states. The functionality of this mechanism is based on the capacity of plants to generate specific resonance effects, which interfere with the suble field of the afflicted organism, restablishing it (Dincă, A., 2006). The devices contain powders extracted from 33 different plants, including the following: elderflower *(Sambucus L.)*, Guelder Rose (Viburnum opulus), parsnip (*Pastinaca sativa),* dog rose *(Rosa canina)*, oak leaf *(Quercus robur)*, field mushroom *(Agaricus campestris)*, fern *(Dryopteris filix-mas)*, peppermint *(Mentha piperita)*, willow leaves and flowers *(Salix alba)*, northern white-cedar *(Thuja occidentalis L.)*, spindle *(Evonymus europaeus L.)*, ivy *(Hedera Helix)*, currant *(Ribes nigrum)*, cypress *(Euphorbia cyparissias)*, common hawthorn *(Crataegus monogyna)*, asparagus *(Asparagus officinalis)*, celandine *(Chelidonium majus L.)*, sessile oak *(Quercus petraea)*, honey fungus *(Armillaria mellea)*, black hellebore *(Helleborus niger)*, downy oak (*Quercus pubescens)*, Hungarian oak *(Quercus frainetto)* etc.

The objective of the study is the identification of alternative therapeutical solutions for minimal chondral defects, utilizing AD biophytomodulators type DIEE, in comparison to classic hyaluronic acid therapy, evaluating the healing process by lameness and histopathological examination.

MATERIALS AND METHODS

The study took place within the Faculty of Veterinary Medicine of Cluj-Napoca, in collaboration with the Centre of biosynergic study and research „Dincă Ancu" in Bucharest. The study was performed under the standards of animal care (ISO 10993, law 43/2014) and the work protocols were approved by the University's Bioethics committee.

Biological materials: 36 Giant German rabbits, 8 months old, coming from a single source, and benefiting from the same living conditions.

Non-biological materials: Instruments and surgical drapes. Sutures, polyglicolic acid 3.0 (Surgicryl®) and silk 2,0 (Silk Braided®) Antibiotics: enrofloxacin 5% (Enroxil® 5%, KRKA) Anesthetics Xylazine (Xilazin Bio 2%®) and ketamine (KETAMIDOR 10%); dental handpiece, single use syringes and needles, physiological serum, hyaluronic acid 10 mg/ml (Hyalgan®, Fidia Farmaceutici); specific reagents for histological processing. Windows GraphPad Prism program, version 5.0, GraphPad Software San Diego California. Olympus® BX41 microscope, equipped with a DP 25 video camera and CELL B – Olympus image processing software.

Technique: Surgeries have been performed under general anesthesia, using 35mg/kg ketamine and 4mg/kg xylazine administered together intramuscularly in an aseptically prepared area. The surgical technique consisted of an incision on the lateral side of the knee joint, followed by identification of the joint capsule, which was incised between the tibial crest and the insertion point of the joint capsule, on the distal femural epiphisis. The joint surface was revealed by medial spraining of the patella, which exposed a large area suitable for the defect placement. Chondral defects on the lateral femural cochleo-condilian junction have been performed using a 0.2/6.4 mm dental handpiece. During cartilage extrusion, bone cooling was done using saline solution. The reconstruction of the anatomical plains was done on two levels: the joint capsule was sutured with a continuous 3.0 polyglycolic acid suture, and the skin was sutured using 2.0 surgical silk, in separate points.

The rabbits have been split into 4 groups, each group containing 9 animals: Group M (control), group BF (treated with biophytomodulators), group H (treated with hyaluronic acid after 5 days), group HS (treated with hyaluronic acid immediately). Animals from group BF had 3 DIEE biophytomodulators applied, according to the manufacturer's indications, in the dorsal region, protected by an adhesive band and fixated to the skin using 3 separate suture points with non-resorbable material, secured with a protection bandage. Each animal had a band-aid applied, in order to induce equal levels of stress. Rabbits from lot HS had 5mg of hyaluronic acid injected intra-articularly, immediately post-surgery, with the same quantity being administered to group H, 5 days post-surgery. All individuals were subjected to antibiotic therapy using 25mg/animal enrofloxacin, with daily clinical evaluation.

Degree of lameness has been evaluated daily by the same evaluator for one week, using the following scale: 0- Normal quadruped position with no lameness, 1- Walking difficulty with discrete lameness, 2- Walking difficulty, with intermittent lameness during faster walking, 3- Obvious lameness during walking, with local pain, 4- Increased sensibility in the suspended limb, while stationary as well as during movement, intense pain, 5- limb completely suspended.

Evaluation of the healing process was performed using histopathological exams. Sample collection for examinations was done after euthanasia at 14, 30 and

60 days after initial therapy. Concerning H group, euthanasia was performed after 5 days, thus equalling the time passed from treatment application. The samples were submerged in 10% formaldehyde for 24 hours, following decalcification using 20% trichloroacetic acid, washed with tap water, dehydrated with ethanol, clarified with butanol, and included in paraffin at 57°C. Histological evaluation was performed on sections, 5 µm thick, stained with Hematoxilin-Eozin, and examined under an optical microscope.

During histological evaluation the following parameters were considered: the cell population, the density of the fibrous plain, the presence or absence of cartilage fractures. Defect centers have been measured on 20 different sections for each collected sample, establishing an average for each defect. The data was then statistically processed for each lot. Statistical analysis of the data and graph generating was done using The GraphPad Prism program, version 5.0 for Windows, GraphPad Software San Diego California. The data is reported as standard deviation, average ± standard error (SEM). For all the applied statistical comparison tests, the significance level $p<0.05$ was considered (95% reliability interval), respectively: ($p<0.05$ but >0.01 – statistically significant difference, $p<0.01$ but >0.001 – very significant statistical difference, $p<0.001$ – highly significant statistical difference, $p>0.05$ insignificant statistical difference).

RESULTS AND DISCUSSION

Lameness degree evaluation

The results obtained after lameness degree evaluation are illustrated as average ± standard error of the average in Table 1.

After the statistical analysis of the data pertaining to the degree of lameness using the "T" Test, the registered values are as follows:

· BF group registered values of p with a statistical significance of T6= 0.014668;

· C group and H revealed a highly significant statistic of p values for all clinical evaluation times T1= 0.00064, T2=0.000131, T3=0.000116, T4=0.000371 T5= 0.005036, T6= 0.002827, T7= 0.014668;

· C group and HS registered a significant p-value only for the 6th evaluation time T6= 0.014668.

Only statistically relevant p values have been mentioned.

Histogram 1 presents the progression of the lameness degree during the 7 days of clinical evaluation, on axis X being registered evaluation times from T1 to T7, and axis Y representing the lameness degree, noted from 0 to 5 according to the evaluation described under materials and methods.

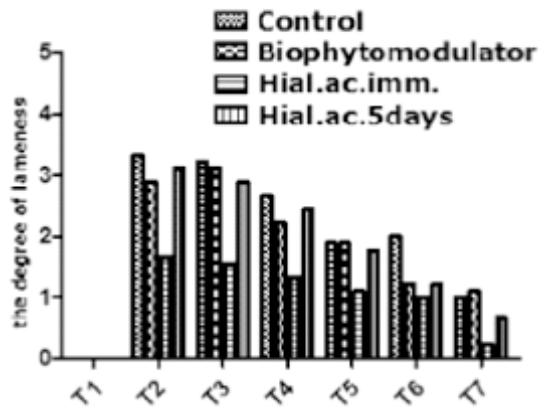

Fig. 1. Variation of lameness degree in all groups

Group M macroscopic and histologic findings

At the 14th day of sampling, macroscopically only one individual revealed a thickening of the tissues at the incision spot, probably due to a reaction to the suture material used for the skin. The defect areas were

Table 1

Descriptive statistics for the degree of lameness in all groups

Lots AVERAGE± SEM	T1 Day 1	T2 Day 2	T3 Day 3	T4 Day 4	T5 Day 5	T6 Day 6	T7 Day 7
Control	0	3.33± 0.10	3.22± 0.10	2.66± 0.10	1.88± 0.09	2± 0.10	1± 0.10
Biophytomodulator	0	2.88± 0.11	3.11± 0.09	2.22± 0.10	1.88± 0.11	1.22± 0.08	1.11± 0.09
Hial.ac.imm.	0	1.66± 0.10	1.55± 0.10	1.33± 0.08	1.11± 0.07	1± o	0.22± 0.08
Hial.ac.5days	0	3.11± 0.09	2.88± 0.11	2.44± 0.09	1.77± 0.10	1.22± 0.08	0.66± 0.11

easily visible, revealing a light fibrosis as well as a bump at the defect level. Microscopically, the defect is observed to be completely filled with a fibrous and fibrocartilaginous mix, with numerous mixoid degeneration zones and fissures (Fig. 2. A). The fissures are created due the low mechanical resistance of the entire matrix which fills the chondral defect (Fig. 2. B).

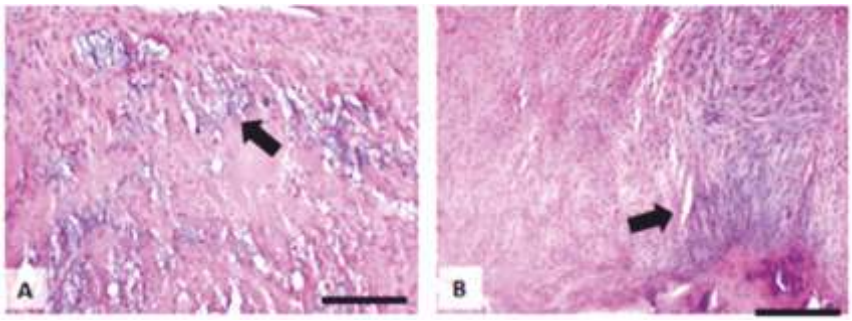

Fig. 2. A. Fibrous pannus and myxoid degeneration, HE X20, scale=100µm
B. Fibro-cartilage and chondral fissures, HE 20X scale=100µm

At the 30th day of sampling, macroscopically the joints presented a slight capsular distension, the synovial fluid being light yellow, more abundant and less dense compared to the previous sampling. The elected zones were identified in all samples but were less embossed than at 14 days.

The location of the arthrotomy was identifiable by a thick fibrous tissue. Microscopically the defect and its borders are morphologically present and filled with a dense fibrous tissue in its lower portion, with lax conjunctive tissue predominating its superior portion and hyaline cartilage laterally (Fig. 3. A).

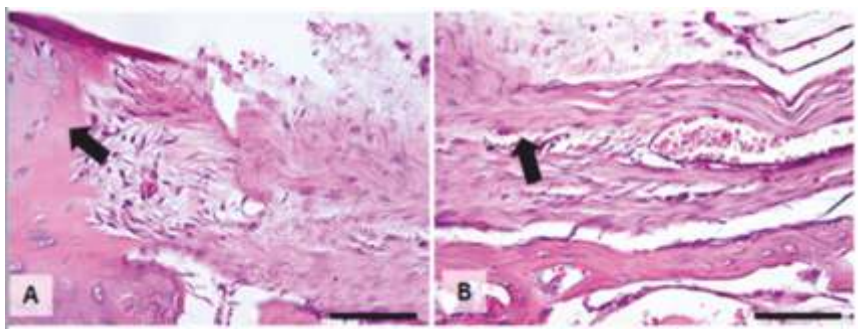

Fig. 3. A. Hyaline cartilage and dense pannus, HE 20X, scale=100µm
B. Connective tissue rich in collagen fibers in the deep part of defect, HE 40X, scale=50µm.

The fibrous tissue as well as the lax tissue are followed laterally by a pannus, much more abundant than at 14 days. Interestingly, fibrocartilaginous metaplasia is absent, which is highly evident in the group treated with biophytomodulators at the time of the sample collection. The profound area of the defect is composed of the dense conjunctive tissue with collagen fibers oriented parallelly to the long axis of the defect, which confers resistance to the entire structure, a phenomenon which was not observed at 14 days (Fig. 3. B).

At the 60th day of sampling the joints were not distended, the synovial fluid was present in a smaller quantity and was of light yellowish colour.

The defect areas were identified in all samples. Histologically the defect filled with fibrocartilaginous tissue in its depths (Fig. 4.A), with a fibrous pannus on the surface, which exceeds the edge of the defect.

Notably, at 30 days the matrix's fibers were horizontal to the long axis of the defect, and at 60 days, they were vertical (Fig. 4. B).

Group BF macroscopic and histologic findings

At the 14th day of sampling macroscopically the joints were symmetrical without effusion, lacking any reaction in the arthrotomy area, with a light yellowish synovial fluid, and identifiable defects in all samples. The defect was filled with connective fibrous tissue which did not exceed its edges. Histologically, the fibrous character of the defect is observed, as well as the fibrocartilaginous metaplasia. Interestingly, this lot does not reveal chondral fissures around the defects, compared to the control lot where they were observed on numerous occasions.

At the 30th day of sampling, macroscopically only one joint presents a cutaneous reaction (thickening of the skin at the incision point) which was not associated with cartilaginous or capsular alterations.

The synovial fluid is thin, yellow, reduced in quantity compared to the contralateral limb. The defects were identified in all collected samples.

Histologically, the defect was completely covered by fibrocartilaginous pannus.

The profound part of the defect and the fibrous pannus are very well anchored to the subchondral bone. The border between the cartilage and the subchondral bone is irregular, ensuring a better connection between the bone and the newly formed fibrocartilage. Notably, the pannus is not as extensive com-

pared to the control lot and no fissures were detected. Interestingly, the tinctoriality of the matrix at the apical part of the material had a similar colour to the hyaline chondroid matrix (Fig. 5.). The tendency of the fibrous tissue to transform into hyaline cartilage started from the lower point, similar to the 14th day transformation, but more evident compared to the control.

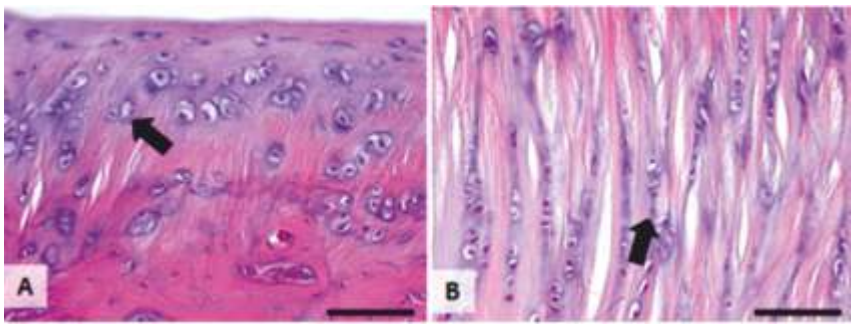

Fig. 4. A. Chondrocytes proliferate with an oblique arrangement, HE 20X, scale=50µm
B. Collagen fibers and perpendicular rows of chondrocytes, HE 40X, scale=50µm

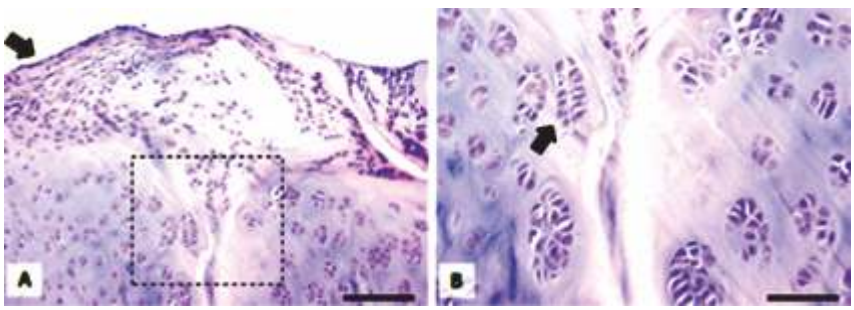

Fig. 5. Group BF 30 days:
A. Coloration similar condroide hyaline matrix, HE 40X,
B. Hyaline cartilage deep zone of defect, HE 40X

At the 60th day of sampling, macroscopically, no alterations have been observed in the joint capsule or the synovial fluid, and the chondral defects were no longer visible. Histologically, the defect was filled with fibrocartilaginous tissue which reaches the articular surface. In one of the samples, between the defect area and the adjacent cartilage, there was an evident fissure which occurred after histological processing. A good anchoring of the defect to the subchondral bone can be observed, as well as cartilaginous metaplasia in its depth, and a band of fibrous tissue at the surface which protect the newly-formed tissue. The fibrous matrix had fibers oriented parallelly to the defect in which numerous viable chondrocytes were present.

Interestingly, the profound section presented low cellularity and the fibers were oriented vertically at the surface and horizontally in depth.

Group H macroscopic and histologic findings

At the 14th day of sampling, the synovial fluid was more abundant compared to lots M and BF, without colour modifications, with a slightly thickened joint capsule for the treated limb. The defects were easily identified for all samples.

Histologically, the defect and its marginal areas showed a minimal resistance area of the newly formed tissue. We noted a hyaline structure of the cartilage and the marginal area of the defect, as well as the layout of the proliferated chondrocytes in oblique and vertical rows. This layout offers physical stability to the newly-formed tissue and a greater resistance to wear. In the central area of the tissue, at a more detailed evaluation, we observed the presence of numerous bone fragments and tissue remains, found in a state of resorption (Fig. 6. A). The marginal and central area of the defect, focusing on the fibrous tissue which completely fills the cavity, and a thin layer of tissue with fibro-conjunctive characteristics are covering the entire defect matrix (Fig. 6. B).

At the 30th day of sampling, the intraarticular formations did not present alterations, the synovial fluid was normal, and chondral defects were easily identifiable. Histologically, at the surface, the defect is covered by a fibro-conjunctive substance, well highlighted, which confers resistance to the underlying tissue,

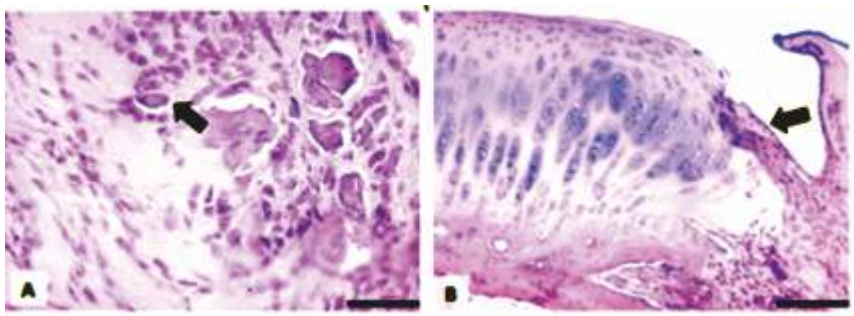

Fig. 6. Group H 14 days: **A.** Bone fragments in resorption, HE 40X,
B. Marginal area of defect, HE 20X.

an aspect which was also present in the previous lots. The chondrocytes around the defect are laid out in rows with a proliferative aspect. The fibrous pannus is well developed as well as the fibrous tissue band at the surface of the defect. At the edge of the defect there were numerous vertical chondral fissures. The cartilage cavity, until the time of the sample collection, was not filled with cartilaginous tissue. Near the defect, a massive chondrocitic necrosis was identified which was not present for lot BF (Fig. 7).

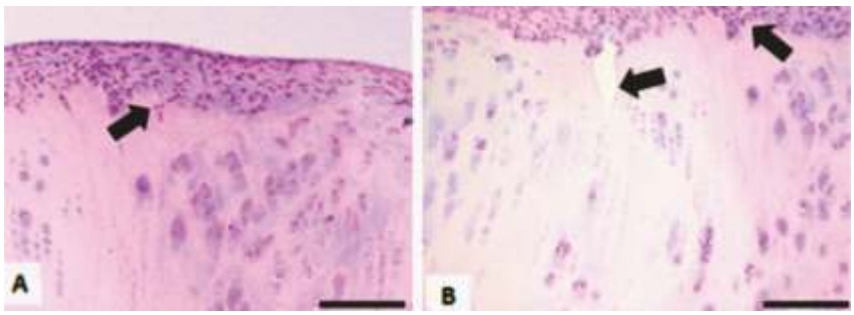

Fig. 7. Group H 30 days:
A. The transition between two types of tissue, HE 20X,
B. Chondral crack and chondrocytes in necrosis, HE 40X

At the 60th day of sampling, macroscopically, the elected areas were identified only in two samples, and very discretely, with a normal articular capsule and synovial liquid. Histologically, the cleavage between the cartilage and subchondral bone was easily observed, unlike in lot BF. The cartilage has a hyaline aspect and a fibrocartilaginous structure at the surface. The tissue surrounding the defect reveals the lack (necrosis) of the chondrocytes. There were also numerous cartilaginous regeneration zones, much more advanced compared to the control lot and similar to those in lot BF, at the time of the sample collection.

Group HS
macroscopic and microscopic findings

At the 14th day of sampling, macroscopically, the chondral defects were easily identified for all collected samples, with a normal synovial liquid and a slightly thickened articular capsule. Histologically, the defect was filled with fibrous conjunctive tissue, granulation tissue in different evolutive stages and isolated fibrocartilaginous islets. Regenerative process are similar to lots HS and BF but more advanced than in group M.

At the 30th day sampling macroscopically there were no alterations observed, all defects were easily visible. Histologically, the upper part of the defect was covered in fibrocartilaginous tissue and the deeper part had a laminar structure. The passage between the two layers was abrupt, aspect that could not be observed in the other lots. In the centre of the defect there was granulation tissue and small cartilage islets. The edges of the defect seemed mostly hyaline, similar to BF lot. A mixed structure was observed, with fibrocartilaginous tissue on the upper side and hyaline on the depth, along with numerous oblique and vertical fissures, more numerous as in the BF group but less than in the M group (Fig. 8). At this sampling, the fissures were very abundant, probably because of the exhaustion of the intraarticular hyaluronic acid reserves, which leads to an increased fragility of the newly formed cartilage.

At the 60th day of sampling the only observed macroscopical modification was the thickening of the articular capsule, and the defect areas were easily identified. The fibrocartilaginous structure of the defect is observed, as well as the dystrophic mineralization (Fig. 9) on its

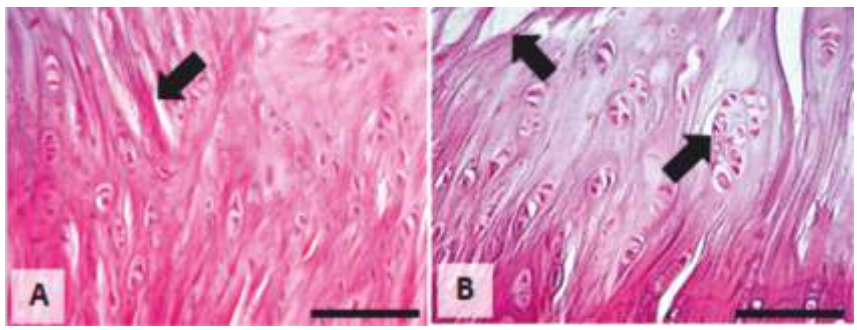

Fig. 8. Lot HS 30 days: Chondral cracks, HE 40X

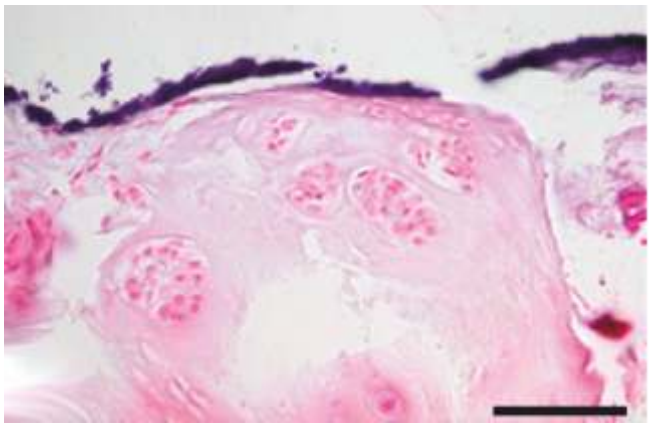

Fig. 9. Group HS 60 days: Dystrophic mineralization, HE X20

surface and newly-formed blood vessels at the defect area. Dystrophic mineralization of the articular surface was only present in this lot and only at the 60th day of sample collection. It is possible that the hyaluronic acid resources have been exhausted, and the articulation did not secrete enough hyaluronate, and the lubrication and nutrition of the cartilage was insufficient, which led to the wearing of the cartilage surface as well as its calcification.

Statistical evaluation results of the size of the chondral defects

Following statistical processing of the data obtained following the measurement of the chondral defects, the average values, the standard error of the average, the standard deviation and "T" test results between the control group and the other three groups are presented in the tables 2-5.

The evolution of the chondral defect horizontally for all groups is presented in histogram 10. The values were registered for the surface of the articular cartilage.

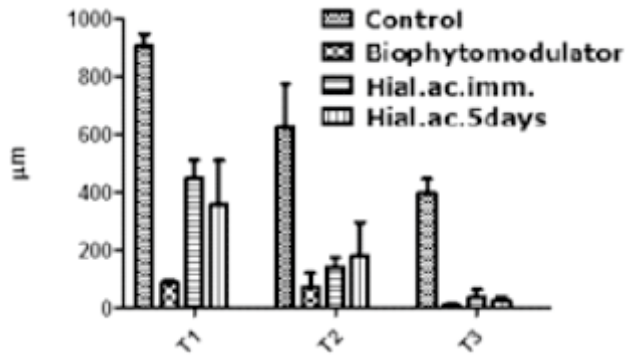

Fig. 10. Chondral defect evolution horizontally in all groups. T1- 14th day of sampling, T2- 30th day of sampling, T3- 60th day of sampling

Table 2

Descriptive statistics of defect depth for the control group

	T1	T2	T3
AVERAGE± SEM	678± 40.21608	436.3333±78.52247	201.6667±44.58076
St Dev	T1: 69.6563	T2: 136.0049	T3: 77.21615

T1- 14th day sampling, T2- 30th day sampling, T3- 60th day sampling

Table 3

Descriptive statistics of defect depth for the group treated with hyaluronic acid the day of surgery (HS)

	T1	T2	T3
AVERAGE± SEM	326 ± 9.539392	40 ± 20	9.666667 ± 5.238745
St Dev	16.52271	34.64102	9.073772
T test M/HS	P=0.009698	P=0.030679	P=0.048164

T1- 14th day sampling, T2- 30th day sampling, T3- 60th day sampling

Table 4

Descriptive statistics of defect depth for the group treated with hyaluronic acid after five days (H)

	T1	T2	T3
AVERAGE± SEM	489.3333 ± 52.09713	211.6667 ± 147.6847	10.66667 ± 5.811865
St Dev	90.23488	255.7974	10.06645
T test M/H	P=0.049109	P=0.270488	P=0.048257

T1- 14th day sampling, T2- 30th day sampling, T3- 60th day sampling

Table 5

Descriptive statistics of defect depths in the biophytomodulator treated group (BF)

	T1	T2	T3
MEDIA± SEM	79.33333± 40.10957	13.66667±6.984109	5.666667 ±5.666667
St Dev	69.47182	12.09683	9.814955
T test M/B	P=0.000458	P=0.031965	P=0.046032

T1- 14th day sampling, T2- 30th day sampling, T3- 60th day sampling

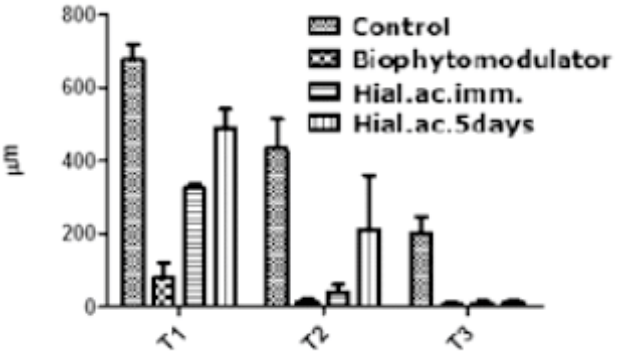

Fig. 11. Chondral defect evolution vertical in all group. T1- 14th day of sampling, T2- 30th day of sampling, T3- 60th day of sampling

Figure 11 illustrates the evolution of the chondral defects for all groups, measured vertically in the histological sample, representing the average distances obtained between the two edges of the chondral defect. Axis X illustrates the sampling times, and axis Y the average size of the defects in µm.

Following the evaluation of the lameness degree, we observed a gradual decrease of the average for all groups, the most prevalent decrease being observed in the group treated with hyaluronic acid immediately post-surgery, followed by the group treated at 5 days, and the lot treated with biophytomodulators. Following statistical processing, with the help of the „T" test, group BF and group H revealed statistically significant values only for the sixth evaluation.

- group M / BF statistically significant values *p : 0.014668*
- group M / H statistically significant values *p: 0.014668*
- group M / HS highly significant statistical values for all sample collection dates.

Strauss *et al* (2009) argue that supplementation with hyaluronic acid has been studied on experimentally induced cartilaginous lesions in rabbits, evaluating articular sensitivity. This proved to be much lower in the treated lot compared to the control group, the administration being made after a certain amount of time form the induction of the defect.

In our study, the hyaluronic acid was administered both immediately post-surgery and at 5 days post-surgery, and the obtained results were satisfactory. It is important that there were no mentions of results to prove the immediate use of hyaluronic acid post-surgery in the literature. This was performed in this study, with results processed by the "T" test, with highly significant statistics for all clinical evaluation dates T1=0.00064, T2=0.000131, T3=0.000116, T4=0.000371, T5=0.005036, T6=0.002827, T7= 0.014668. Other experimental studies have given good results following the usage of hyaluronic acid for the purpose of chondrocyte regeneration and improving cellular viability, following a model that is similar to our study. The association is possible due to the references to partial chondral lesions on a rabbit model used in this study (6).

At the evaluation of the chondral defects, the values obtained for the group treated with biophytomodulators were much smaller compared to the other three groups. Apparently, the reenergizing of the organism by using biophytomodulators for chondral lesions gives good results, fact proven by the histological processing of the samples. The usage of plants in cartilaginous regeneration is cited in the literature. Hougee (2008) used a product named Carathron® for the treatment of osteoarthritis, having as ingredients dry *Trichosanthes kirilowii Maxim roots* (*Cucurbitaceae*), dry *Clematis mandshurica Rupr.* (*Ranunculaceae*) roots and dry flowers from *Prunella vulgaris L*. (*Laminaceae*) and thus proving the possibility of the reducing the inflammatory symptoms with their usage (5).

Jung *et al* (2001, 2004), in a clinical study on human patients with osteoarthritis, demonstrate that the same products, beside their analgesic efficiency, also helps with the functional recovery of the affected articulations (6, 7). Choi *et al* (2002) demonstrate the protective qualities of this product on rabbit articular cartilage (3). All the aforementioned studies are based on the anti-inflammatory effect of the plants administered orally, but no one presented information concerning the reenergizing effect of the product following topical therapy, an effect which was evaluated in our study. Beneficial effects were also discovered by Oros *et al* (2013) for bone defects experimentally produced in rats. The application of alternative therapy was done topically, on the organism, at the exterior, by fixing the biophytomodulators on the skin. The energizing effect has given certain results, demonstrated by the size of the chondral defects mentioned in the previous section. At the 14th day of sample collection, in group M, macroscopical studies revealed a thickening of the tissues at the incision spot for a single individual, subsequently proving that this was only a reaction to the suture. Histopathological studies have been performed for the respective area, and a fibrosis process for the periarticular fascia was identified. Group BF did not present any macroscopical modifications and the groups treated with hyaluronic acid presented only a larger quantity of synovial liquid com-

pared to groups M and BF.

Microscopically, for all groups, the defects were filled with fibrocartilaginous tissue, and the control lot presented a persistence of the chondral fissures.

Alongside this aspect, detachment areas between the neighbouring cartilage and the fibrocartilaginous pannus have been identified in a large number of samples. Both the chondral fissures and the cleavage between the two types of tissue are formed because of the low mechanical resistance of the newly-formed pannus. Curiously, the lot treated with biophytomodulators had no such modifications in any sample.

Strauss *et al* (2009) demonstrated that in the case of chondral fissures, treatment with 3 doses of hyaluronic acid weekly leads to the filling of the fissures with tissue, improving the macroscopical aspect. Alongside these, the degenerative modifications in the cartilage were reduced using hyaluronic acid. This result was also present in this study in the lots treated with hyaluronic acid, the tissue structure being similar to the structure of the other lots, but with more advanced chondral metaplasis. Microscopically, the chondrocytes were arranged in rows at the base of the defect, the tissue being better attached to the subchondral bone compared to the control lot. Interestingly, this structure of the articular cartilage was also discovered in the lot treated with biophytomodulators, where there were also a few spots of incipient chondral metaplasis. At the 30th day of sample collection, there were no pathological microscopic modifications concerning the anatomical articular formations. In all the samples, the defects were filled with dense fibrous tissue, which at the surface was covered by a lax fibrous tissue with collagen fibers aligned in a way that conferred more resistance to the pannus in the defect. In lot BF the pannus was well anchored to the subchondral bone, the chondral metaplasia processes at the 14th day being already significantly advanced.

The pannus was not exuberant as it was in the control lot where it exceeded the articular edges of the defect. A similar study to the one we conducted could not demonstrate that treatment with hyaluronic acid has a significant regenerative effect, in the case of chondral lesions in rabbits, 3 months after administration. Moreover, no differences between the histological and macroscopical characteristics could be observed in the newly-formed tissue in the case of treatment with a single dose per week or 3 doses per week. The chondral defects were filled with mixt tissue formed from fibrocartilage and hyaline cartilage with presentation differences between the studied samples. This fact was also met in our study, groups H and HS presenting a significant difference concerning cell population and evolution of the intralesional pannus. The precise way in which the hyaluronic acid acts in the regeneration process is not completely understood.

This notwithstanding, some studies say that the effect does not consist only of the mechanical properties of the hyaluronic acid but also of the stimulation of endogenous production of hyaluronic acid, inhibiting the degradation of proteoglycans and anti-inflammatory effects studied in vitro (22). Strauss *et al* (2009) made a comparison after 3 months of treatment on human patients with microfractures, administering 3 doses of hyaluronic acid per week and 5 doses per week. Statistically, the treatment with 3 doses per week proved to be more efficient, even after 6 months of therapy. At the 60th day of sample collection, there were no registered macroscopic modifications, group BF presenting more evident cartilaginous metaplasia compared to other groups, the differences between the samples being presented in the results section.

CONCLUSION

The experimental model used here is suitable for studying articular cartilaginous defects. The reparatory processes in lot BF, evaluated by histological exams, unwinds faster compared to the control group, which demonstrates that biophytomodulators AD type DIEE stimulate chondral healing. In groups H and HS the reparatory processes were similar with those in group BF and more advanced compared to the control group. The results obtained in this study are encouraging for the pursuit of further research, concerning the effect of AD biophytomodulators in articular therapy. Hyaluronic acid administered immediately post-surgery diminishes articular pain, reducing the degree of lameness.

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