Practical Experiment: The Use of Biological Glue Made of the Local Material for Wound Healing

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Abstract: The article shows the results of experimental studies of biological glue invented based on the sturgeon swimming bladder. The glue composition and its gluing ability in connecting strips of natural skin was studied using the IR-spectroscopy; the glue structure was studied using the methods optic and atomic force microscopy. We have the planimetric research results gained from the skin wound experimental model held on laboratory animals using the invented tissue adhesive. We found that covering the wound with a biological filter with medical fillers gives a good adhesion on the top of the wound. There is no irritating effect and it stimulates the dermatic wounds' healing process.
Keywords: biological glue, sturgeon swimming bladder, skin wound surface, experiment.

Introduction

At present medical tissue glues of different nature are actively developed and introduced, making revolutionary changes in surgery [4,6]. Nevertheless the existing glue has its own advantages and defects. In particular, biological glues on fibrous base do not bear high power, syntetic glues are toxic from the point of histology, are weakly clamped with human tissues. The above mentioned defects of tissue glues limit the possibility of the using these composition in surgery. All stated is indicative of big urgency of the question of the development new optimum physiological active non-immune composition for increasing efficiency for surgical technology and conservative treatment, stimulates the conducting of joint studies among chemists, morphologists and surgeons.

Recently the treatment of skin wounds became actively developed and introduced the biodegrading materials on the base of biological polymer - collagen, gelatine, chitosan. The main advantage of these data material is high adsorbing ability, high adhesive in wound, absence of toxicity and irritating actions, favourable influence upon healing processes of the skin. The available literature has little studies on using biological composition on the base of fish collagen in wounds treatment. The works by V.I. Lukiyanenko shows that fish glue completely consists of collagen, related to human collagen [1,2,3,7].

So then we chose biological polymer from swimming bubbles of north sturgeon fish for making tissue glue. For a long time the people of sakha used sturgeon as food, boiled the glue from their air bladders, used them for glueing together timber, pasting skins of the deer to ski and other home necessities, as well as in ethnic medicine.

Purpose of the study: the working of biological tissue glue on the base of swimming bubbles of the north sturgeon fish and conducting of experimental treatment studies of skin wounds among animals.

In accordance with the target goal the following problems were defined:

To create the technology of making materials and compositions on the base of biopolymer (collagen of Siberian sturgeon swimming bladder).

To study a chemical composition as well as physical and chemical properties of biological composition material.

To model the dermal wound surface of experimental animals.

To research the influence of new-made tissue adhesive, based on biopolimer , on the repairing process of the dermal wound surface of experimental animals.

Materials and testing methods

The experimental research included several stages: the first stage was to study a chemical composition as well as physical and chemical properties of biological composition material of Siberian sturgeon swimming bladder. The second stage was to create the technology of making materials and biological films on the base of the fish collagen. The third stage consisted of experiments on animals. The laboratory rats weighing 180-220 grams served as test animals. All of them were females since they have less coarse undercoat. All experiments were made under ether anesthesia according to "The rules of work with experimental animals". Before the experiment, during a week, all the animals were kept in a special room of the North-Eastern Federal University Medical Institute for the new conditions adaptation. They had a standard food ration with a free access to food and water. All the animals were weighed beforehand. They also underwent a close medical examination. The ill animals did not participate in the experiment.

The experimental model of the dermal wound was made after thorough shaving the upper dorsal part of the animal body. The boundary of the future wound was made with a marker in the interscapular region. Then the skin of the animal was cut with the lancet up to subcutaneous fat. Two kinds of biological films were made to study the repairing ability of the new-made biological glue on the base of the collagen of Siberian sturgeon swimming bladder: the first one did not have any medical components, while the second kind had medical additives of dymethylsulfoxide, calcium chloride, glycerin. The animals were divided into three groups. The first group was the control one, with experimental dermal wound surface without correction; the second group was a trial group, in which the wound surface was covered with a biological film without medical components; the third group was a trial group, in which the wound surface was covered with a biological film, containing medical components.

To estimate the repairing effectiveness, the thorough dynamic trial observations of the animals' general state, the local flow of the wounding process, the repairing path of the wound were held. The experimental model of the dermal wound was investigated on the third, seventh, tenth, seventeenth, twenty third days from the beginning of the experiment. The clinical judgement of the results was made on the base of dynamic visual and planimetric approaches. To calculate the daily speed of the wound surface reduction the formula, suggested by L.N.Popova in 1942,was used: $V=(S-Sn)x100/S \times t$, where S is the value of the wound surface at the previous sizing, Sn is the value of the wound surface at the moment of the next control, t is the number of the first and the last measurement [5].

The result of the research

The fish swimming bladder almost completely consists of pure collagen. Collagen is a fibrous protein, the basis of the animals' connective tissue that makes it durable and elastic; it makes up about 1/3 of the total amount of all the proteins in an organism. It supports the extracellular frame of all the metazoan animals and it is a component of any animal tissue [4]. On the microscopic photos that we took on an optical microscope Olympus Bh-2, we can point out single collagen fibrilla, 19-23 microns in size, that are group in tracts in the form of fiber from 300 to 400 microns in size (Fig. 1).

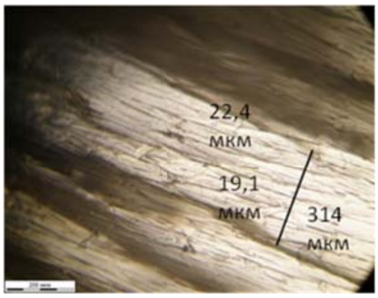
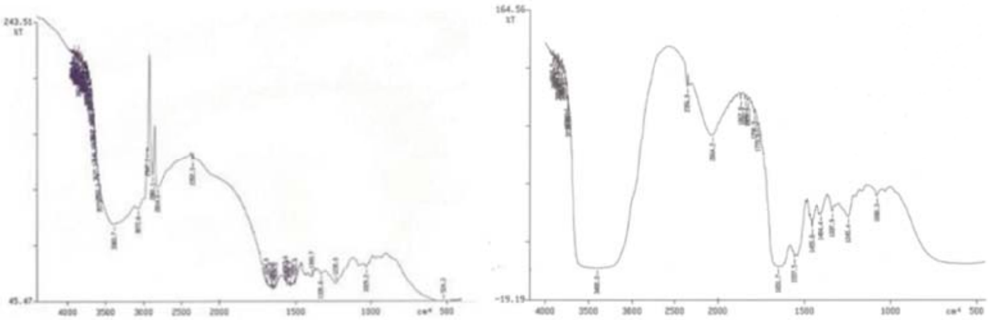


Figure 1. The structure of a sturgeon swimming bladder, taken on an optical microscope (1-200 microns).

As the results of the atomic and absorption analysis show, in our research of the swimming bladder, the concentration level of heavy metals did not exceed the maximum allowable concentration, i.e., it can be used in contacting with the human body.

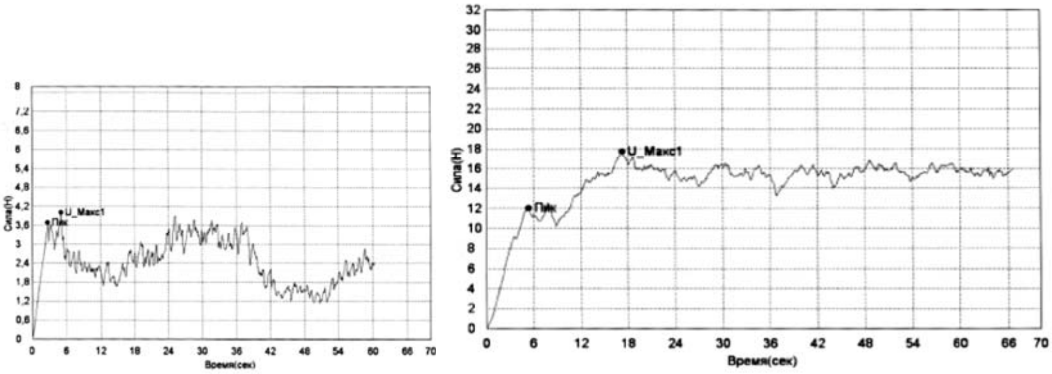
As for the chemical composition, the glue made of sturgeon swimming bladder is an aqueous solution of the protein collagen, the polymer molecule of which consists of over 20 amino acids. In the spectra of the swimming bladder and glue, taken from an IR-spectrometer Paragon-1000, there were a great number of relatively strong absorption bands that, as a rule, belong to the vibrations of the peptide group, the common structural element of the protein molecules. In the swimming bladder at the range of 1660,1 cm^{-1} there is a strong band that belongs to the CO group valence vibration and is called amide I; also at the range of 1510-1570 cm^{-1} the amide II band can be seen. The amide I and amide II bands show that there is α – spiral. In comparing the spectra of the swimming bladder (native collagen) and glue made from it (denaturated collagen) there was seen a disappearance of the absorbtion bands amide I and amide II, i.e., there is a complete destruction of the α-spiral, which happened due to denaturation of native collagen during thermal treatment (Fig.2).



а) б)

Fig. 2. IR spectra of the sturgeon swimming bladder (a) and glue from it (b)

In order to evaluate the adhesion ability of the produced glue, there were model experiments carried out on the sample of natural skin, glued sheets by created glue and glue BF-6. The glue BF-6 is an alcohol solution based on polyvinyl butyral and bakelite varnish, which is one of the most famous types of medical glue in Russia, based on synthetic polymers. The force of delamination of the premature glued sheets was defined in accordance with the Unified State Standards №6768-75 "The method of defining the force of connecting between to sheets during delamination"

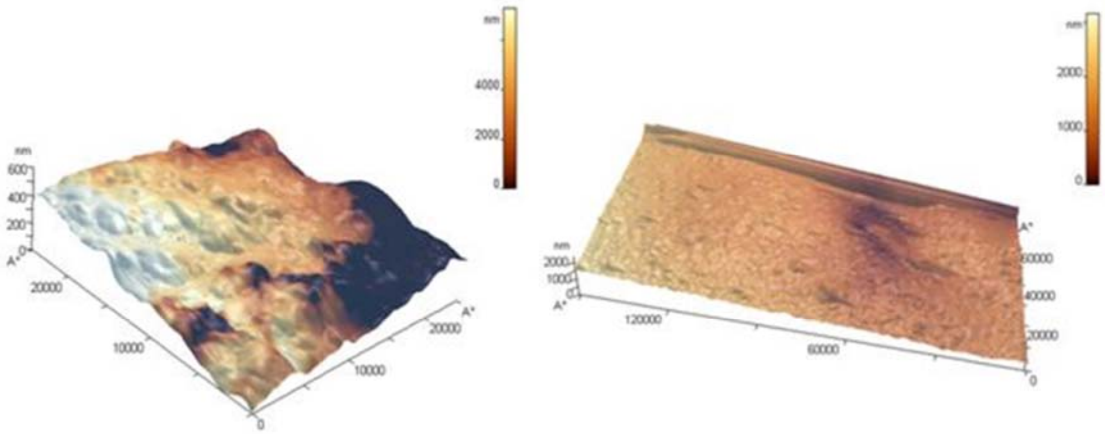


(a) (b)

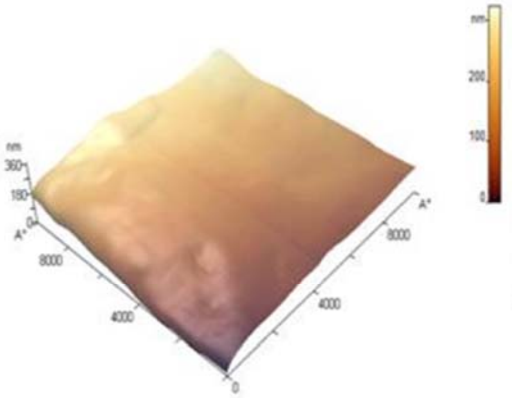
Fig. 3. The correspondence of the BF-6 glue specimens' connecting force (H) to the time (a) and the swimming bladder glue (b).

The analysis of the force of delamination of the samples (table 1), glued together using medical glue BF-6 (Fig.3,a) and the glue made from swimming bladder (Fig.3,b) shows that the produced glue has a high gluing ability. When the samples of natural skin were glued together, the force of delamination of glue made from swimming bladder was 4.5 times higher, than the same rate for medical glue BF-6.

Studies of the collagen structures and its changes during the glue preparations using the method of atomic force microscopy show that there is a difference in the surface texture of the different component film samples in comparison with the initial swimming bladder (Fig.4). On the other hand, the surface of the swimming bladder is more embossed (Fig.4, a) and it corresponds to the fibrillar structure, which in turn, corresponds well with the optic microscope data. After a thermal treatment the structure of the films is amorphic, which corresponds to the collagen's transition into gelatins (Fig.4,b). The introduction of medical components does not influence the gelatin structure and the other qualities of the films practically, but the glue stickiness increases. (Fig.4,c).



a) swimming bladder c) film made of glue, glycerol, dimethylsulfoxide and $CaCl_2$



b) Plain glue film

Fig. 4. Illustrations of the swimming bladder surface texture and different component film samples based on the initial swimming bladder in 3-D mode, that was made using AFM.

We held a through dynamic and visual research and observed the general state of the animals, the local wound appearing and healing process, in order to evaluate the effectiveness of the cure. On the third day of the experiment we observed that the first (or the control) animal group without the cure had a thin half-transparent red-colored layer, covering the wound and the skin adjoining the edges of the wound was swollen and congested. We also noticed a curved pink-colored interface 1 mm wide around the perimeter of the wound, which demonstrates the beginning of the boundary epithelialization process. The wound surface decreased by 43 mm^2, which gives an area 98.27 mm^2. In the same time period, the second animal group's wound is covered with the biological maroon filter with the medical components. The biological filter is hard and curved. The skin, adjoining the wound has no visible pathologies. There is a vivid pink interface, 1 mm wide around the perimeter of the wound. The wound surface decreased slightly (from 141-109.5mm^2 correspondingly) in comparison with the first day. The wound of the third group of animals is covered with biological filter with medical components. It is non-curved, maroon and there is a pink interface, 1 mm wide, which also shows the beginning of the regeneration phase process. The wound surface decreased by 49.46 mm^2, which makes up 91.84 mm^2.

On the seventh day of the experiment, the wound surface of the first (control) group was covered with a hard maroon wound crust. There is a pink interface around the perimeter of the wound, 2mm wide. There is small swelling and congestion remaining in the adjoining tissue. The area of the wound was 43.55 mm^2, which is 54.72 mm^2 smaller, than on the third day. The wound of the second group of animals was closed by a thin curved maroon layer. There is a vivid pink-colored strip, 3 mm wide around the perimeter of the wound. The wound surface decreased from 109.5 mm^2 to 52.35 mm^2 in comparison with the third day. A flat thin maroon layer covered the wound of the third group of animals with a biological filter, modified by medical components. There is a pink interface 3-4 mm wide around the perimeter of the wound. The wound surface decreased from 91.84 mm^2 to 38.85 mm^2 in comparison with the third day.

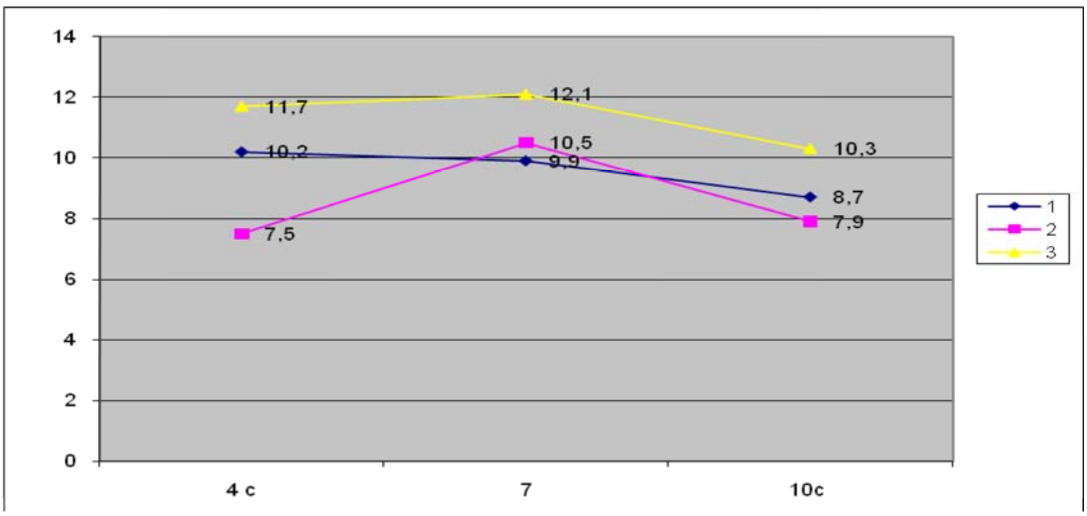
On the tenth day of the experiment the base of the wound defect of the first group is under a thin hard curved maroon wound crust, which can be easily removed with a lancet. Underneath it, there is a wound surface, covered by a transparent red-colored layer. If the wound was slightly damaged, there was a strong punctuate bleeding. The adjoining tissue has no visible pathologies. The wound surface area decreased from 43.55 mm^2 t9 17.89 mm^2 in comparison with the seventh day. The wound of the second group of animals with a biological filter without medical components is covered with a thin flat maroon layer; the layer is adherent with the wound surface. There is a small strip of scar tissue around the border of the wound. The are of the wound surface decreased insignificantly in comparison with the control and the third group and it is 40.42 mm^2. The wound surface of the third group of animals with a biological filter modified by medical drugs, is covered with a thin flat maroon layer, which is adherent with the wound surface. There is a light-pink strip, 4 mm wide around the contour of the wound. The width of the wound corresponds visibly to the epithelium of the intact dermatic surface, which shows the beginning of border epithelialization. The area of the wound surface decreased to 10.59 mm^2, which is 7.3% less, than the one of the control group and 29.8% less, than the second group.

Table 1. The area of the wound surface in the different time limits of the experiment (in mm^2)

Group No	The wound area on Day 1	The wound area on Day 3	The wound area on Day 7	The wound area on Day 10
I	141.3	98.3(30.5%)	43.6(69.2%)	17.9(87.3%)
II	141.3	109.5(22.5%)	52.4(62.9%)	40.4(71.3%)
III	141.3	91.8(35%)	38.9(72.5%)	10.6(92.5%)

The analysis of the daily speed of the dermatic wound healing among the groups of animals under study, showed that in the third group of animals with a biological filter, modified by medical fillers, the figures are bigger, than the in the control group without corrections and in the second group of animals with a biological filter without medical fillers. On the third day of the experiment, the wound healing speed in the third group was 11.7% per day, whereas the figures showed 10.2% per day for the control group and 7.5% per day for the second group. Provided that, we can see that the wounds of the animals of the second group heeled slower on the third day, than the ones of the control group. On the seventh day of the experiment the third group figures were also higher, that is 12.1%, whereas the control group had only a rate of 9.9% and 10.5% for the second group of animals. On the tenth day of the experiment, the daily wound healing speed was 8.7% for the control group. The third group with the biological filter, modified by medical components had the highest rates (10.3%) in comparison with the control group and the group of animals with biological filters without medical components.

Diagram 1. The dynamics of the experiment animals' daily wound healing



In conclusion, the visual observation and planimetrical figures analysis of the experiment wound surface showed that covering the wound with a biological filter with medical fillers shows good adhesion. There is no irritating effect. The filter also stimulates the dermatic wounds healing processes and gives a basis for further experiments.

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