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Dynamics of Histological Parameters in Treating Dogs' Festering Wounds with Extracellular Matrix

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***Abstract*— The study describes ways of obtaining extracellular matrix from livestock organs and using the obtained powder and gel in practical veterinary medicine and presents the description of the obtained histological results.**

***Keywords*— tissue engineering, extracellular matrix, organs, histology, biotechnology**

I. INTRODUCTION

In our days, the interest in small pet animals has increased significantly and their numbers among the urban population have grown several times [1]. Injuries of pet animals in urban areas are a frequent reason for addressing a veterinary physician. The main causes of injuries are being run over by automobiles, bites by other animals, and improper care. Damage to owners of injured animals is determined not only economically, but emotionally as well.

The advancement of knowledge about the regularities in the healing of wounds has led to the introduction of differentiated treatment depending on the depth of the damage, phase of the wound process, wound location, as well as a number of other factors. If the first phase, hydration, requires the provision of antimicrobial, sorbing, and analgesic effect, while the second phase, dehydration, demands the conditions for the optimal reparative processes [1,5,10].

At the moment, the rapid development of biotechnology in the last decades brought forth new means for quick recovery of any damaged animal organ. The task of a veterinary physician is to use the most efficient modern means, techniques, and medication to treat a wound pathology [8,9,10].

Presently, the extracellular matrix is not as well-studied and common in Russia as it is in the West. Products based on the extracellular matrix (ECM) are only starting to make their way around and are not yet widely accepted. Western countries have made a large step forward in developing new technologies in veterinary medicine. Russian biotechnology is yet at the beginning stages of learning and researching this universal material produced from livestock and human organs.

Goal and objectives of the research. The goal of this paper is to study the effect of an ECM-based powder on the healing of animal wounds. The following objectives were set to achieve this goal:

- Obtain a decellularized organ and use it to produce an ECM-based product for treating festering wounds of subject animals;
- Evaluate the effect of the ECM-based powder throughout the course of the entire process in subject animals.
- Study the histological parameters of the treatment and control groups during wound healing.

II. RESEARCH MATERIALS AND METHODS

Clinical and experimental work was carried out at Professor G.P Serdsev Department of Internal Noncontiguous Diseases, Pharmacology and Tocology of the Veterinary Medicine Faculty, FSBEI HE "Yakutsk State Agricultural Academy", in partnership with GAU RS (Ya) "Technopark Yakutia" (laboratory). "Technopark Yakutia" produced the ECM-based powder while the Department carried out the clinical and experimental part of the study.

A. Obtaining powder based on the extracellular matrix from pig bladder

Decellularized ECM was produced using a pig urinary bladder frozen at the temperature of -70° C.

The blader was thawed at room temperature and washed in running water to remove blood clots, streaks of fat, and dirt.

The prepared material was placed in a specially prepared 500 ml container with distilled water and left for 1 hour, stirring continuously with a shaker.

The process was repeated 2-3 times, additionally using 70% alcohol for 5-10 minutes for disinfection. Further, the material was washed with distilled water several times.

After thorough washing, the material was placed in solutions: 1x PBS 250 ml with 3% of Triton X-100-7 ml, 1% Pen/street antibiotic 0.5 ml, and 250 ml of distilled water for 24 hours with continuous stirring with a shaker.

After the material was obtained, it was washed several times in distilled water during several hours to remove residual solutions.

In the end, the obtained material was kept in a freezer for one night at -70° C. The completely frozen material was placed in vacuum to produce the ECM.

Histological confirmation of the "purity" of the ECM.

Histological analysis is carried out in order to ascertain the complete absence of cells.

Samples sized around 0.7-1.0 cm were taken from the obtained material and fixed in 10% neutral buffered formalin for 24 hours.

Pieces of material were transferred from formalin to 75% alcohol and further on, gradually increasing alcohol to 100%.

Then, the samples were put into chloroform for 12 hours.

A slurry consisting of paraffin and chloroform was prepared and put into a thermostat for 1 hour under at the temperature of +37˚ C. The material was placed into basins with cold water. Next, paraffin blocks suitable for working with a microtome were prepared. An MS-2 microtomed wasused to cut 5 µm-thick sections. The classical hematoxylin and eosin coloring was used [12].

The sections were analyzed through an AxioVertA1 inverted microscope.

After carrying out the microscopic study, it was determined that the obtained extracellular matrix is ready for further use.

On the next day, the ECM was lyophilized in vacuum for 3 days. After that, it was reduced to the state of powder in a special device through the addition of liquid nitrogen.

B. Using ECM-based powder to treat dog wounds

The experiment was carried out with dogs that were divided into two groups: control: treatment using conventional techniques; treatment: ECM-based powder was added in the course of treatment.

The animals were treated every day. The wounds were pretreated with cotton gauze swabs soaked in 3% hydrogen peroxide solution and a short 0.5% antibiotic procaine blockade with Bicillin-3 prolonged antibiotic was introduced to prevent the inflammatory response and development of pathogenic microorganisms. Balsamic liniment was used in the first stage of hydration, Laevomecolum skin ointment was used in the second stage.

The ECM-based powder was continuously applied to the wound surfaces of the treatment group animals.

The physiological parameters of these animals were studied according to conventional techniques. Temperature was taken using clinical thermometers (rectally); heart rate was determined at beats per minute, respiratory rate was recorded by counting respiratory movements per minute.

Blood was taken from the saphenous vein in the forearm in the morning before feeding, and further samples were taken: before treatment, during treatment, and after treatment. Amounts of formed elements in blood were identified. Hematological studies were carried out using an Abacus junior 30 automated hematology analyzer.

Histological studies of tissues were carried out using optical microscopy. Wound surface biopsy was performed after 1 hour and on the 5th, 10th, and 14th days to see the growth of tissue in different treatment groups of dogs. Prepared histological specimens were photographed using an AxioVertA1 inverted microscope with a built-in camera.

C. Hematological and histological studies r

Blood samples were taken from the saphenous vein in the forearm on the 1st, 5th, 10th, and 14th days. Hematological studies were carried out using an Abacus junior 30 automated analyzer.

To confirm the efficiency of treating wounds using the ECM-based powder, tissue samples were taken after 1 hour and on the 5th, 10th, and 14th days to confirm the healing of wounds in the treatment group.

Samples sized around 0.7-1.0 cm were taken from the obtained material and fixed in 10% neutral buffered formalin for 24 hours.

Pieces of material were transferred from formalin to 75% alcohol and further on, gradually increasing alcohol to 100%.

Then, the samples were put into chloroform for 12 hours.

A slurry consisting of paraffin and chloroform was prepared and put into a thermostat for 1 hour under at the temperature of +37˚ C. The material was placed into basins with cold water. Next, paraffin blocks suitable for working with a microtome were prepared. An MS-2 microtomed wasused to cut 5 µm-thick sections. The classical hematoxylin and eosin coloring was used. The sections were analyzed through an AxioVertA1 inverted microscope.

III. RESEARCH RESULTS

A. Comparative analysis of the treatment of the treatment and control groups

The courses of wound process were compared depending on the treatment using the ECM-based powder applied topically (treatment group n=3).

The animals from the control group were treated in a "conventional" way, that is surgical treatment of wounds and short 0.5% antibiotic procaine blockades with Bicillin-3.

From day 1 to day 3 both treatment and control group animals were developing inflammatory edema in the wound with festering occurring later on. Clinical changes were observed in the wound area manifested by the increase in local

temperature, painfulness, hyperemia, increase in swelling without marked borders, and exudation of pus. The first stage of the wound process (hydration) occurred in the wound and it was being cleaned of necrotic tissue.

On the 5th day, the inflammatory edema started to abate, pain was present at palpation, local temperature increased moderately, and redness and moderate exudation of serous exudate were observed. The second stage (dehydration) of the wound process set in, which means that the processes of regeneration and restoration started. ECM powder started to be used with the treatment group, applying it to the wound surface.

On day 7, the condition of the dogs in the control group was satisfactory with moderate hyperemia, insignificant exudation of serous exudate, and inflammatory edema of the surrounding tissue. In the control group, the processes were comparatively more pronounced.

After day 10, the state of the treatment group animals improved. The inflammatory edema abated, redness decreased, there was weak exudation of serous exudate, pain at palpation was not pronounced. That day, the ECM-based powder was used on the wounds again.

On the 12th day, treatment group animals did not display inflammatory edema or redness, painfulness at palpation was insignificant. Isolated isles of granulation tissue were visible on wound walls. In the treatment group, granulation tissue appeared 2 days earlier than in the control group.

On day 14, the treatment group displayed no signs of inflammation and wound cavities were filling with granulation tissue. Swelling or inflammation were absent in the control group. Wounds started to show granulation.

This way, healing of wounds in the treatment group occurred 2 days earlier than in the control group.

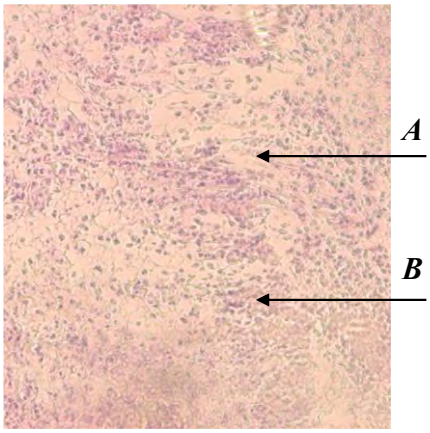

Fig. 1. Tissue sample after the injury (60 min). *A* – destroyed epithelial cells; *B* – intact cell. Hematoxylin and eosin coloring, magnification x40

B. Histological parameters

Tissue samples for histological assessment were taken before treating the wound and on the 5th, 10th, and 14th days.

Histological studies 1 hour after the injury in the treatment group revealed partial destruction of epithelial cells, which is confirmed by tissue section Fig. 1. An isolated necrotic area was observed on day 5 (Fig. 2).

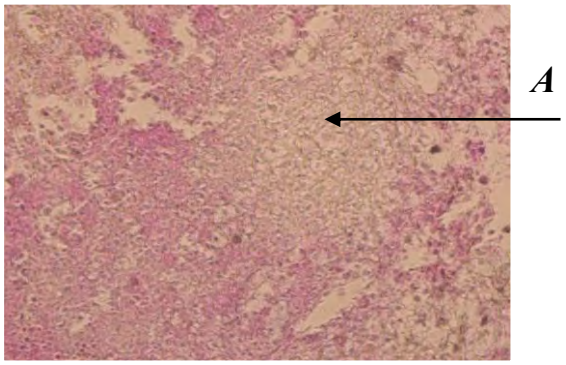

Fig. 2. Wound surface in 5 days. *A* – necrosis area. Hematoxylin and eosin coloring. X40 magnification

On day 10, minute capillaries form in the area of damaged tissue, and connective tissue and epithelial cells have formed (Fig. 3)

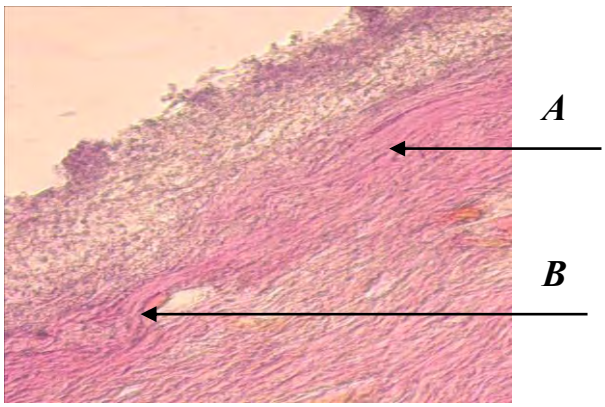

Fig. 3. Wound surface on day 10. *A* – capillary vessels; *B* – connective tissue. Hematoxylin and eosin coloring. X40 magnification.

On day 14, the initial new formations of basal cells and the keratinizing layer are visible (Fig. 4).

IV. Conclusion, Recommendations

The studies confirm the fact that using the powder based on the extracellular matrix to treat wounds of the animals in the treatment groups made healing more intense and caused it to complete 2 days earlier than in the control group. The decrease in healing duration can be explained by the formation of granulation tissue.

As a result of histological studies, it can be noted that capillaries formed in the wound areas of the treatment group, which leads to the intense formation of connective tissue. Initial basal layer formations were visible on the 14th day.

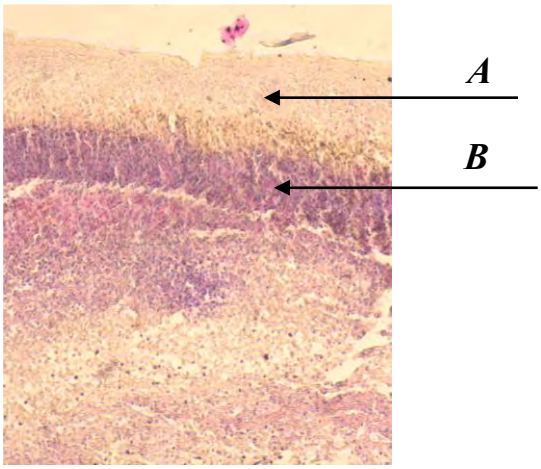

Fig. 4. Wound surface in 14 days. A – keratinizing layer; B – formation of basal cells. Hematoxylin and eosin coloring. X40 magnification.

This way, using the powder based on the extracellular matrix to treat animal wounds yields positive results. In order to achieve more effective treatment results, it is necessary to meet the following requirements:

- hygiene and sanitary conditions for subject animals during their treatment must conform to standards;
- to prevent licking of the wounds, animals must be provided with protective collars and bands for the period of treatment;
- it is recommended to use the powder based on the extracellular matrix in combination with antibiotics to prevent infection and ensure the efficient healing of wounds.

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