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Chapter

A Study of the Use of Modified Collagen of Freshwater Fish as a Material for Personal Care Products

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Abstract

The work is devoted to the use of collagen materials produced on the basis of connective tissues of hydrobionts. Their characteristics as potential means of personal hygiene and wound healing are investigated. The results of studies of physical and chemical characteristics of collagen materials from freshwater fish with a modified structure obtained in this paper indicate the prospects of their use as an absorbent layer of personal hygiene products, due to the high moisture absorption capacity, which is an order of magnitude higher than the moisture capacity of untreated collagen and higher moisture capacity of superadsorbing polymers required for use in personal hygiene products. The resulting material meets the requirements of sanitary and epidemiological safety, allergenic action on the skin, as can be concluded from the results of tests on animals, it does not. This material accelerates reparative processes to the same extent as collagen used in standard hemostatic sponges with the simplicity of its production technology, low cost, and availability of raw materials.

Keywords: hygiene, human, safety, hydration, moisture capacity, sorption capacity, deodorization, collagen, substances, fish raw materials

1. Introduction

Absorbing and deodorizing properties are especially important when improving and creating new medical means and materials, including those for individual use (personal hygiene).

Presently, the improvement of, for example, the napkins is focused primarily on the modification of their shape and design [1–3]; however, less attention is paid to a selection of moisture-absorbing layer materials for personal hygiene means, and their domestic production (in Russia) is absent. These materials can be divided now into three categories, i.e., the synthetics—for example, polyvinyl alcohols, polyethylene oxides, cross-linked polyacrylic esters [2]; natural materials—cellulose, guaric or xanthan gum; and modified natural materials—the cross-linked starches, sulphitic cellulose, Kraft cellulose [1]. The mixes of polymers of either synthetic or natural origin can be used. Particles of these polymers (they are called the superadsorbing polymers too) may have the form of powder, grains, granules, or fibers. Their use in the form of fibers is met more often because in this case the extensive capillary network is formed capable of binding a significant amount of moisture in macrocapillaries.

In case of choice of materials for functional layer, the preference, as we think, should be given to natural or modified natural materials. They do not, as a rule, cause allergy; they are environmentally friendly; and in general, they are perfectly recognized by the human body. Of all, collagen, particularly, the protein connecting tissues of animals, fish, and birds in whose organisms its content is the highest [4], belongs to natural materials, which could be used in personal hygiene.

At the same time, collagen is concentrated in the by-products and waste of the processing industries of agro-industrial complex [5] that are based on processing poultry, meat, and fish. In this regard, the sources of collagen can be considered as easily available and inexpensive, and their use for receiving collagenic substances decreases environmental pollution. The wide range of technological processing and a variety of sources [6–8] give the chance to use collagen not only as a part of the absorbing layers but also in other layers of personal hygiene. Collagen plays an important role in the implementation of reparative function of connecting tissue [9], it is fully compatible with human skin [10]; therefore, effects of irritation, discomfort, and other negative effects on skin may be expected as the system will be minimized.

Collagen is the fibrillary protein that forms fibrous materials [11–13], i.e., similar to cellulose, its fibers can form locks and gaps where water pours and remains entrapped. Moreover, like cellulose, collagen can swell and bulk up in water due to penetration of water into a structure of the material due to a large number of functional groups and their increased hydration ability [14, 15]. This property creates the prerequisites for use of collagen in the absorbing layers of personal hygiene means as the best material capable to compete with cellulose presently in use as well as other known materials.

Fish collagen in this regard is of the greatest interest, due to its special rheological characteristics facilitating the technological processing (its structure is low molecular in comparison with collagen of animals, it does not require obligatory hydrolysis when processing raw materials, and the final materials made of fish collagen are more elastic) [16]. However, the question of methods of its processing is open yet for saving and increasing its water-absorbing ability, and the question of sorption ability, especially to aromatic substances, and also its wound healing activity are not properly investigated yet.

The wide use of collagen in the cosmetic, medical, and food industries is interfered by its possible allergenicity. Thus, when holding mesotherapeutic procedures with collagen injections, allergic reaction develops in ~6% of women [17]. However, in this case, it must be taken into consideration that allergic effects are, as a rule, found in collagen of animal or sea fish origin. At the same time, for example in [18, 19], separate data are given that collagen of fresh-water fish shows minimum allergenicity or its total absence in comparison with collagen of sea fish. Yet, the results of both fresh-water fish collagen allergenicity research and its processing to increase its water-absorbing ability are unknown to the authors of this article. Moreover, there are but a few researches of microbiological and toxicological indicators of collagen of fish of internal natural reservoirs.

One more very interesting scope of collagen use is the creation of antidecubital bandages and sheets for people with limited mobility. High requirements of water-absorbing ability are obvious in this case. The hemostatic collagenic sponges are rather popular [20] having both styptic and aseptic effects and stimulating the processes of tissue regeneration. However, they are manufactured by way of hydrolysis of the collagen extracted by splitting cattle skin. The process of hydrolysis is extremely prolonged, and it demands the use of expensive reactants, which makes such sponges a very costly product. These shortcomings are eliminated in the products from fish collagen but the question of whether they will be able to compete with standard sponges' wound-healing properties requires a separate research.

The purpose of this work is to study the water-absorbing and sorption ability of collagen of fresh-water fish, search for opportunities of its increase, and assess the effect of a wound-healing, sanitary, and epidemiologic safety and allergenicity of the material received through collagen from fresh-water fish from internal natural basins of Russia.

2. Materials and methods

The objects of research were the collagenic product received by special processing of skins of pond fish using the author's technology (cf. the Russian Federation Patent No. 2614273). Skins of the silver carp were used, which is a valuable source of proteins among which the prevailing fraction is collagen [21]. Skins of fish were processed in weak solutions of alkalis and organic acids, and the received preparations were dried up with lyophilic process, in the course of which the material got a sponge form.

For the research of swelling capacity of the received material, the preset amount of water was used, and in our case, it was 20 ml, previously weighing the cup for processing and the studied material, measured the change of mass of water without sponge was measured when the constant volume of free liquid was eached, then the change of mass of a sponge was recalculated.

The research on the effect of a wound-healing property was made by histologic and histochemical methods [22] on animals of the same age received from a vivarium and the laboratories of the Voronezh State Medical University of N. N. Burdenko.

The first stage of research was carried out on animals and then on the human patient. All animals passed the quarantine, and they had no symptoms of diseases and received a standard diet. The average mass of an animal was 300 ± 25 g, and the dispersion of initial weight did not exceed 15%.

The research was made on 168 laboratory white rats divided into three groups: two of them are control and one is the main (see **Table 1**).

Wound modeling was carried out with the modified Sychennikov's technique (1974). Under ester anesthesia in aseptic conditions on the skin spot shaved from wool, after processing of skin with antiseptic solution, using the disposable medical scalpel a linear section of skin 1 cm long was made on the external surface of an average one-third of a hip, a fastion and the muscles. Soft tissues were clipped to extend the cut and warmed up. The area of wounds before an initiation of treatment in all groups averaged 26.0 ± 0.5 mm^2 without meaningful distinctions

Groups	Quantity of test animals	Group characteristics
No. 1, control	56	Without treatment
No. 2, control	56	Treatment with standard collagenic sponge
No.3, Tested species	56	Treatment with collagenic substance prepared from fish raw materials

Table 1.
Test group characteristics.

between the groups. The treatment of the second control group and the main group was applied right after the modeling of wounds.

In the first control group, the treatment was not used. In the second control group, the treatment of wound was made by washing it directly after modeling with 5 ml of 0.9% solution of sodium chloride and introduction into a wound of a standard collagenic sponge, which was cut out to fit the area of wound for its full closing. In the main group unlike the second control group used an analog of a standard sponge made of fish raw materials was used.

The dynamics of wound process arresting was estimated on the basis of the research methods listed below:

1. Clinical methods: the general condition of animals, indicators of a course of wound process (defect closing speed, exudation, presence of necrotic masses, emergence of granulations, epithelization, etc.).

2. Planimetric methods: the control of the area of a wound by the technique of Popova/the tsellofanografic method/and its dynamics. For calculation of percent of a daily change of the wound area, the following formula was used: ***% S = (S–Sn/S)*100%,*** where *S* is the area of a wound at the previous measurement; *Sn* is the area of a wound at the current measurement.

3. Histologic methods: to study the dynamics of histologic changes, the excision of tissue was made on the first, third, seventh, and eleventh day after the initiation of treatment with capture of an intact skin, a bottom and edge of a wound to a size 1.0 × 1.0 × 0.5 cm. The received material was fixed in the 10% solution of neutral formalin, dehydrated, further the 6 microns thick paraffin cuts were made and painted with hematoxylin-eosin and studied in a light microscope according to Van-Gizona. The images were obtained using the cross view of several fields of vision and analyzed in the image analysis system LeicaQwinStandartV2.6 (Leica, Germany), consisting of LeicaDRM microscope equipped with the "LeicaDC 300F" digital camera and a computer with the LeicaQ 550W software. Micrographs were processed using the Microsoft applications.

3. Histochemical methods

For histochemical research, the painted cuts of collagen samples were photographed at four hundredfold increase, and then contrasting of the reaction product was made equivalent to the histogram using the Threshold function filter allocation. For the allocated areas, the area in pixels was determined, which was recalculated in micrometers by the photograph with the same device with which the image was obtained, and the final object with an area of 1 micron was recalculated into pixels.

Investigation of the content of ribonucleic acid (RNA) with the technique with Azur V by Shea was made, which provided the selective identification of nucleatic and cytoplasmic RNA and made it possible to carry out the quantitative research of RNA content within the Malpighi sprout layer of epidermis.

Processing of cuts in 100% acetic anhydride at room temperature allowed to block the potentially reactive amino groups of protein, and the differentiation in tertiary butyl alcohol provided the removal of the molecules of Azur V that were not connected with RNA.

The content of sulph-hydrylic groups of proteins (SH group) was studied by Chevremont and Frederick's method. This reaction represents the biochemical cysteine test at which the insoluble deposit of the Berlin azure is formed. Quantitative assessment of maintenance of SH-group within a Malpighi layer allowed to define the extent of maturing and the differentiation of epidermis at various methods of wound treatment.

Research of sanitary and epidemiologic safety was made at "The Center of Hygiene and Epidemiology in the Voronezh Region" Federal establishment (see the Test report No. 9627p of 09.02.2017). Determination of the content of heavy metals—arsenic, mercury, and lead—was made by GOST 26927-86, GOST 30178-96, and GOST 29188.2-9, and hydrogen indicator was measured according to GOST 26930-86. The skin-irritant effect of collagenic substances and its impact on mucous membranes were determined by the instruction 1.1.11-1352004, and the general toxic effect was determined by the alternative *in vitro* methods according to the document No. 29FTs1394 of January 29, 2002. Microbiological indicators were determined by the state standard specifications: (GOSTs) as follows: the total of mesophilic aerobic and facultative and aerobic microorganisms—by GOST ISO 21149-2013; *Candida albicans*—by GOST ISO 18416-2013; *Escherichia coli*—in accordance with GOST ISO 21150-2013; *Staphylococcus aureus*—in accordance with GOST ISO 22718-2013; and *Pseudomonas aeruginosa*—in accordance with GOST ISO 22717-2013.

The research of allergenicity was made in the same conditions (The Protocol on technical and toxicological research within the preclinical tests of porous medical materials from fish raw materials of July 01, 2014). Allergenic properties of collagenic substance of fish origin were studied on guinea pigs with skin applications.

Provocative skin tests were made as follows: before the applications, the sensitization of animals was made by repeated drawing of collagenic dispersion on skin. Daily applications were made on three guinea pigs with water dispersions of the modified collagen in cultivations on the cut-off site of skin, 1:1; 1:10 and 1:100, within 14 days (which corresponds to the duration of the incubatory period).

During the entire period of the experiment, the observed guinea pigs underwent measurement of body temperature, thickness of a skin fold on the place of application, and temperature on the place of introduction.

4. Results

The graphic dependences reflecting the kinetics of swelling capacity of collagen made of fish skin are presented in **Figure 1** (curve 1). The swelling capacity, i.e., the amount of water, absorbed by the unit of mass at hydration, is the characteristic that most adequately reflects the water-absorbing properties of the absorbing layer material of personal hygiene means.

In **Figure 1**, it is clear that the established value of swelling capacity of fish skin is approximately 3 g of moisture per 1 g of solid. After fish skin processing and receiving the material, its swelling capacity and, respectively, the moisture capacity increase approximately by 10 times (**Figure 1**, curve 2). At the same time, the amount of moisture bound with the sample is equal to 30 g per 1 g of solid. According to the data [2], the required value of this indicator for the superadsorbing polymer used in personal hygiene means is equal to 10 g of water per 1 g of solid. The processing we offered here allows to receive the material comparable or surpassing in moisture capacity of the polymers that are traditionally used in the absorbing layers of personal hygiene. Producing the collagenic materials from skin of fresh-water fish by modification is expedient because it allows to increase a material's moisture capacity.

The results of histologic analysis of experimental animals in the wound healing research are the following:

One day after the initiation of treatment: In all groups, the skin around the wounds was edematous and hyperemic; the palpation in the projection of a wound caused anxiety in an animal. The serous and hemorrhagic discharge was noted. In the groups where the collagenic sponges and collagenic substance from fish raw materials were used (the second control group and the main group), less expressed inflammatory reaction was noted visually (i.e., the reduction of hypostasis and hyperemia).

In the first control group, the histologic picture of a traumatic inflammation came to light: an injury of epidermis, hemorrhage, necrotic masses, or infiltration of a significant amount of neutrophilic leukocytes. Muscle fibers were displaced owing to intermuscular hypostasis. Connective tissue was inflamed. Hypostasis of tissue increased in paravual tissue and was followed by a compression of capillaries and venules, interfering with the blood outflow. The increased permeability of the vascular wall with an exit in tissue of forming elements and proteinaceous components of blood was observed. The most intensive basophyly in the RNA identification was observed within the basal and spike layers that indirectly indicate the most active metabolic processes at this level. The product of reaction (RNA) was sedimented in the cells of epidermis in the form of a disperse basophyly in cytoplasm, and the cells with larger granules were found.

In the second control group, the defect of epidermis was infiltrated densely with polymorphonuclear leukocytes, lymphocytes, plasmocytes, and macrophages. The expressed plethora and swelling of collagenic fibers were observed. Muscle fibers were moved apart owing to hypostasis, and some of them had signs of expressed dystrophy and phenomena of myolysis. In intermuscular spaces, the inflammatory infiltration was observed, expressed mainly with polymorphonuclear leukocytes.

In the main group in the area of the wound inflammatory reaction, moderate necrosis and hypostasis of soft tissue with leucocyte infiltration were observed.

Three days after the initiation of treatment: The behavior of experimental animals practically did not differ from the behavior of healthy species.

In the first control group, moderate inflammatory and edematous reactions of surrounding tissue were observed, which were less expressed in comparison with the first days. Inflammatory infiltrate contained cellular components: leukocytes

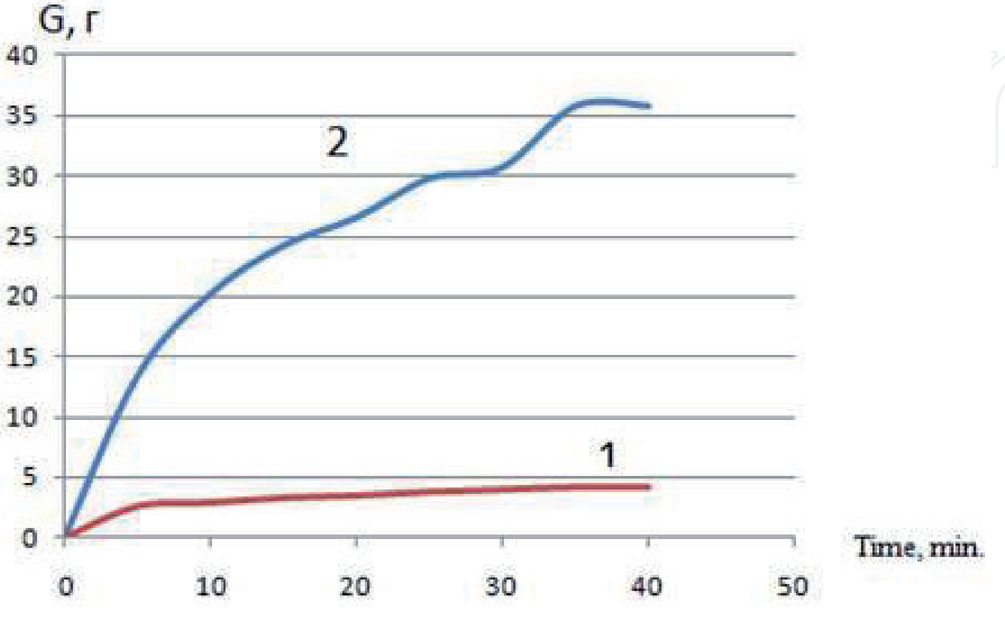

Figure 1.
Kinetics of swelling capacity G of fish skin before processing in organic acids (curve 1) and after processing (curve 2).

with decaying kernels and isolated tissue basophiles, macrophages, and lymphocytes; muscle fibers in the periphery of infiltrate had extended necrosis sites; single fibroblasts were visible; and in the wound, bottom isolated centers of granulated tissue were observed. Loss of fibrin was noted, which was connected with the walls of the wound defect. Insignificant amount of isolated collagenic fibers was observed in the mesh dermic layer. The intensity of epithelial cell coloring increased, especially in the deep layers of epidermis where uniform adjournment of basophile material or its localization in a perinuclear zone was observed.

In the second control group, moderate inflammatory and edematous reaction in a paravualny zone was observed, and granulation tissue and vascular and capillary network was formed on the surface, containing the visible organized (regular) fibrin, fibroblasts, histiocytes, and endoteliocytes, small congestions of polymorphic-nuclear leukocytes.

In the main group, the defect of epidermis was reduced, and edges of the wound were edematous. Restoration of skin integrity was observed, which was seen in the thickening of regional epidermis as a result of activation of reparative regeneration. The divergence of muscle fibers was noted due to the inflammatory infiltration filling the intermuscular spaces, and young granulations were formed in the periphery of the wound, which was the proliferative reaction of epidermis and skin elements. Collagenic fibers contained numerous infiltrated macrophages, fibroblasts, and eosinophilic leukocytes.

Seven days after the initiation of treatment: In the first control group, inflammatory reaction became less expressed, and infiltration with inflammation cells remained. The surface of the wound was filled with granulation tissue, and microabscesses of various localizations came to light. In the hem formation area, the increased quantity of fibroblasts, collagenic fibers wavy with the primary horizontal direction, and small capillaries in the formation phase were observed.

In the second control group, minimum signs of inflammatory reaction were observed, and in some wounds, there were leukocytes still, together with the expressed collagenic and angiogenesis. The surface of the wound was covered with epidermis with underlying granulation tissue enriched with completely filled blood vessels and capillaries, with a large number of eosinophils, fibroblasts, histiocytes and tissue basophiles, and a significant amount of collagenic fibers. On the periphery, there were the corpulent cells. In the depth of the wound, in intermuscular spaces, there was the formation of small capillaries.

In the main group, the restoration of tissue in a zone of wound defect was observed, and in the dermic layer, there were the well-formed collagenic fibers and granulation tissue.

Eleven days after the initiation of treatment: By the eleventh day, a hem had been formed completely in all groups, and the newly formed granulation tissue was clinically observed, which completely covered the wound.

In the second control group, the new epithelized hem was formed.

In the main group, the wound closed completely, and in the dermic layer, new collagenic fibers had been formed.

The wound area of all animals decreased in the main group, and in the second control groups, it was quicker, compared to that in the first control group (see **Table 2**).

Proceeding from clinical signs (**Table 3**), it is possible to conclude that after the initiation of treatment by the second day in the second control group and in the main group, and by the third day in the first control group, complete cut off of inflammation signs was noted with healing of wounds under a scab strip. It should be noted that the signs of inflammation or the first phase of wound process was stopped in the main group on average for 34–65% earlier compared to the first control group.

Animal groups	Initial wound size (area)	Time elapsed after wounds modeling, days			
		1	3	7	11
1 control group	26,1±0,5	18,5±0,5[1]	9,2±0,4[1]	4,5±0,4[1]	Well-formed hem
2 control group	26,0±0,5	12,8±0,6[1,2]	5,7±0,3[1,2]	1,5±0,3[1,2]	
the main group	26,1±0,5	12,6±0,6[1,2]	5,8±0,3[1,2]	1,5±0,3[1,2]	

[1]*Reliability of the distinctions in comparison with basic data;*
[2]*Reliability of distinctions in comparison with data of the 1st control group.*

Table 2.
Wound area change dynamics, for animals, mm^2.

Clinical signs	Groups		
	1 control	2 control	the main
Cut off of skin hyperaemia	1,8±0,5	1,3±0,5	1,3±0,4
Cut off of tissue hypostasis	2,2±0,3	1,7±0,4	1,6±0,3 *
Reduction of the quantity of separation to minimum	2,7±0,4	1,7±0,4 *	1,7±0,4 *

**Reliability of distinctions in comparison with 1-control group r < 0.05.*

Table 3.
Clinical signs of a course of wound process, days.

Groups	Content of ribonucleic acid (RNA)				Maintenance of SH-group			
Time, days	1	3	7	11	1	3	7	11
1 control	0,25	0,25	0,31	0,30	0,25	0,26	0,30	0,29
2 control		0,27	0,30	0,30		0,27	0,29	0,28
The main	0,28	0,29		0,31	0,31	0,29		0,30

Table 4.
Histochemical indicators of wound process.

Histochemical indicators of wound process are shown in **Table 4**, which shows that in all stages of its healing in the main group, the RNA level and SH-group are higher than in the first and second control groups. It prompts the actively going metabolic processes and approximately high rates of maturing and differentiation of epidermis.

When comparing the morphology of wounds, more expressed positive dynamics of healing of wounds in the second control and main groups is noted, revealed by the earlier decrease in tissue puffiness, organization of fibrin, and formation of collagen. It is established that the collagenic material imposed on a wound surface promoted local hemostatic action.

The analysis of toxicity of active ingredients showed that the acute state is not revealed in the experiment. Deviations of frequency of respiratory movements and warm reductions were within the norm regardless of the group of animals. Body temperature varied within ±0.7–0.8°C regardless also of the group of animals. The neurologic and behavioral status did not change. The total "Open field" test was 52.4 s, and the gravity 'sagging' test was 11.2 s. The qualitative analysis of immunoglobulin E showed the lack of sensibilization effect on the seventh day of the experiment.

Thus, during the course of the experimental study of the wound healing process, no distinctions were found between the application of a standard collagenic sponge and collagenic substance from fish raw material. In comparison with the control group of the animals who did not receive additional treatment the data confirming the acceleration of reparative processes in soft tissue under the influence of a standard collagenic sponge and collagenic substance from the fish raw materials which are especially expressed in the first phase of wound healing process that was shown in the stimulation of a collagenic genesis, activation of the metabolic processes which are followed by the increased level of reactions of RNA and SH-group identification. The conducted pilot studies did not reveal toxic and allergic effects of collagenic substance made of fish raw material both in the analysis of clinical data and when studying the results of histologic and histochemical analysis.

The results of the research of sanitary and hygienic safety of collagenic substance made of fish collagen are shown in **Table 5**, which goes to show that deviations from safety standards were not revealed.

In the allergenicity tests during the entire period of the experiment, there were no changes in the clinical status of animals and no changes in the skin condition over the area of collagenic substance applications. Based on the received results, the response on allergenicity was estimated negatively.

The main mechanism of influence of collagen is the implementation of reparative function of connecting tissue. Introduction of collagen-based composition of medicines promotes the extension of the medicines' effect due to high osmotic activity providing the dehydrating action on a tissue in the inflammation. Such medicines can be used as an effective wound covering [7]. The napkins based on collagen impregnated with antibiotics have good compatibility with skin tissue, thanks to low allergenicity and biodestruction of collagen. For example, the best results of surgical treatment were received for the approbation in Maykop, the Republic of Adygea, at the Adygeian Republican Clinical Hospital. During the tests, 10 patients with different diagnoses were observed, i.e., acute paraproctitis, acute osteomyelitis, postoperative hems, burns, and trophic ulcers. When imposing

No.	The defined indicators	Analysis results	Size of admissible level	Units of measurements
1	Arsenic	less than 0,025	below 5,0	mg/kg
2	Mercury	less than 0,03	below 1,0	mg/kg
3	Lead	less than 0,25	below 5,0	mg/kg
4	Hydrogen indicator	4,6	2,5-8,5	-
5	Skin irritant action	0 (not found)	0 (not found)	points
6	Impact on mucous membranes (once)	0 (not found)	0 (not found)	points
7	The all-toxic action determined by the alternative in vitro methods	absent	absent	-
8	Total of mesophilic aerobic and facultative and aerobic microorganisms	85	Less than 10^{-3}	
9	Candida albicans	no	not allowed	-
10	Escherichia coli	no	not allowed	-
11	Staphilococcus aureus	no	not allowed	-
12	Pseudomonas aeruginosa	no	not allowed	-

Table 5.
Results of tests of the modified collagen for sanitary and hygienic safety.

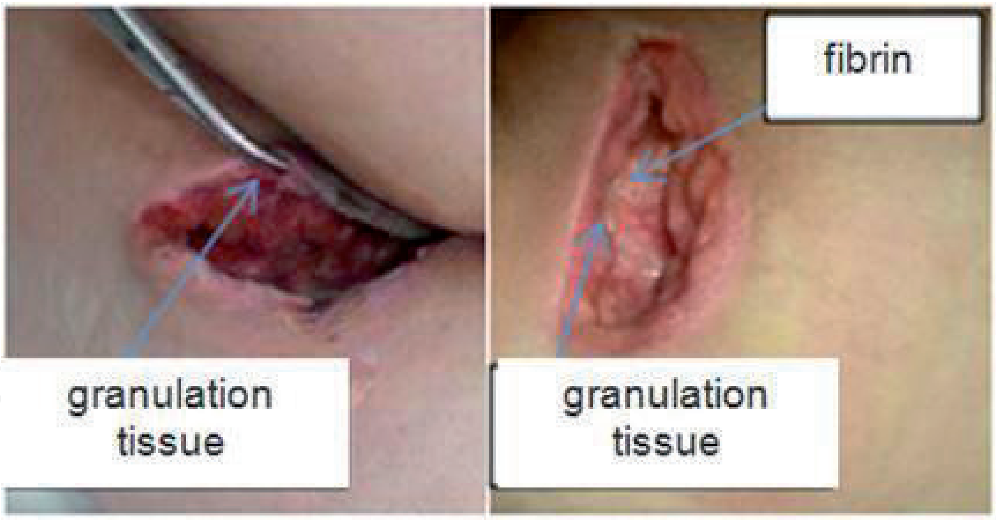

Figure 2.
Dynamics of treatment with application of collagen-based napkins (3 days): (a) result before use of collagen; (b) result after use.

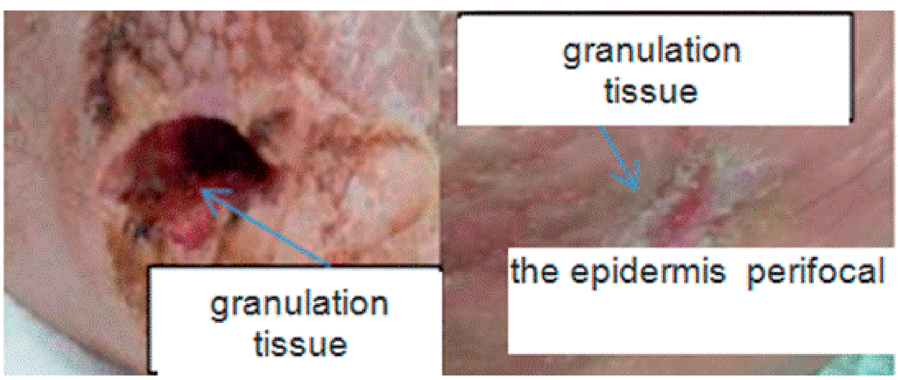

Figure 3.
Dynamics of treatment with collagen-based wipes (3 days): (a) the result before the collagen application; (b) the result after the application of collagen.

a collagen-based napkin as a bandage to patients with a sharp paraproctitis, the satisfactory condition of the patient, without therapeutic side effects, the lack of necrosis, and the formation of granulation tissue, with positive wound healing results (**Figure 2**), were observed.

Collagen-based napkins were applied on the acute osteomyelitis wounds, which arose after foot amputation (on the lower extremity, see **Figure 3**). The results are positive, viz., the patient's satisfactory dynamics without therapeutic side effects and stable formation of granulation tissue were observed.

Collagen film covering was applied for the treatment of postoperative hems. The results are positive, viz., the patient's satisfactory dynamics without therapeutic side effects and stable formation of granulation tissue and wound healing were observed on the fifth day. Film application is shown in **Figure 4**.

The offered covering allows the wounds, burns, and ulcers to be treated well and quickly, and it can be used for treatment of the bleeding traumatic damages, flat granulating slow wounds to a stage of regeneration, trophic ulcers, decubituses, and when healing donor sources. The of wound healing films demonstrate the stabilizing properties that remain reliable for a rather long time. Results of healing on the sixth day with the use of the collagen-based materials of fish origin are shown in **Table 6**.

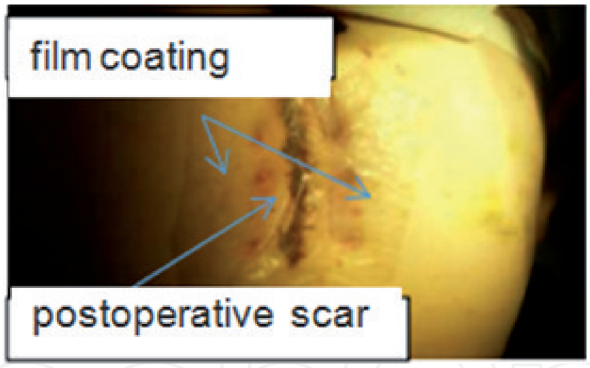

Figure 4.
Application of a collagen film.

Patient	Wound area (cm)	Wound size 1st day	Wound size 2nd day	Wound size 3rd day	Wound size 4th day		Wound size 5th day	Wound size 6th day
№1 Chronic osteomielitis)	5×3×3	5×3×3	4,5×2,5×3	4,5×2,5×2,5	4×2×2,5		4×2×2	2,5×1×1
№2 Acute paraproctitis)	9×5×5	9×5×5	8,5×4,5×4,5	8,5×4×4	8×4×4		7,5×3×3	6,5×2,5×2,5
№3 Acute paraproctitis)	7×4×4	7×3,5×4	7×3×3,5	6,5×3×3,5	6,5×3×3		6×3×3	6×2,5×2,5
№4 ECH without abscellation	13	Hem reddened	Hem reddened		Restoration, reddened			Complete restoration

Table 6.
Results of healing for the 6th day.

5. Discussion

Processing of biopolymers in organic acids is quite often applied for the purpose of modification of biomacromolecules and adding to a material the necessary physical and chemical properties [23–25]. There are also mentions of processing of fish collagen organic acids for simplification of its dispergating [26, 27]. It is possible due to the processes of destruction of fibers of collagen [28] beginning when the processing in acids starts, apparently, it is connected with a rupture of communications between polypeptide chains of collagen. As a result of change of structure, the extensive capillary network where moisture is caught increases the general moisture capacity of the studied material.

Identical wound healing action of a standard collagenic sponge based on modified fish collagen can be explained as follows:

The molecule of collagen of mammals consists of three polypeptide α-chains, mutually coiled in a structure of the threefold right twirled superspiral like a three-vein rope [11] that gives this structure high durability and big molecular weight. These chains are connected among themselves by cross links. Distinctive features of fish collagen are the 'single-coil' structure and the parallel arrangement of polypeptide chains, in which molecular mass is much lower than of collagen of mammals and it is preserved at the level of a tropocollagen [19]. As a result, fish collagen is not strong enough, and the collagen of mammals is possibly not so strongly connected with other elements of tissue. Therefore, fish collagen does not require preliminary hydrolysis, and when processed in weak alkaline and acid solutions, it is allocated easily from fish skins. Then, when in contact with the wound surface, the polypeptide chains of fish collagen are built into a structure of a regenerate, similar to the fragments of a molecule of animal collagen after its long hydrolysis in the rigid modes.

From the submitted data on a sanitary and epidemiologic condition of examinees of samples it follows that the studied collagenic substance, contains ions of heavy metals in concentration tens times less than the admissible level, it does not render the skin irritating and all-toxic action, and an effect on mucous membranes, and moreover it contains much smaller amounts of mesophilic aerobic and facultative and aerobic microorganisms below the admissible threshold. In the studied samples, there were no pathogenic microflora at all. Thus, the obtained collagenic materials of fish origin are biologically safe, they have an expressed effect on wound healing, and the technology of their preparation differs favorably from the process of production of standard materials.

Except the excellent moisture-absorbing and wound-healing properties, the modified fish collagen has the essential occluding ability. Thus, in [29, 30], the high sorption capacity of fish collagenic substances have been proven due to the presence of various functional groups, hydrophilic and hydrophobic sites, in a molecule structure. This fact allows to assess positively the prospects of collagenic substances' use as a part of personal hygiene means with a deodorizing effect.

6. Conclusion

The results of research of physical and chemical characteristics of collagenic materials received in the real work from fresh-water fish with the modified structure testify to prospects of their use as the absorbing layer of personal hygiene means, in view of the high moisture-absorbing ability which is 10 times higher than a moisture capacity of the raw collagen and is higher than the moisture capacity of the super-adsorbing polymers demanded for use in personal hygiene means. The material thus obtained conforms to the requirements of sanitary and epidemiologic safety, allergenic action of skin as it is possible to conclude by results of tests on animals, it does not render. This material accelerates the reparative processes as much as the collagen used in standard hemostatic sponges, having the simplicity of technology of its production, low cost, and availability of raw materials. These results altogether give the grounds for perspective considerable development of domestic production of fish collagenic materials including their use as a part of personal hygiene means and surgery.

New environmentally friendly sources for production of collagen—an urgent scientific and technical problem of the present as it is popular and proved **conclusions** in biomedical technologies, structure of specialized food for rehabilitation and during the post-operational and post-traumatic periods. It has an extraordinary demand in cosmetology for the production of cosmetics of various functionalities: as a part of shampoos, creams, etc.; for restoration and improvement of structure of hair; increase in their volume; and rejuvenation of skin. The research of properties and the characteristic of unique biopolymers of the collagenic nature shall give way to operate respective technologies in the production of food, cosmetics, and medical products and medicines; to extend this segment and to stabilize the situation of Russia in the international market; and to expand the range and scopes of products of cutting of fish. Thus, it is possible to claim that production of collagen from collateral fish raw materials answers the principles of rational environmental management and, at last, it is economic.

Its application is competitive and perspective both in its original form and for the production of foodstuff, medical supplies, and cosmetics in the internal and world markets.

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