DEVELOPMENT OF MEDICAL MATERIALS ON THE BASIS OF BIOLOGICAL POLYMERS

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Abstract: *Technology of production of glue from sturgeon swimming bladder has been developed. Beside collagen adhesive film contained special additives and drugs, which assisted in blood coagulation and had bactericidal properties. Glue composition adhesive properties in tying two strips of leather were investigated. Glue composition and structure was investigated by light and atomic force microscopes, IR-spectroscopy, determination of the delamination force surfaces bonded by developed composition. Results of the experiment "in vivo" on rats show promising use of composite developed materials for wound healing.*

1 Introduction

Collagen is the main structural protein of the connective tissue that performs important biological functions. It has a great number of properties, but its main property is biodegradation, which allows to leave the material in the place application and not to be concerned about its further excision. At present, scientists are doing some intensive research on the properties of this protein. For example, there are ways of applying collagen in order to prepare various kinds of drugs, films and cosmetics [1]. For example, in their work Takashi Sekine, Tatsuo Nakamura and others used porcine collagen to prepare biodegradable glue for connecting parenchymal organs together [2]. Now researchers are developing and implementing different kinds of tissue glue, making revolutionary changes in surgery. In addition, the existing tissue glue has their advantages and disadvantages. For instance, biological glue based on fibrin is not exposed to high exertion. Synthetic glue (cyanoacrylate) is histotoxic and it doesn't have a strong hold on the tissues glued together. The disadvantages of the existing tissue glue that we mentioned above, do not allow using these compositions in surgery on a regular basis. All the points mentioned above, show the importance of the problem of creating new compounds for increasing the efficiency of surgical technologies and conservative ways of treating; it also serves as a stimulus to carry out co-operated chemical, morphological and surgical research.

In this research paper, we tested a new source of collagen, that is, collagen from the swimming bladder of siberian sturgeon. The people have long since used sturgeon in food and

have made glue from their swimming bladder, which they used to glue together wooden parts and for other household necessities. We used the swimming bladder of siberian sturgeon to develop medical materials. On the initial stages of research we worked on solving primary questions of the perspectives of using materials based on the siberian sturgeon swimming bladder. Unlike the animal collagen, fish collagen has genetic immunity close to that of the human. Both fish and human beings have almost the same amount of immunoglobulin-gamma because it is a carrier of antibodies. For this reason, the fish have immunity (insensibility) to a large range of microflora, toxins and peculiar antigens [3].
The aim of this research is to study and define the properties in the main areas of using materials based on the siberian sturgeon swimming bladder.
To reach our goal we have to finish following tasks:
1. Development of production technology and composition of materials based on the biopolymer.
2. Research of a chemical composition, physico-chemical properties of biological composition material.
3. Evaluation of developed medical materials experimental usage and identification of a perspective application sphere. Selection of optimal forms of biological materials application for certain medical purposes.

2 Materials and testing methods

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 Olimpus 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).

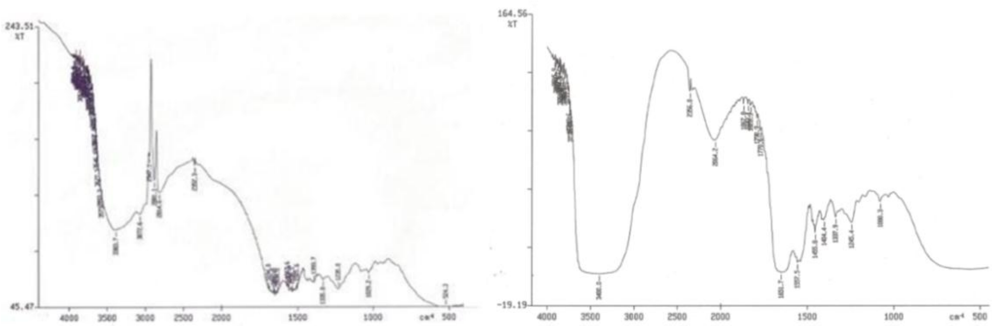
Figure 1. The structure of a sturgeon swimming bladder, taken on an optical microscope (1-200 microns).

Depending on the fields of applying it in surgery, swimming bladder can be used as glue (in surgeries on parenchymal organs as a stitching material; in traumatic surgery to glue together bone fragments) and as film (for external use in healing wounds and burns). On the initial stage, the glue was tested as film to cure animal wounds.
In this research we extracted in the following way: we made glue out of inner layer of the swimming bladder using thermal treatment. Then we injected adjuvant substance: plasticizer, antiseptic and hemostatic. After that we formed the films by evaporating the dissolvent in the Petri dish. In one of the experiments we put a diamond-containing load of a specific brand into the glue formula; the diamond-containing load has nanocrystals of non-processed diamonds the size until 10 nanometers. In further experiments nanodiamonds can be used in adsorption of pharmaceuticals and their prolonged extraction into the surface of the wound.

We also studied the technical properties of glue, such as viscosity, concentration as well as the level of swelling in water.

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 [5]. 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).



a) б)

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

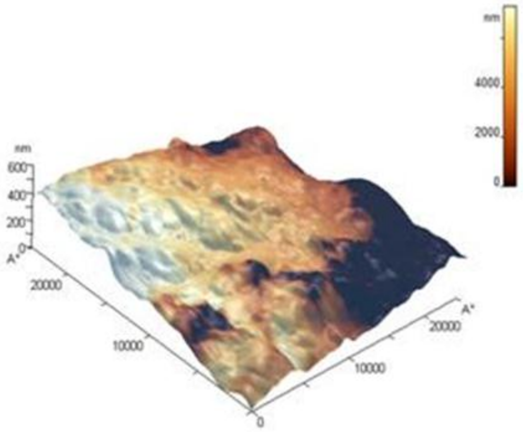
The developing materials should have high adhesion to biological tissue. In order to evaluate the adhesion ability of the produced glue, there were model experiments carried out on the sample of natural skin, glued sheets by created glue and glue BF-6. The glue BF-6 is an alcohol solution based on polyvinyl 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” (Testing Instrument AUTOGRAPH AGS-J, SHIMADZU, Japan).

	force	strain	force	strain	force	strain
Parameters	maximum peak		first peak		mean	
Unit	P, кN	R, кN/mm	P, кN	R, кN/mm	P, кN	R, кN/mm
Medical glue BF-6	4,0±0,1	0,2±0,01	3,7±0,07	0,2±0,01	3,4±0,06	0,2±0,01
Swimming	17,7±0,1	1,1±0,05	12,0±0,06	0,8±0,03	15,9±0,09	1,0±0,03

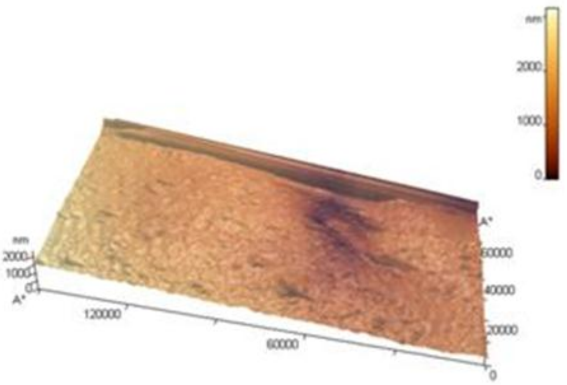
bladder						

Table 1. The results of determination of force delamination when bonding sheets of leather

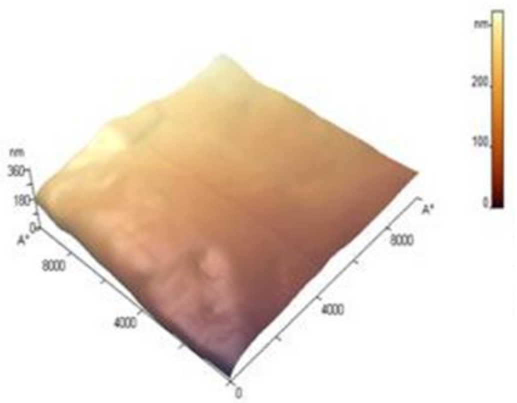
The analysis of the force of delamination of the samples (table 1), glued together using medical glue BF-6 and the glue made from swimming bladder shows that the produced glue has a high gluing ability.
When the samples of natural skin were glued together, the force of delamination of glue made from 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. The insertion into the nanofillers hardly affects the gelatins structure and the films other properties, but the viscosity of the glue increases.



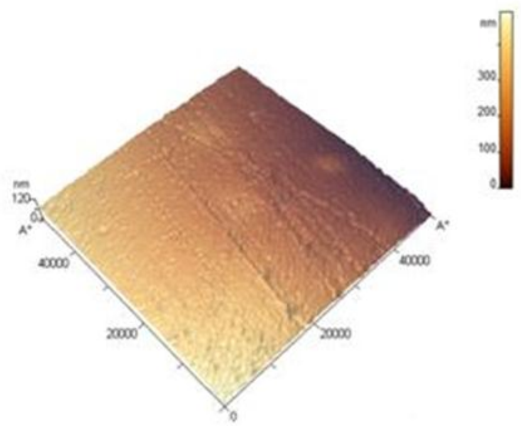
a) swimming bladder



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



b) Plain glue film



d) film made of glue and nanodiamonds

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.

In order to define the ability to use the produced films for medical purposes, a medical experiment in vivo was carried out; so that we could evaluate the film of different component structure effect on the process of wound healing among experimental animals. Male rats of the Winstar link, 180-200 gr. In weight were used in the experiment. After removing the hair-covering in the paravertebral and lumber region a wound surface was created. The clinical judgment of the results was carried out, based on visual dynamic observation in the course of 23 days. The process of wound healing starts right after the tissue damage and includes three main phases: inflammatory, the phase of granulation tissue formation; phase epithelialization and the cicatricial tissue formation. On the 17^{th} day after the surgery, the wound surface, without the film, healed slower, than the wounds covered with the films. During these days the area of the wound covered with films has significantly grown smaller, than the wound without the film. On the 23^{rd} day of the experiment all the wound surfaces were healed. During the whole time of the experiment using the films, no diapyesis of the wounds or any allergic process was observed.

Conclusion

In conclusion, a perspective of using the siberian sturgeon swimming bladder to produce medical materials was shown. The formula and technology of making films out of sturgeon swimming bladder glue has been worked out; the technical properties of the materials and their chemical properties have been studied. The invented glue has a high gluing ability in gluing natural skin samples; the force of delamination of the swimming bladder glue is 4.5 times higher, than the similar data of the medical glue BF-6. Using the method of optical and atomic force microscopy, we studied the collagen structure and its changes during the production of glue. There is a transition of fibrillar structure, typical of native collagen, to an amorphic structure during its denaturation. An in vivo experiment was carried out on laboratory animals, which allowed to evaluate the effect of the films content on the time necessary for blood coagulability and wound healing. When films were used, the area of the wound decreases and the time of granulation tissue formation as well as the time of wound healing decreases.

The given work needs further research in order to develop ways of using the invented glue, based on biopolymer to solve various medical problems.

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