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STUDY OF COLLAGEN STRUCTURE AND TISSUE REACTION DURING IMPLANTATION

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Abstract

This paper discusses the method of obtaining and studying collagen isolated from cattle skin using alkaline-salt hydrolysis. The physicochemical properties of collagen, its structure and biocompatibility were studied. The use of IR spectroscopy and SEM made it possible to confirm the preservation of the fibrillar structure of collagen after processing, which is important for its use in medicine. Morphological histological studies on male Wistar rats showed that collagen stimulates metabolic processes and promotes an increase in the number of fibroblasts and collagen fibers, indicating its active participation in tissue restoration. The results showed that collagen has high biocompatibility and is promising for use in medicine.

Keywords: *collagen, fibrils, IR spectroscopy, SEM, morphohistology, biocompatibility*

Introduction

The development of chemical science made it possible to develop a method for isolating a biopolymer, collagen, from collagen-containing tissues, which retains its basic biological properties. The absence of toxic and carcinogenic properties in collagen attracted the attention of specialists in various fields and stimulated the study of this natural material for its introduction into wide medical practice (Ivanova L. A., 1990; Radjabov O. I., Gulyamov T., Turaev A. S., 2011; Chaikovskaya E. A., Istranov A. P., 1990).

The role of collagen in people's lives has not diminished even today. And perhaps it is the continuous emergence of new synthetic polymers that will allow us to once again highly appreciate the properties of this natural polymer and admire it. Practically without giving up its positions in traditional areas of application – leather, clothing, food industry, collagen begins to penetrate into other areas of industrial production; its role as a polymer material in medicine in general, and in surgery in particular, is very great (Radjabov O. I., Turaev A. S., Gulmanov I. D., Otajanov A. Yu., Azimova L. B., 2022).

Collagen types are widely expressed, but have tissue-specific roles, many have specific functions. Collagen type I is mainly found in

the skin, tendons, blood vessels and cornea. It is highly expressive and its dermal microfibrillar network influences the rigidity and compactness of the substructures beneath the skin surface, which underlies the extensible biomechanical function of human skin. Therefore, its loss or damage can significantly reduce the strength of the skin and lead to failure of various skin functions (Shahrajabian M. H., Sun W., 2024).

Type I collagen is the most common and important. It is a raw material for a number of technical industrial sectors. Pharmaceutical felts occupy an important place and foams, surgical suture material, collagen membranes, filters, etc. (Radjabov, O.I., Otajonov, A.Y., Baratov, K.R., Azimova, L.B. 2023; Muydinov, N.T., Radjabov, O.I., Gulyamov, T., Turaev, A.S., Atajonov, A.Yu., Barotov, K.R. 2023; Radjabov, O.I., Turaev, A.S., Gulmanov, I.D., Otajanov, A.Yu., Azimova, L.B., 2022).

The purpose of the research work is to determine whether the natural structure of collagen extracted from cattle skin by alkaline-salt hydrolysis is preserved and its biological activity.

Materials and Methods

Objects of study: lyophilic collagen isolated from cattle skin by alkaline-salt hydrolysis.

IR spectroscopy. IR spectra of polysaccharides were recorded on a Perkin Elmer 2000 IR Fourier spectrometer in the frequency range of 400–4000 cm^{-1} in a tablet with KBr. To record the spectra, 10 mg of the studied samples were ground in a ball mill with 100 mg of potassium bromide for 1 min, then about 100 mg of KBr were added to the mixture and the mixture was ground again in the mill, after which the remaining potassium bromide was added (300 mg in total), ground for another ~30 sec and the tablets were pressed.

Molecular structure determination. The molecular structure of collagen was examined by scanning electron microscopy (SEM). For this purpose, the sample was coated with carbon in a Q 150 RES (QUORUM. USA) device at a vacuum voltage of 15 kV, and its morphological structures were studied in an EVOMA 10 (Zeis, Germany).

Implantation of biomaterial. The experiments were carried out on male Wistar rats weighing 150–200 g. Collagen was implanted under the skin in the area of the thigh and back of the rats in the operating unit of the Department of Anatomy and Clinical Anatomy of the Tashkent Medical Academy.

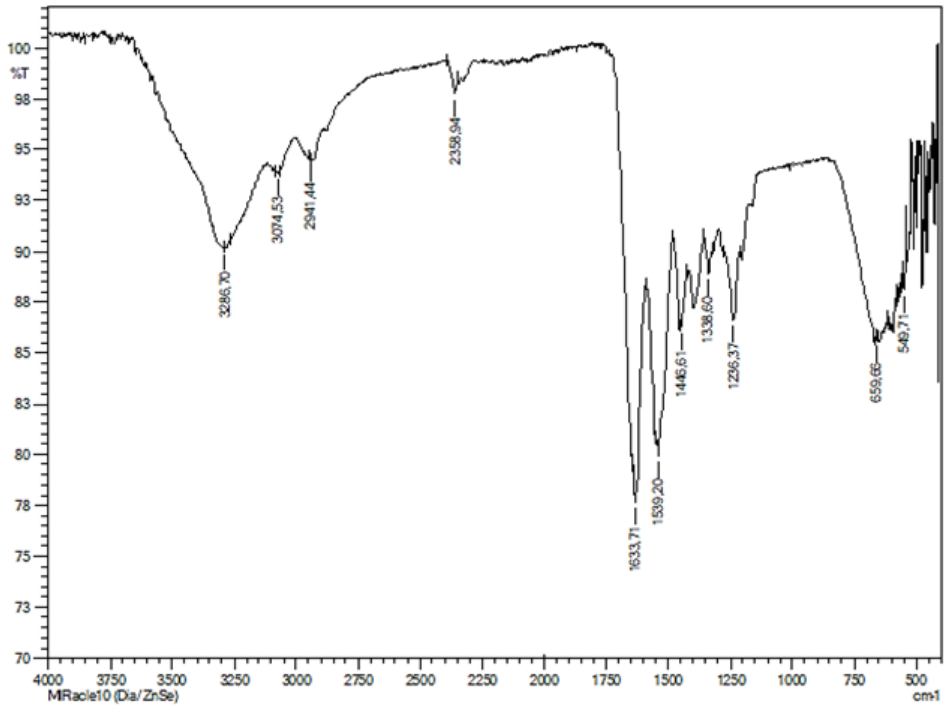
Morphohistological studies. Pieces of skin in the area of the implanted biomaterial were taken on the 5^{th}, 10^{th} and 15^{th} days after the introduction of the biomaterial. For this purpose, a marked area of skin measuring 0.5 × 0.5 cm was excised under ether anesthesia, after which the wound was sutured with interrupted sutures. The material was fixed in 10% neutral formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin, picrofuchsin according to van Gieson.

Results and discussions

Infrared spectroscopy is one of the most informative methods for structural analysis of biopolymers such as collagen. The spectrum obtained from a lyophilized collagen sample shows a number of characteristic absorption lines indicating the presence of typical functional groups corresponding to its amino acid composition and polypeptide structure. The IR spectrum of lyophilized collagen is shown in Figure 1.

As can be seen from Figure 1, the most intense and wide absorption band is observed in the region of 3286 cm^{-1}, which indicates the presence of extended systems of hydrogen bonds caused by stretching vibrations of O-H and N-H groups. This region corresponds to vibrations of amino groups ($–NH_2$) and hydroxyl groups (–OH) located in the side chains in the collagen structure. The width and intensity of this band is due to the overlap of signals from intermolecular and intramolecular hydrogen bonds, which play a key role in the formation of a stable three-helix structure of the collagen molecule. In the region of 3000–2800 cm^{-1}, peaks are recorded at 3074 and 2941 cm^{-1}, corresponding to stretching vibrations of C-H bonds in CH_2^- and CH_3^- groups. These signals are associated with aliphatic hydrocarbon chains of amino acid residues such as proline and hydroxyproline, amino acids that play a key role in stabilizing the collagen helix.

Figure 1. *IR spectrum of collagen*



The most significant bands indicating the protein nature of the sample are found in the following ranges:

–1633 cm^{-1}: an intense Amide I band arising from the stretching vibrations of the carbonyl group (C = O) in the peptide bond. This band is especially sensitive to the type of secondary structure of the protein: for collagen it is characteristic of an organized triple helix;

–1539 cm^{-1}: the Amide II band caused by bending vibrations of the N-H and stretching of the C-N bond. Together, the Amide I and II bands serve as a reliable indicator of the preservation of the native collagen structure and the absence of deep denaturation;

–1338 cm^{-1}: the band corresponds to Amide III vibrations arising as a result of combined movements of the C-N and N-H bonds. This region provides an idea of the configuration of amino acid side chains and the spatial organization of the protein. Peaks in the low-frequency region, in particular 659 and 549 cm^{-1}, correspond to deformation vibrations of the molecule skeleton and reflect the stability of the spatial configuration of collagen. The absence of significant shifts and blurring of the amide bands indicates the preservation of the triple helical structure of the protein, which is especially important when assessing the functional suitability of collagen as a biomaterial.

Also in our research work, SEM images of lyophilized collagen were obtained to determine whether the fibrillar structure of collagen was preserved after hydrolysis.

Figure 2. *SEM of collagen*

The presented SEM image demonstrates the microstructure of fibrillar collagen isolated by hydrolysis of cattle hide. The figure clearly shows the fibrous structure of collagen, where the fibrils have a characteristic helical organization. Such a structure is typical of native collagen, which confirms the preservation of its natural characteristics after hydrolysis. The surface of the collagen fibers has microroughness, possible ruptures and small fragments, which may indicate partial degradation of the fibrillar network due to the hydrolytic process. However, in general, the fibers retain their integrity, indicating a gentle hydrolysis mode. According to the scale (20 μm) of the figure, the fibril diameter varies in the range of 10–20 μm, which corresponds to natural fibrillar collagen. This parameter plays a key role in medical applications, since the fibril size affects the mechanical properties of the material, its biodegradation and cellular adhesion.

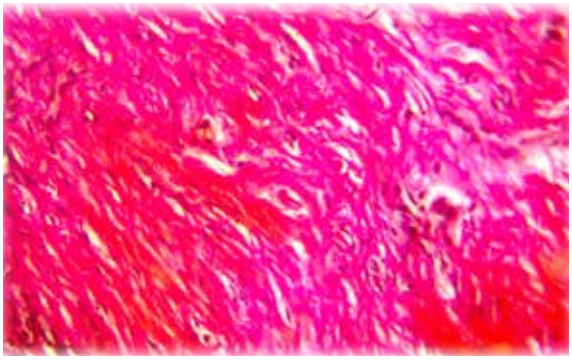
In the course of our research, experiments were conducted to determine the biological activity of collagen with a natural fibrillar structure isolated from cattle skin.

According to the results of the experiment, the general condition of the experimental animals is satisfactory. No pronounced allergic reactions were observed during the entire study period, either from the skin or from other organs and systems of the body. On the 5th day of the experiment, in the area of collagen injection, the epidermis is multilayered, flat, keratinized, consists of two to three layers of cells, with loose connective tissue under the epidermis. The dermis and hypodermis are represented by formed connective tissue, contain a large number of collagen fibers with pronounced edema and single cells of connective tissue. The preparation shows an area where the remains of the injected collagen have been preserved. Focal collagen fibers are tightly adjacent to each other in the form of parallel layers. Next to this zone, the dermis consists of young formed connective tissue. There are especially many cells under the epidermis. The closer to the intact zone in the dermis and hypodermis, a moderately pronounced stimulation of collagen fibers is noted. A large number of connective tissue fibers, small vessels with an expanded lumen are noted.

After 10 days, a macrophage reaction is observed in the hypodermis. The hypodermis contains many collagen fibers, loosely located with intense staining, as well as fibroblasts. The vessels are dilated and filled with blood. Next to this zone, young developing connective tissue with the presence of connective tissue cells is visible in the dermis and hypodermis.

On the 15 th day, the number of fibroblasts and collagen fibers increases in the collagen injection zone, which form a network between the mature collagen fibers of the dermis itself. In the hypodermis, there is formed connective tissue with single connective tissue cells, that is, there is development of collagen fibers and a large number of connective tissue cells (Fig. 3).

Figure 3. *Collagen implantation zone, day 15*



Conclusion

The conducted analysis of physical and chemical characteristics of powdered biomaterial based on collagen and polysaccharide demonstrates its high technological effectiveness and biocompatibility. Optimal indicators of humidity, pH and particle size ensure stability of the material, and a high degree of

grinding indicate its prospects for medical applications. According to SEM analysis, it was established that the obtained collagen from cattle skin retains its fibrillar structure. According to morphohistological studies, it was proven that collagen is included in metabolic reactions, stimulates metabolic processes, starting from the first day. Subsequently, on the 5th, 10th and 15th day, metabolic processes are enhanced, which is manifested by an increase in the number of fibroblasts and collagen fibers, which is confirmed by bright coloring according to van Gieson.

References

Ivanova L. A. Collagen and prospects for its use in the technology of dosage forms // Pharmacy. 1990. Vol. 1. – P. 81–82.

Radjabov O. I., Gulyamov T., Turaev A. S. Collagen medical, obtaining and research // Uzbek chemical journal. Special issue. – 2011. – P. 94–97.

Chaikovskaya E. A., Istranov A. P. Collagen in the technology of dosage forms and products for ophthalmology // Pharmacy. 1990. Vol. 4. – P. 82–83.

Radjabov O. I., Turaev A. S., Gulmanov I. D., Otajanov A. Yu., Azimova L. B. Obtaining Collagen and Morphological Studies of Injection Solution on Its Basis // International Journal of Materials and Chemistry. – 12(3). 2022. – P. 39–43. DOI: 10.5923/j.ijmc.20221203.01.

Shahrajabian M. H., Sun W. Mechanism of action of collagen and epidermal growth factor: A review on theory and research methods // Mini Reviews in Medicinal Chemistry. 2024. – Vol. 24(4). – P. 453–477.

Radjabov, O. I., Otajonov, A. Y., Baratov, K. R., Azimova, L. B. Local hemostatic biomaterial based on native collagen // E3S Web Conference., 2023. – 420 p. URL: https://doi.org/10.1051/e3sconf/202342009004.

Muydinov, N. T., Radjabov, O. I., Gulyamov, T., Turaev, A. S., Atajonov, A. Yu., Barotov, K. R. Developing composition and studying physico-chemical and antiadhesive properties of biopolymer films based on collagen and Na-CMC // Khimiya Rastitel'nogo Syr'ya. 2023. – Vol. 4. – P. 81–88.

Radjabov, O. I., Turaev, A. S., Gulmanov, I. D., Otajanov, A. Yu., Azimova, L. B. Obtaining Collagen and Morphological Studies of Injection Solution on Its Basis. International Journal of Materials and Chemistry. 2022. – Vol. 12(3). – P. 39–43.

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