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Study of Biodegradable Properties of Beeswax Membrane in the Focus of Ectopic Implantation

SUMMARY

The aim of this study was to investigate the response of muscular tissues upon implantation of beeswax membrane into the muscles of the anterior abdominal wall in 50 rats, and the way of its dissolution.

The beeswax membrane was an inert to surrounding muscles and did not produce inflammation in the focus of ectopic implantation, promoting normal regeneration of the tissues. Dissolution time of beeswax membrane of 35-40μ width lasted 5-6 months. The data obtained point out a possibility of using beeswax membrane as a membrane for guided tissue regeneration.

Keywords: Beeswax; Ectopic Implantation; Tissue Response

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Introduction

At present, there are over 3 dozens of various membranes that are used for bone defect isolation from penetrating unwanted cells during the regeneration period. There are several studies that investigate various types of membranes[1,5,6,12,13,16,20,23,24,26,27]. A number of researches reveal negative influence of bacteria contaminating the membrane, and the decrease of bone tissue augmentation[4,9,18,19,21,25]. Bacterial contamination of the regenerating wound significantly leads to a compromised outcome. Bacterial species, bacterial count and the area of bacterial contamination present on the guided tissue regeneration (GTR) membrane are some of the factors that may affect GTR outcome[7,11,22]. Comparing various membrane types, the researchers came to the opinion that the effect of their use significantly depends on treatment planning concerning indication for reconstructive procedure and its performing, as well as local conditions[15,24]. The point of insufficient efficacy of the membranes due to infection in the wound remains disputable[28]. For the reduction of bacterial contamination and prevention of wound infection, some authors recommend the use of antibiotic-lead membranes[7,11,22]. Numerous experimental and clinical investigations proved a significant improvement if membranes and transplanting materials are used[2,3,8,10,12,14].

The aim of this study was to investigate the response of muscular tissues upon implantation of beeswax membrane into the muscles of the anterior abdominal wall in 50 rats, and the way of its dissolution.

Materials and Methods

Beeswax is a tough wax, formed from a mixture of several compounds. Beeswax has a high melting point range, 62-64°C (144-147°F). Its main components are palmitate, palmitoleate, oleate esters, aliphatic alcohols, hydroxypalmitate and pro-vitamin A. Beeswax has anti-inflammatory, bactericidal and anti-oxidative features, stimulates regeneration and growth of tissues. Beeswax is an ingredient in surgical bone wax.

The experiments were carried out on 50 white mongrel rats with average body mass of 150g. The animals were divided into 2 groups: group 1 - controls and group 2 - animals with ectopically implanted beeswax membrane (BWM). Under ether anesthesia, a pocket 1x0.5 cm in size was formed in the oblique muscles of the anterior abdominal wall with blunt tool corresponding to BWM size - 1x0.5cm and thickness - 32-40 mk (Fig. 1). In the 1st group of animals the pockets remained empty, and in the second, BWMs were packed up in sterile condition, with one membrane in each pocket. The BWMs were sterilized by gamma-irradiation. The wounds were completely sutured, layer by layer, and treated by 5% iodine solution (Fig. 1). The animals were

withdrawn from the experiment by the excessive dose of ether in the 7th, 15th, 30th, 60th, 90th 120th and 180th days. The tissues obtained in these terms were fixed in 10% formalin neutral solution and filled in paraffin blocks. The slides sized about 7-8μ were dyed with haematoxylin-eosin (HE).

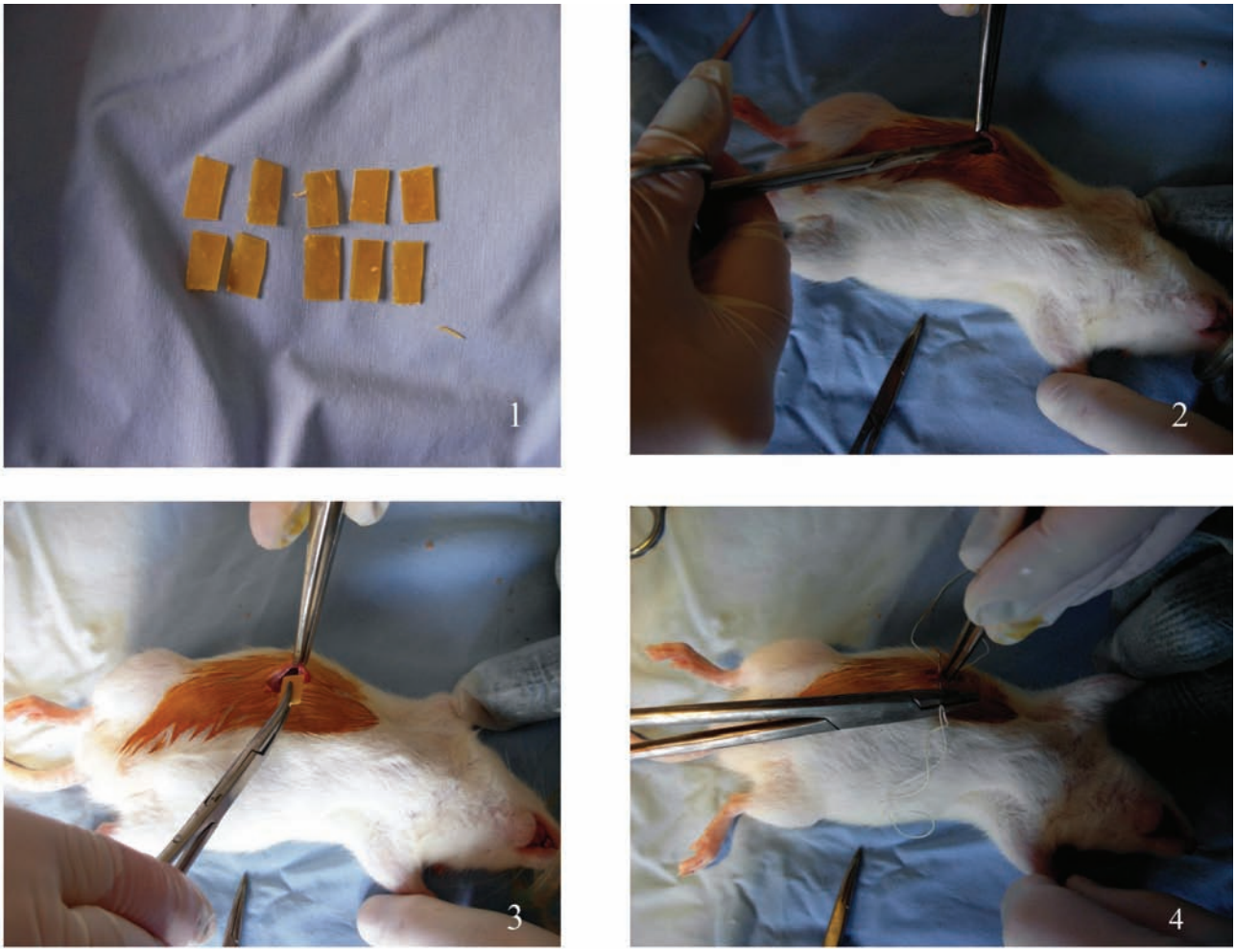

Figure 1. The stages of bee wax membrane implantation into the anterior abdominal wall in rats. 1) beeswax membranes; 2) pocket formation corresponding to the membrane size; 3) membrane implantation into the formed pocket; 4) layer-by-layer suturing of the wound.

Results and Discussion

On the first day and the following days of observation of animals, no significant deviations of wound healing processes after surgery were observed. In all the animals wounds healed uneventfully, by first intention.

At the 7th day after surgery, exploring the area of surgical intervention in control animals, some haemorrhagic foci and disturbance of blood vessel bed in the surgical area was observed in the anterior abdominal wall (Fig. 2.1). In the experimental group animals the contours of bee wax membrane were obviously determined (Fig. 3.1). In both groups a certain decrease in translucency of the anterior abdominal wall was observed in the area adjacent to the zone of surgical intervention, probably due to developing post-operative oedema. Microscopically, regenerative-granular processes were expressed in the control group as a reactive-inflammatory response, with newly generated vessels. Defibrillated areas of the muscular fibres were penetrated by plasmatic cells and connective, presented by polymorph cells, including endothelial cells, fibroblasts, histocytes, as well as accumulation of neutrophils and macrophages, and the net of fibres located between the vessels (Fig. 4.1). In the preparations of the experimental group, no macrophage response was revealed; the observed connective tissue elements were mainly fibroblastic, though wide lucidity areas between the muscular bundles were found out, defining the presence of the membrane (Fig. 5.1).

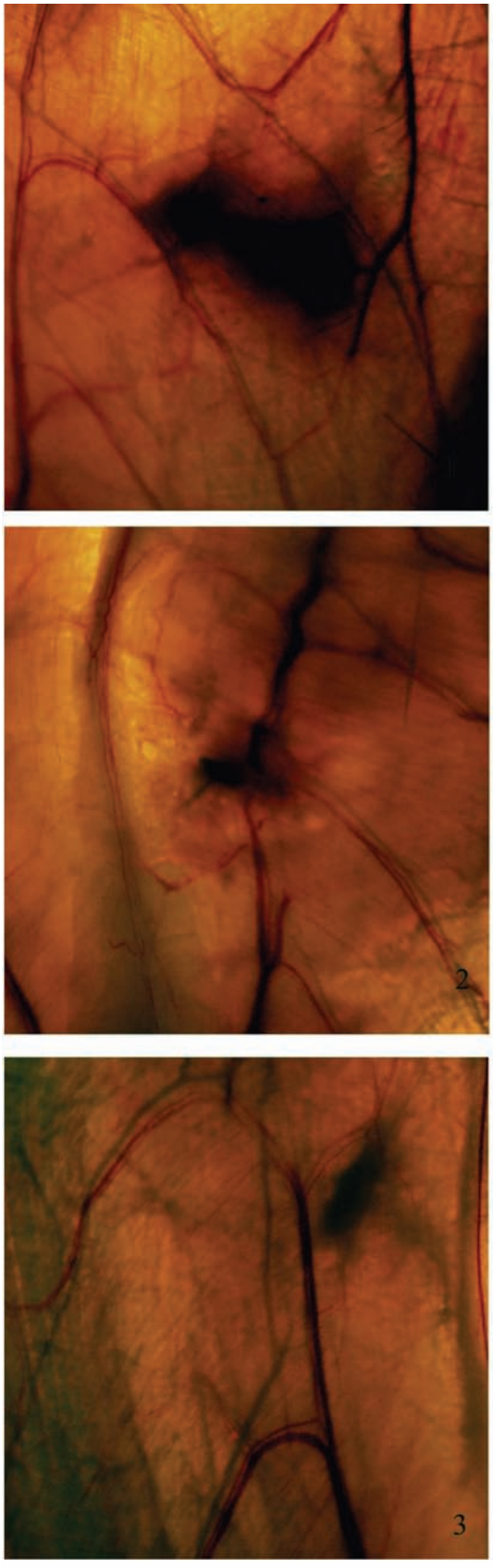

Figure 2. Macroscopic picture of surgical intervention in the oblique muscles of the anterior abdominal wall of the control group animals (explanation in the text)

At the 15th day after the surgery, findings hardly differ from those obtained on the 7th day. Macroscopically, transparency increased in the anterior abdominal wall, and signs of tissue oedema disappeared. However, surgical area in the control group animals was more transparent. In micro-preparations, a moderate reactive inflammatory process with densely located mononuclear cells of the same type and partly preserved infiltrations of plasmatic type was observed, including the presence of thin-walled capillaries type. Rough fibrous connective tissue strips were formed in the junction points of the torn fibres, with muscular fibre regulation (Fig. 4.2). In the experimental group of animals, the formation of fibrous-granular tissue around the main vacuolar cavities was observed (Fig. 5.2). The latter could be the evidence of triggering BWM biodegradation process, so that the BWM is fragmented into separate parts and wrapped by fibroblastic elements.

At the 30th day after membrane implantation in the muscular bed, macroscopically, the membrane edges became more transparent and irregular, resembling mosaic (Fig 3.2). The muscles surrounding the membrane had the structure typical of the cross-striated ones. Microscopically, homogenization of the affected myofibrils and bordering areas of the muscles was observed, with continuation new blood vessels and capillaries formation (Fig. 5.4). In histograms of experimental animals, the fibroblast number continued progressively to increase, enlarging the granular tissue area and decreasing size of the space occupied by the membrane, i.e., meaning dissolution of BWM and its replacement with fibrous-reticular tissue (Fig. 5.4). Increase in the amount of collagen fibres and formation of dense rough fibrous connective tissue with the developed capillary net was also observed.

At the 60th day after membrane implantation in the muscular bed, macroscopically, the area of surgical intervention was practically invisible in the control group, and vascular bed was restored almost completely (Fig. 2.2). In the experimental group, the membrane resembled mosaic picture with dark and lighter areas. The membrane edges were irregular and ragged (Fig. 3.4). Microscopically, vessel infiltration with cellular elements was observed here and there, which confirms the angiogenesis process. The spaces occupied by the membrane varied in shape and size, and were surrounded by the connective tissue with capillaries, including collagen and elastic fibres accumulated in bundles. The absence of connective-tissue capsule in the zone of the implanted membrane represents the evidence of the material biocompatibility and complete dissolution of the BWM.

At the 90th day of study, the intrusion zone in the control group animals macroscopically could not be revealed and capillary bed restored completely (Fig. 2.3).

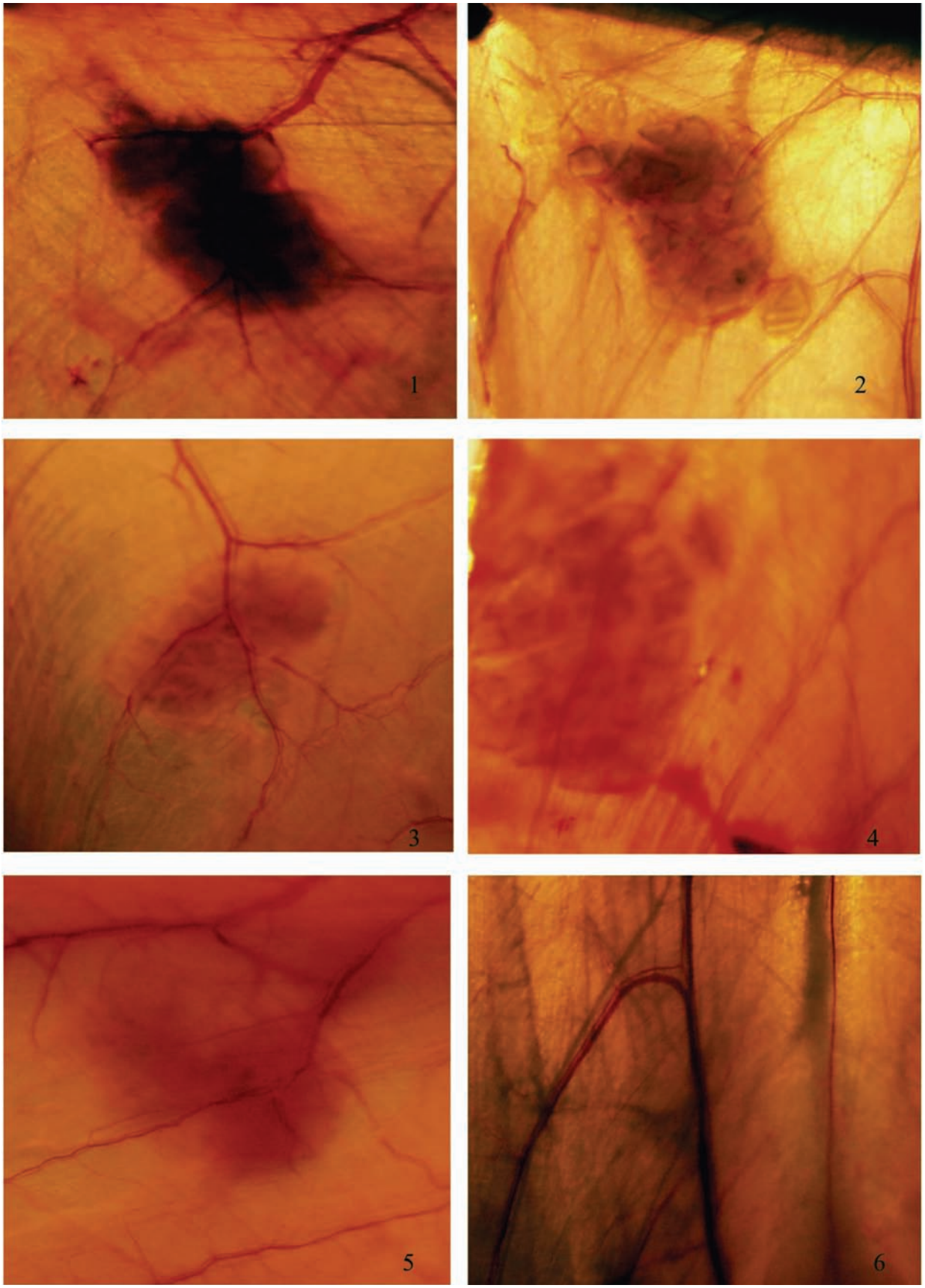

Figure 3. Macroscopic picture of the intervention area in the oblique muscles of the anterior abdominal wall in the experimental group animals (explanation in the text)

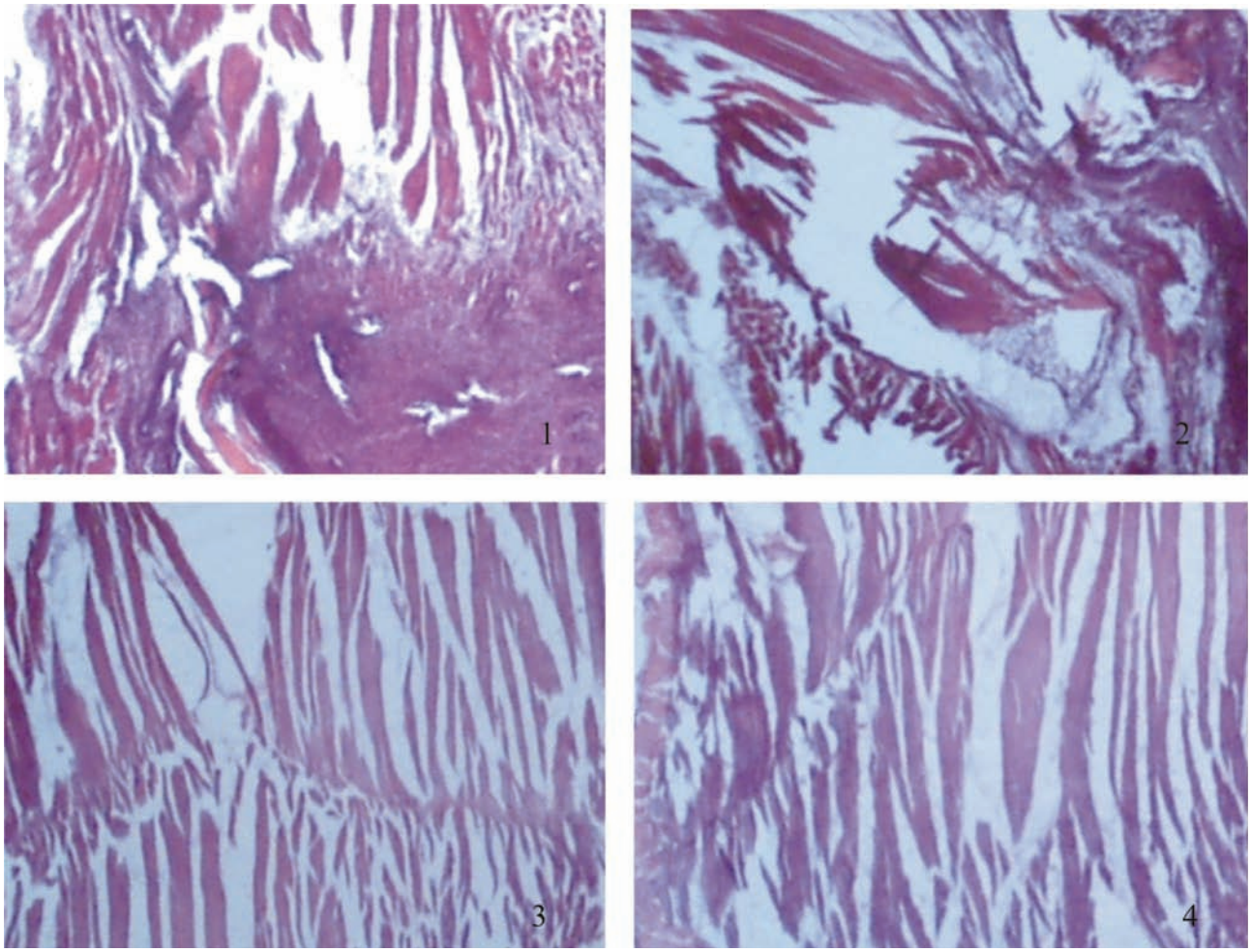

Figure 4. Morphological picture of intervention in the control animals (staining by hematoxillin-eosine , x60 magnification, explanation in the text)

In the histograms, only in some preparations, junction lines of the muscular bundles were traced. (Figs. 4.3 and 4.4). In the experimental group, macroscopically, the picture was almost the same as at the 60th day of the study. The process of collagen and elastic fibre formation actively continued though the decrease in the amount of fibroblasts and fibro-granular tissue.

By the 4th month of observation, the membrane thinned fragments with ragged and uneven edges were observed. In the surgery zone, vascular bed was practically restored (Fig. 3.5). Microscopically, an unordered location of muscular fibres and, here and there, preserved micro-cavities with uneven edges, with penetration of fibrous cord and a delicate net of connective tissue, were observed in the experimental group of animals (Fig. 5.5).

By the end of the experiment, at the 180th day after surgical intervention, the presence of the membrane in the muscular bed in the experimental animals was not observed, which indicated a complete dissolution of the membrane. The vascular bed and the transparency of the abdominal wall completely restored (Fig. 3.6). Microscopically, in all the preparations, a restored tissue with cross-striated orientation of the fibres and the vascular system with fibrous tissue net areas, including muscular fibre fragments, was present (Fig. 5.6). Microscopically, muscular fibres, a delicate net of fibrous tissue with the areas of rough fibrous tissue were observed, which means that cicatricle tissue has been completely formed in the intervention zone.

Thus, based on the results of the presented research, we concluded that the BWM prepared by us is not only an inert material for implantation; it did not cause inflammatory reaction, but promoted normal regeneration. It should be noted that bee wax is a biologically active product possessing high bactericidal properties. Due to the presence of pro-vitamin A and other biologically active substances, it is able to stimulate regeneration processes, which results in quick healing of the affected tissues.

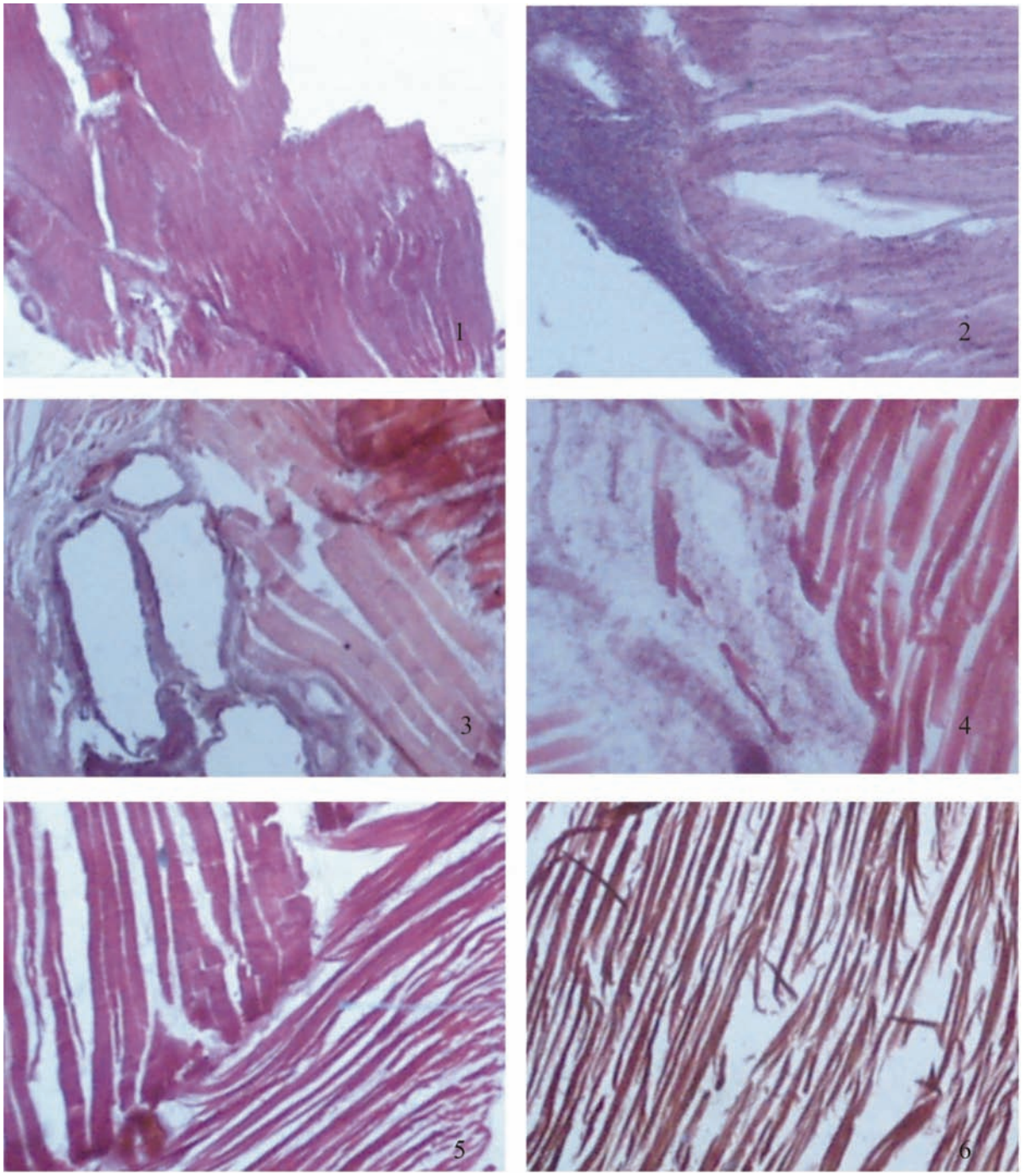

Figure 5. Morphological picture after beeswax membrane ectopic implantation (stained by hematoxillin-eosine, 1.3.4.5.6. x60 magn., 2 – x200 magn, explanation in the text)

The membrane dissolution terms are such that it could be applied as an alternative to other known membranes for isolation of bone defects and bone augmentation; it creates favourable conditions for successful formation, reliable preservation and normal transformation of various substitutes of bone tissue and blood clot in the area of bone defect. Summarizing the research data on regeneration processes after BWM ectopic transplantation, the results indicate that the applied BWM is involved into the biodegradation process by the 15th day after the implantation and, by gradual fragmentation and dissolution, it is substituted with connective tissue of various degree of maturity. The dissolution terms of the BWM 32-45 μ thick vary from 5 to 6 months, which is acceptable to be used as a membrane for guided tissue regeneration.

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