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Histological Study of the Healing of Bone Defects in Rats Treated with AD-DIEE Type Biophytomodulators

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Abstract. Healing of bone lesions lasts longer compared to healing of soft tissues. Bone reconstruction can be finalized as early as 5 to 6 weeks unless disturbing factors intervene. This study aimed to histologically assess the healing of surgically induced bone defects in rats treated with AD-DIEE type biophytomodulators, compared to control group. Adult male Wistar rats were used. Surgeries were performed under general anesthesia and consisted in drilling a bone defect in the femoral shaft, using a dental drill. The animals were divided into 2 groups, control group and test group, treated with AD (DIEE) type biophytomodulators. Bone samples for histological examination were collected at 2, 4 and 6 weeks after surgery. The samples were histologically processed, stained by Masson's trichrome method and examined under the optical microscope. The findings show that the chosen experimental model corresponds in every aspect, as it provides complete information about the bone defect, its edges, adjacent tissues, femoral shaft and how the periosteum is involved in bone proliferation consolidation. The use of AD (DIEE) type biophytomodulators proved somehow beneficial, stimulating bone proliferation, without any large differences compared to the control group.

Key words: rat, bone, healing, biophytomodulator.

INTRODUCTION

The AD-DIEE Biophytomodulators are devices for energetic loading and balancing, patented by physicist Ancu Dincă. The effect of these devices upon the bone healing phenomena has been previously studied in sheep (Oana et al., 2010, Onisor-Gligor et al., 2010), but the experimental model needed an improvement, due to the impossibility of histologically assessing the perilesional tissues, because the sheep were not euthanized and only the newly formed callus was harvested for further study. Oana et al. (2011) described an experimental model for the histological investigation of bone healing phenomena in rats. The model mimics stable fracture repair, by drilling a partial defect in the femoral shaft of Wistar rats, without interrupting the continuity of the bone. *Stable fracture repair* means that the fracture ends have been immobilized to give relative clinical stability (not necessarily weight-bearing ability), but have not been rigidly fixed by surgery (McGavin and Zachary, 2007). Oros et al. (2011) have conducted a biochemical study of bone healing in rats treated with AD-DIEE Biophytomodulators. The biochemical tests revealed a higher postoperative increase in the values of osteocalcin and alkaline phosphatase in the animals treated with AD-DIEE type biophytomodulators compared to values obtained in the control group.

MATERIAL AND METHODS

The biological material used in this study was represented by 24 white Wistar rats, males, 8 months old, clinically healthy, average weight of 250 g. The bone defects were done using a technique

described by Oana et al. (2011). The animals underwent general anaesthesia, xylazine 8mg/kg + ketamine 40 mg/kg. The femoral shaft was exposed surgically, after the incision of the skin and the dilaceration of the thigh muscles. Bone defects were made using a dental drill of 1.6 mm diameter, adapted to a dental micromotor at a speed of 3500 rpm. The drilling was done through the whole thickness of the compact, reaching the medullary canal. The skin was sutured, using 4-0 surgical silk.

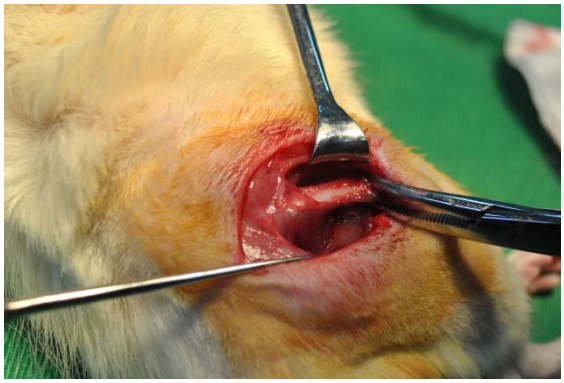

Fig. 1. Exposing the femoral shaft

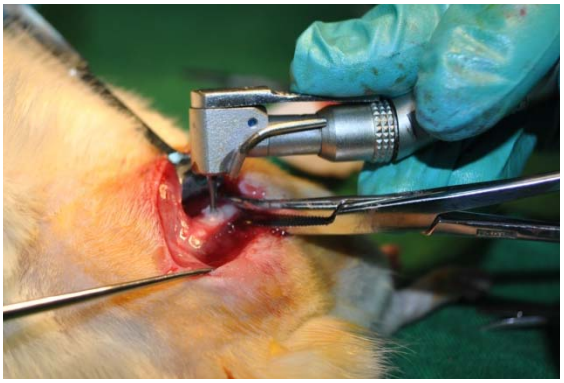

Fig. 2. Drilling the defect

The rats were divided into two groups, control and test group. The control group received no medical treatment. The animals from the test group were treated with AD-DIEE type Biophytomodulators. Three Biophytomodulators were attached to the bottom of the cages, in a triangular shape. The histological study was also done using the protocol described by Oana et al. (2011). Bone samples containing the defect and the healthy bone in the vicinity of the defect were taken 2, 4 and 6 weeks after the surgery. The rats were euthanized in accordance with the laws in force and the bone fragments for the histological study were chosen in such way that the defect was framed by perilesional healthy bone tissue.

For the fixation of the bone samples, 10% formalin was used. The decalcification was done with trichloroacetic acid. After the inclusion in paraffin of the decalcified bone samples, serial sections of 5 µm thickness were performed. The histological slides were then stained by Goldner`s Trichrome method and examined under the optical microscope.

RESULTS AND DISCUSSIONS

At 2 weeks postoperatively, ongoing bone repair processes are present in the cortical defect area, even if not yet in a very advanced stage. They consist in proliferation of young bone tissue which tries to cover experimental bone defect area (Fig. 3). The bone tissue here consists of young bone trabeculae. Reparative processes are present both in the animals from the test group and the control, and their intensity is similar in both groups. In this stage the limit between the bone from the edge of the defect and the newly formed trabeculae can be easily noticed. (Fig. 4) The area of the defect is completely covered with a continuous layer of newly formed bone tissue at 4 weeks postoperatively. The layer of bone is coated in its inner part with a series of young trabeculae, which shows that the reparative processes are ongoing.

There is a slight difference between the control and the test group; in the control group there are discontinuities in the central part of the defect (Fig. 5), but in the test group the newly formed bone layer is thick and continuous (Fig. 6). At 6 weeks postoperatively, the repair processes are

appear more advance, but are far from over. The young bone trabeculae occupy about half of the medullary canal in the samples from the control group (Fig. 7) and a larger area in the samples from the test group (Fig. 8). In the test group there is a tendency of the trabeculae to inundate the whole medullary canal, but this aspect is in a relatively early stage.

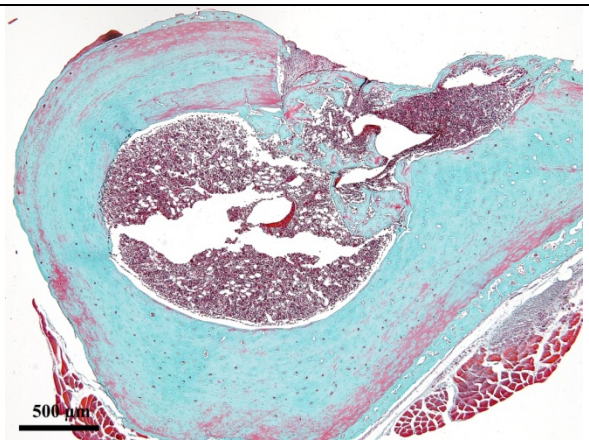

Fig. 3. Histological image – test group, 2 weeks postoperatively, Goldner's Trichrome

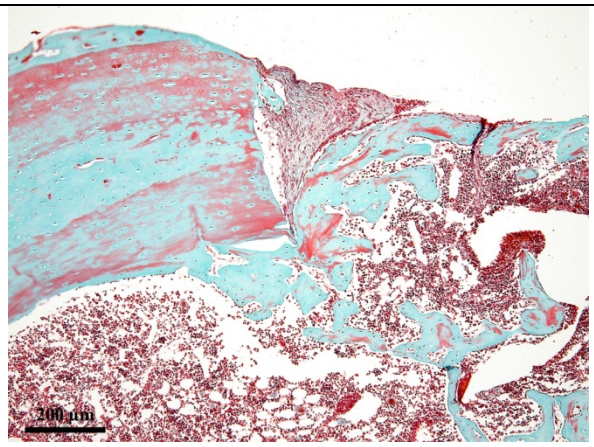

Fig. 4. Histological image – test group, 2 weeks postoperatively (detail), Goldner's Trichrome

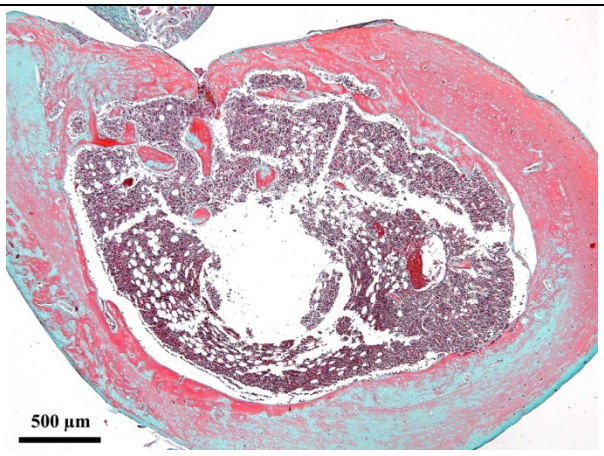

Fig. 5. Histological image – control group, 4 weeks postoperatively, Goldner's Trichrome

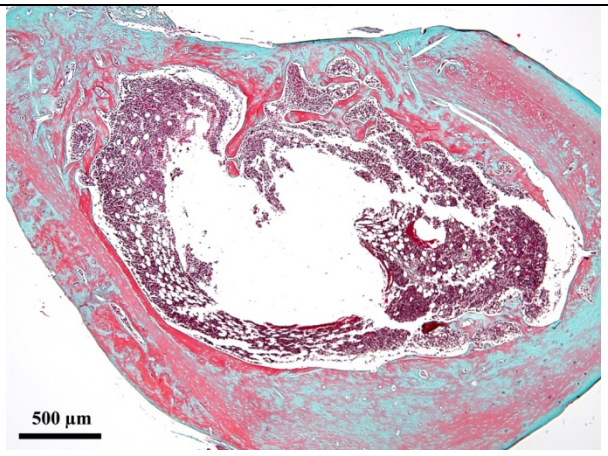

Fig. 6. Histological image – test group, 4 weeks postoperatively, Goldner's Trichrome

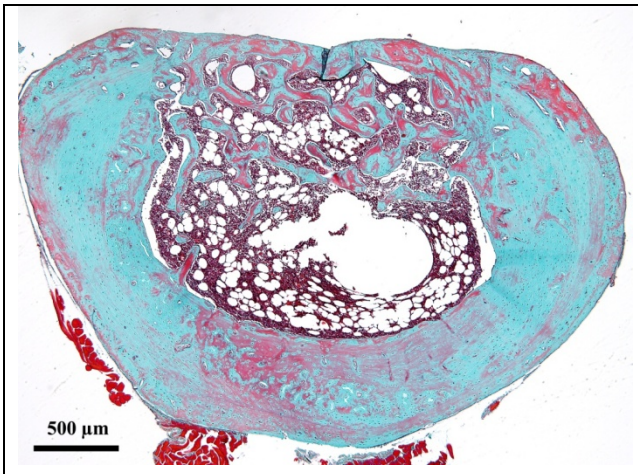

Fig. 7. Histological image – control group, 6 weeks postoperatively, Goldner's Trichrome

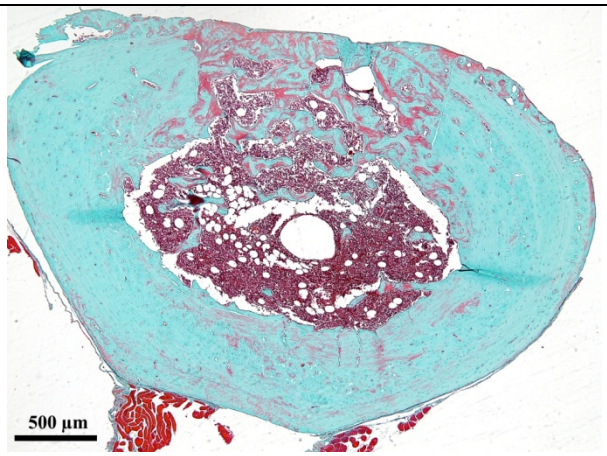

Fig. 8. Histological image – test group, 6 weeks postoperatively, Goldner's Trichrome

The findings of the histological study are in concordance with the biochemical study performed by Oros et al. (2011). The slightly higher number of young trabeculae found in the animals treated with AD-DIEE Biophytomodulators are proportional with the higher values of bone markers found in the previous study.

CONCLUSIONS

All the aspects prove that the experimental model offers complete information about the area of the defect. In a histological slide one can assess the whole surface of the defect, its margins, the adjacent tissues, the bone wall, the implication of the periosteum in the process of bone proliferation, to cover and consolidate the defect area.

The use of AD-DIEE Biophytomodulators proved useful, enhancing the bone proliferation, but without major differences between the control and the test group.

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