Comparative analysis of collagen from different sources for wound and burn management

Yuliya Kulikova*, Stanislav Sukhikh, Anastasiia Zhikhreva, Svetlana Noskova, and Olga Babich

ABSTRACT

Burns are serious injuries commonly treated using dressings and tissue-engineered biological or synthetic skin substitutes. Collagen is a promising biomaterial for tissue engineering due to its biochemical composition and structure. This study comparatively examined the properties of collagen derived from the jellyfish *Aurelia aurita* and *Rhopilema hispidum* under varying temperature conditions, alongside collagen from other animal sources. The potential application of jellyfish-derived collagen in burn and wound treatment was assessed based on these analyses. The molecular weight of jellyfish collagen ranged from 105 kDa to 240 kDa. The isoelectric point was 5.19 for *R. hispidum* and 4.90 for *A. aurita*. Compared to animal and avian collagen, jellyfish collagen exhibited a lower denaturation temperature. It was inferior in hydrophilicity, hydration degree, mechanical strength, and solubility, indicating a need for additional modification before use in tissue engineering. Microscopic analysis revealed a highly porous structure in both jellyfish species. Pore sizes for *A. aurita* ranged from 57.1 μm to 256.7 μm with wall thicknesses of 58.2 – 241.7 μm; for *R. hispidum*, pore sizes ranged from 57.1 μm to 337.6 μm and wall thicknesses from 48.7 μm to 163.6 μm. *In vitro* studies using human umbilical vein endothelial cells demonstrated enhanced migration on the 1st day in the presence of jellyfish collagen, indicating a lack of cytotoxicity. *In vivo* mouse model experiments showed rapid collagen assimilation when sutured subcutaneously. Minor inflammation observed in *R. hispidum*-based sponges was likely due to inadequate sterilization. These findings indicate that *A. aurita* and *R. hispidum* are viable marine sources of collagen and hold significant promise for future applications in regenerative medicine and wound healing.

Keywords:
Jellyfish; regenerative medicine; collagen; elastic modulus; oxygen permeability; membrane

***Corresponding author:**
Yuliya Kulikova,
IVKulikova@kantiana.ru

How to cite this article:
Kulikova Y, Sukhikh S, Zhikhreva A, Noskova S, Babich O. Comparative analysis of collagen from different sources for wound and burn management. *Biomater Transl.* **2025**

doi: 10.12336/bmt.25.00013

1. Introduction

Burns and wounds are becoming more common due to changing lifestyles and the faster pace of life.[1] Burns are serious injuries that often require intensive care, prolonged hospitalization, and rehabilitation. According to the World Health Organization, burns cause about 180,000 deaths each year.[2] Extensive tissue damage from burns can lead to severe scarring, pain, weakened immunity, infections, hyperglycemia, muscle atrophy, and severe physical and mental impairments.[3,4] Given the physiological, psychological, and social consequences of burn injuries, it is important to accelerate recovery and wound healing. Present treatments include dressings, systemic and topical medications, wound excision and skin grafting, biological and synthetic skin substitutes, dermal tissue engineering, and surgical techniques.[3] Although recent advances have significantly improved burn care outcomes, challenges remain regarding effectiveness, and many treatment strategies are relatively expensive and inaccessible in some countries.

Collagen is considered a promising biomaterial in bioengineering and tissue engineering due to its chemical composition and structure.[5,6] It is a major component of specialized and non-specialized connective tissues in the human body, comprising about one-fourth of total

body protein, three-fourths of the dry weight of skin, over 90% of tendons and corneal tissue, and nearly 80% of the organic content of bones.[7] As a biomaterial, collagen offers key advantages by directly stimulating cell adhesion and growth through multiple attachment sites.[8] Furthermore, it can self-organize into collagen fibrils, supporting cell survival and preserving the tissue's mechanical flexibility.[7] Collagen is widely used in pharmaceutical delivery systems, skin substitutes, and substrates for tissue engineering applications.[6] At present, to address the challenges faced in the field of biomedicine, collagen and its derivatives are predominantly sourced from the processing waste of animal and avian carcasses. However, such sources frequently carry a high allergenic potential. In addition, bovine and porcine-derived collagens pose a risk of disease transmission to humans.[5,9] and may face religious and dietary restrictions.[5] Furthermore, studies suggest that collagen from traditional sources exhibits lower water solubility compared to marine-derived collagen.[10] In light of these facts, processes for obtaining collagen from marine organisms such as fish and scallop waste, whole jellyfish carcasses, and sponges are becoming increasingly popular.[11] Collagen scaffolds derived from marine animal biomass have high resorptive properties and low allergenic potential.[12,13]

Given that the collagen content of jellyfish exceeds 60% of their dry weight,[14] they represent an attractive source of collagen for biomedical applications.[13,15] In addition to their low immunogenicity and resorptive properties, collagens from marine animal biomass significantly promote the proliferation of human keratinocytes, suggesting their potential utility in addressing challenges in regenerative medicine.[16] Marine organism-derived collagen has also been successfully used to produce dental composites and scaffolds for cardiovascular surgery, orthopedics, urology, neurology, and ophthalmology.[17]

Despite these advantages, several limitations have prevented the widespread use of marine organism-derived collagen as the main raw material for the production of medical devices in regenerative medicine and tissue engineering. In particular, Abedi *et al.*[18] highlighted that the thermal stability and mechanical properties of terrestrial animal collagens are generally superior to those of fish-derived collagens. As the biological function of collagen is primarily determined by its mechanical properties,[19] which are influenced by various factors, including habitat conditions, body temperature, and molecular composition,[18] it is essential to thoroughly study its physicochemical and mechanical characteristics.

In this study, we performed a comparative analysis of the properties of collagen extracted from the jellyfish *Aurelia aurita* and *Rhopilema hispidum*, which inhabit different temperature conditions. Our findings suggest that jellyfish collagen is a promising candidate for the development of wound and burn treatment products. In addition, the present study compared the physical, mechanical, and biological properties of jellyfish, animal, and avian collagens. These findings are important, as a thorough understanding of the basic physicochemical, mechanical, and biological properties of jellyfish collagens could support the development and standardization of collagen-based formulations for use in bioinks and products intended for tissue engineering and regenerative medicine.

2. Methods

2.1. Research specimen

Adult specimens of *A. aurita* caught in the Baltic Sea, Russia, in August 2024, and adult specimens of *R. hispidum* caught in the Black Sea in August – September 2024, were used as the samples in this study. Species identification was confirmed by a staff at the Immanuel Kant Baltic Federal University (protocol No. 16/24).

2.2. Collagen extraction from *A. aurita* and *R. hispidum*

Collagen was extracted following a previously described method.[14,20] Briefly, jellyfish biomass was chopped and placed in a flask. Extraction was performed by adding 0.5 M acetic acid at a ratio of 1:10 (wt/volume) to the jellyfish and incubating for 3 days at 4°C. The mixture was then filtered, and the insoluble residue was subjected to a second extraction. The filtrates from both extractions were combined, and 52.6 g/L of sodium chloride (NaCl) was added to precipitate the collagen. The resulting precipitate was separated from the liquid phase by centrifugation at 3,600 g for 10 – 20 min. After draining the supernatant, the precipitate was dissolved in two volumes of 0.5 M acetic acid. The solution underwent dialysis at +40°C in two stages: initially, for 72 h against 0.1 M acetic acid solution, followed by 72 h against distilled water. The collagen clot obtained was then frozen and freeze-dried.[21]

2.3. Molecular weight analysis of collagen

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed according to the Laemmli method.[22] One milliliter of dialyzed collagen was dissolved in a 0.5 M Tris-hydrochloride buffer (pH 6.8) containing 1% SDS, 10% glycerol, and 0.01% bromphenol blue, and denatured at 70 – 80°C for 5 min. Then, 15 μL of the denatured sample and 7 μL of molecular weight marker (10 – 250 kDa, Protein Dual Color Standards, BioRad, USA) were loaded into the wells at the top of a polyacrylamide gel composed of a 4% concentrating gel and a 12.5% separating gel, with a thickness of 1 mm. A voltage of 15 mA was applied for 30 min; once the proteins entered the separating layer, the voltage was increased to 30 mA for one hour to allow protein separation based on size. After electrophoresis, the gels were stained overnight in a dye solution containing 0.25% Coomassie Brilliant Blue G-250 (JT Baker, USA), 10% acetic acid, 40% ethanol, and 50% deionized water. The gels were then washed in a solution with the same composition (without Coomassie Brilliant Blue) and incubated for at least 1 h.

2.4. Isoelectric point of collagen

The isoelectric point of collagen was determined by preparing acidic and alkaline solutions of varying pH, to which different

Scientific and Educational Center of Industrial Biotechnology, Institute of Living Systems, Immanuel Kant Baltic Federal University, Kaliningrad, Russia

amounts of protein were added. Two milliliters of ethyl alcohol were then added to each tube. The samples were then mixed and incubated at room temperature for 40 min.

The zeta potential of collagen solutions was measured using a Photocor Compact-Z zeta potential analyzer (Photocor LLC, Russia). Aliquots of 2 mL were transferred into cuvettes with universal electrodes. The pH of the solutions was adjusted using 0.1 mol/L hydrochloric acid or 0.1 mol/L sodium hydroxide. Zeta potential measurements were performed in quintuplicate for each solution.

2.5. Thermogravimetric analysis of collagen using differential scanning calorimetry (DSC)

A Netzsch DSC 214 Polyma instrument (Netzsch GmbH, Germany) was used to study the denaturation and degradation temperatures of collagen. The measurements were performed at a very low heating rate of 10°C/min in a nitrogen atmosphere. Aluminum crucibles were used for the heating process.

2.6. Solubility of collagen

The solubility of collagen was evaluated using the method described by Montero *et al.*[23] Solubility was tested in a range of commonly used solvents, including alcohols (ethanol, methanol), hexane, ethyl acetate, methylene chloride, distilled water, and an alkaline solution (pH = 12), and acidic solutions (hydrochloric acid and acetic acid). All solutions were subsequently subjected to ultrasound using an ultrasonic unit (Elamed, Russia).

2.7. Water absorption capacity of collagen

The water absorption capacity of collagen was studied using a gravimetric method.[24] A 0.025 g sample was placed in a 15 mL centrifuge tube, followed by the addition of 10 mL of distilled water. The sample was suspended by shaking at 150 rpm for 40 min at room temperature (21°C). It was then centrifuged for 5 min. The supernatant was carefully removed from the tube using a pipette, and the sample tube was weighed. The experiment was performed in triplicate. Water absorption capacity was calculated using the formula in Equation I,

$$\% = \frac{m_{wet} - m_{dry}}{m_{dry}} \quad (I)$$

Where, m_{wet} is the weight of wet collagen, and m_{dry} is the weight of dry collagen.

2.8. Degradation of collagen

Collagen degradation by collagenase was assessed using the colorimetric ninhydrin method.[25] Four tubes were each filled with 25 mg of collagen and 5 mL each of N-[tris(hydroxymethyl) methyl]-2-aminoethanesulphonic acid (TES buffer) solution (CAS 70331-82-7, Sigma Aldrich, USA), and incubated at 37°C for 15 min. The incubation process was performed without stirring. Following incubation, 0.1 mL of collagenase solution (with an activity of 12 E/mg collagen) was added to two of the four tubes, and 0.1 mL of TES buffer solution was added to the remaining two control tubes. All tubes were incubated at 37°C for 30 min. To stop the enzyme reaction, the solutions were heated to 90°C and maintained at this temperature for 20 min. The samples were then cooled to 21 – 25°C, after which 5 mL of 50% n-propanol was added to each tube. Absorbance was measured at 600 nm using a spectrophotometer. A calibration curve was generated using L-glycine solutions of varying concentrations (0.2, 0.5, 1.0, 1.5, 2.0, and 4.0 mg/mL) prepared in TES buffer.

2.9. Drying of collagen

To study the hydration degree (hydrophilicity), oxygen permeability, microstructure, and mechanical properties of collagen, the samples were air-dried in a laminar flow cabinet (BAVnp-01-"Laminar-S"-1.5 LORICA, Laminarnye Sistemy, Russia) at 25°C. The thickness of the dried collagen was 0.4 – 0.5 mm, measured using an Inforce caliper (VseInstrumenty, Russia) with a measurement error of ±20 μm.

2.10. Degree of hydration (hydrophilicity) of collagen

The degree of collagen hydration was assessed by measuring the marginal wetting angles. Measurements were performed at room temperature using a Biuged BGD 190 optical device (Biuged, Germany). Each sample was tested in five replicates.

2.11. Study of collagen microstructure

The microstructure of the collagen was examined using scanning electron microscopy (SEM) after vacuum gilding. SEM was conducted at an accelerating voltage of 5 kV, with magnifications of ×100 and ×500.

2.12. Study of oxygen permeability

Oxygen permeability was measured using a coulometric detector (also known as the equal pressure method) in accordance with the ASTM D3985 and ISO 15105-2 standards. A Labthink C201B device (China) was used for this purpose. Films with a thickness of 0.4 – 0.5 mm were prepared, fixed, and tested in a dry state. Two samples of different thicknesses were examined to evaluate the effect of sample thickness on oxygen permeability.

2.13. Assessment of mechanical properties

For mechanical testing, collagen products were prepared in two types of membranes and tested under both dry and wet conditions (after soaking in a 0.9% sodium chloride solution). The tensile strength, elongation, and Young's modulus of the collagen membranes were evaluated using a universal testing device (Instron 68SC-5) equipped with an AVE2 video extensometer and a 500 N force transducer. A tensile force was applied at a rate of 1 mm/min until sample failure. All tests were performed at room temperature. Young's modulus was calculated as the slope of the linear elastic portion of the tensile curve. Ultimate tensile strength and elongation at break were calculated using the following equations in Equations II and III:

Tensile strength (MPa) = Maximum breaking load (N)/Cross-sectional area (m^2) (II)

Elongation (%) = (Breaking length - Initial length)/Initial length × 100 (III)

2.14. Evaluation of human umbilical vein endothelial cell (HUVEC) proliferation and viability

HUVECs were seeded at 20,000 cells per well in a 24-well plate. Cells were cultured for 24 and 72 h in the presence of collagen samples to assess the cytotoxic properties of collagen. After incubation, cells were detached from the surface using a trypsin solution, which was subsequently neutralized with serum. The cell suspension was carefully pipetted and mixed with a 4% trypan blue (BD) solution in a 1:1 ratio, incubated with the dye for 1 – 3 min; during this time, the dye stains non-viable cells with damaged membranes. The total number of cells, as well as the number of live and dead cells, was counted using a Luna-II counter (Logos Biosystems, South Korea).

2.15. Evaluation of cytotoxicity using the resazurin test

The HUVEC cells were seeded at a density of 3,000 cells per well in a 96-well plate. The medium was replaced with a medium containing collagen on the next day. The cells were cultured for 24 and 72 h in the presence of collagen samples. After incubation, the collagen and nutrient media were removed, and 100 µL of a 1× resazurin solution (Abisens LLC, Russia), diluted in phosphate-buffered saline, was added to each well. The cells were incubated at 37°C for 2 h. For colorimetric analysis, optical density was measured at 570 nm and 600 nm using a microplate reader (BioTek, USA). The results are presented as fluorescence intensity/absorption dependence graphs.

2.16. Evaluation of collagen regenerative properties (scratch test)

The HUVEC cells were cultured in a culture medium composed of M199 (PanEco, Russia) supplemented with 10% fetal bovine serum (Hyclon, USA), 1% Glutamax (Gibco, USA), 1% insulin-transferrin-selenium (ITS) (Gibco), 1% penicillin-streptomycin (Gibco), 0.25 ng/mL of isoproterenol, 0.5 µg/mL of hydrocortisone, 10 ng/mL of epidermal growth factor (EGF), 2.5 ng/mL of basic fibroblast growth factor (FGF), 15 units/mL of heparin, and 1% sodium pyruvate in a Galaxy CO170R CO_2 dry-air incubator (New Brunswick, Canada) at 37°C. The medium was changed every 2 – 3 days, and cultures were passaged by detaching cells from the surface of culture vials with a mixture of trypsin solution and Versen solution (1:2) (PanEko, Russia) when confluency was reached, usually every 2 – 3 days. Cells were seeded at a ratio of 1:3. Cultures of primary HUVECs at early passages (up to passage 5) were used in the experiments.

The HUVEC cell line was seeded at 20,000 cells per well in a 24-well plate, with three replicates. After the cell culture reached 100% confluency (Day 3), vertical lines resembling dashes were drawn in the center of each well using a Pasteur pipette, disrupting the integrity of the endothelial layer to simulate a wound in this assay. The medium was then replaced with a medium containing a collagen suspension diluted at a ratio of 1:5. Medium without collagen was used as a control. The results are presented as the percentage of wound healing.

2.17. *In vivo* experiments

A total of 35 male Balb/C laboratory mice weighing 18 – 20 g were used as the animal model. All animals were divided into groups "1" and "2" (each 12 mice), a "control" group with surgery (eight mice), and a group of three healthy animals for control without surgery.

All manipulations with animals were performed under gas and local anesthesia, in accordance with the International Guidelines for Biomedical Research Involving Animals. The ethical principles of the European Convention for the Protection of Vertebrates used for Experimental and Other Specific Purposes were strictly observed. Animals were housed with free access to food and water. The research was reviewed and approved by the Bioethics Commission at the I. Koltsov Institute of Biomedical Problems of the Russian Academy of Sciences (protocol no. 82, dated April 25, 2024).

For subcutaneous administration, round fragments of 6 mm in diameter were cut from collagen sponge samples using a punch, corresponding to approximately 1 – 1.5% of the animal's body area. Fur was removed from the back in the shoulder blade area, followed by an incision using surgical scissors, and the skin was lifted to create a pocket in the subcutaneous space corresponding to the size of the prepared fragments. The sample was then placed in the pocket, and the wound was sutured using Vicryl surgical thread. Necropsy material for analysis was removed on Days 3, 7, 14, and 21 in three biological replicates. Animals were euthanized with chloroform.

Mouse skin fragments with subcutaneous connective tissue obtained from the area of sample implantation were fixed in 4% formalin for 24 h at room temperature, then washed in running water and dehydrated by successive immersion in 30%, 50%, 70%, and 100% alcohol for 30 min each. After dehydration, the tissue was cleared by successive immersion in xylene solutions mixed with alcohol in reverse order of alcohol concentration: 70%, 50%, and 30%, followed by 100% xylene. The tissue was then impregnated with paraffin, and histological blocks were prepared. Sections with a thickness of 20 microns were cut using a microtome and stained with hematoxylin and eosin. After staining, the samples were mounted using a Vitrogel embedding medium (Biovitrum, Sweden).

2.18. Statistical analysis

The data were subjected to analysis of variance using Statistica 10.0 (StatSoft Inc., 2007, USA). *Post hoc* analysis (Duncan's test) was performed to identify samples that differed significantly from each other.

3. Results

The molecular weight of collagen isolated from the jellyfish *A. aurita* and *R. hispidum*, presented in **Figure 1**, indicates that collagen isolated from jellyfish is a high molecular weight compound, about 240 kDa.

It was also determined that collagen isolated from *R. hispidum* consists of α-chains and β-chains (with molecular masses of ~135 and ~240 kDa, respectively). In contrast, collagen from *A. aurita* has only α-chains.

The pH value at which the positive and negative charges on

a protein molecule in an aqueous system are equal, resulting in a total charge of zero, is called the isoelectric point.[26] The magnitude of the isoelectric point depends on the amino acid composition of the protein. Amino acids with different charged side chains contribute to different surface charges in proteins.[27] According to the titration curve (**Figure 2**), the surface charge of collagen isolated from *R. hispidum* was zero at pH 4.90, while the isoelectric point of collagen isolated from the Baltic Sea jellyfish was 5.19.

The denaturation temperature of collagen is species-specific and is related to the content of amino acids (proline and hydroxyproline) in the molecule;[28] therefore, this index was studied here. The results presented in **Figure 3** indicate that

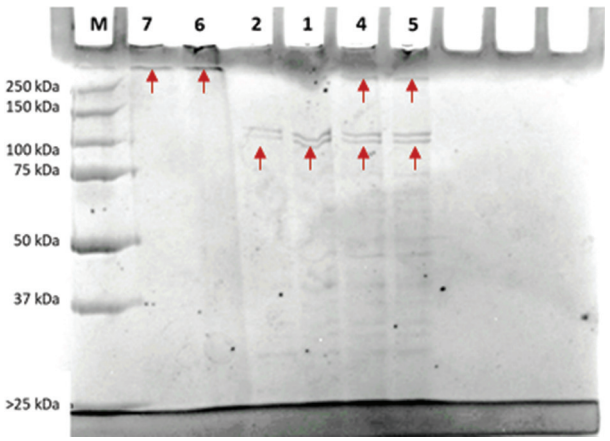

Figure 1. Electrophoregram of the collagen extracts. Notes: 1 and 2: *Aurelia aurita*, 4 and 5: *Rhopilema hispidum*, ispidum: d 2: extracts. llagen extracts.em.2017.12.083.

the denaturation temperature of collagen isolated from *A. aurita* is 32.23°C, while that of collagen isolated from *R. hispidum* is 33.15°C. It was also demonstrated that the studied collagen samples underwent degradation in the temperature range of 40.00 – 150.00°C.

Given that solubility, water absorption capacity, degradation, oxygen permeability indices,[29] and degree of hydration are important for collagen fibers (as medical agents for regenerative medicine), further studies were aimed at evaluating these indices. The solubility study revealed that the collagen samples did not completely dissolve in any of the solvents. Moreover, collagen solubility did not increase when exposed to ultrasound (duration 20 s, power 130 W, frequency 20 kHz). The results of the analysis of water absorption capacity, degradation indices, oxygen permeability, and degree of hydration of collagen isolated from *A. aurita* and *R. hispidum* are presented in **Table 1**.

Based on the table data, the mean value of the water absorption capacity of collagen from jellyfish *A. aurita* was 7.03 ± 0.57 mmol/mL, while that of collagen from *R. hispidum* was 13.25 ± 1.1 mmol/mL. The surface of dried jellyfish collagen was found to be non-hydrophilic (**Table 1**). The degree of degradation of collagen isolated from *R. hispidum* was 0.057 mmol/mL, whereas, for *A. aurita*, it was 0.015 mmol/mL.

Jellyfish collagen films did not have significant gas barrier functions. As shown in **Table 1**, oxygen permeability ranges from 26 to 42 × 10^{-14} $cm^3/(m{\cdot}s{\cdot}Pa)$, compared to polyvinylidene chloride film, which has a permeability of 0.4 – 5.8 × 10^{-14} $cm^3/(m{\cdot}s{\cdot}Pa)$, and chitosan film, with a permeability of 4.9 × 10^{-14} $cm^3/(m{\cdot}s{\cdot}Pa)$.[22,30]

An ideal three-dimensional scaffold for tissue regeneration

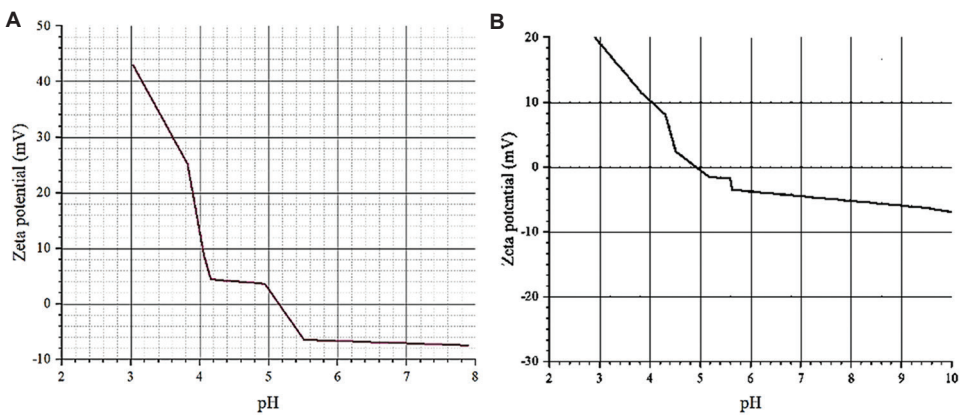

Figure 2. Zeta potential of collagen isolated from (A) *Aurelia aurita* and (B) *Rhopilema hispidum*

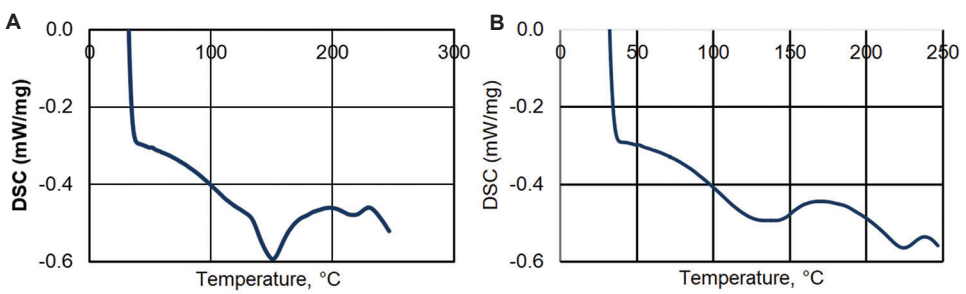

Figure 3. Differential scanning calorimetry plot for collagen isolated from (A) *Aurelia aurita* and (B) *Rhopilema hispidum*

should closely resemble native tissue in both chemical composition and physical nanostructure.[31] Therefore, it is essential that the material used to produce the fibers, as a medical device for regenerative medicine, possesses a well-defined structure. The results of the microstructure evaluation of collagen isolated from jellyfish *A. aurita* and *R. hispidum* are presented in **Figures 4** and **5**.

Table 2 and **Figure 6** present the results for tensile strength, Young's modulus, and elongation at failure for all test types. As shown, the collagen membrane sample exhibited relatively weak mechanical properties, with a tensile strength of 0.54 ± 0.09 MPa. However, it was notably highly elastic. Soaking the membrane in a physiological solution led to a decrease in tensile strength, with a particularly significant reduction in Young's modulus (**Figure 6**). A similar trend was observed when membranes made from *R. hispidum* collagen were soaked in solution.

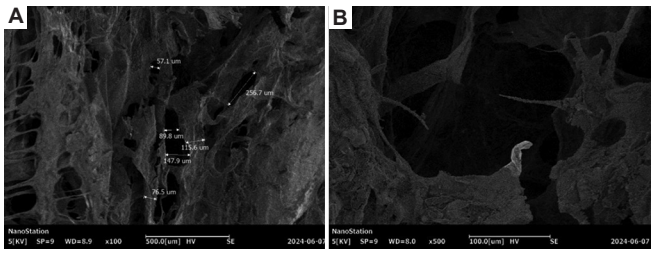

Figure 4. Scanning electron microscopy images of collagen from *Aurelia aurita* at (A) ×100 magnification (scale bar = 500 μm) and (B) ×500 magnification (scale bar = 100 μm).

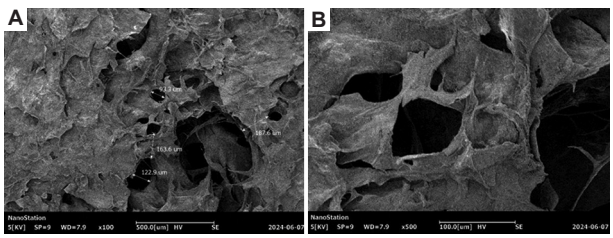

Figure 5. Scanning electron microscopy images of collagen from *Rhopilema hispidum* at (A) ×100 magnification (scale bar = 500 μm) and (B) ×500 magnification (scale bar = 100 μm).

Table 1. Parameters of water absorption capacity, degradation, and degree of hydration of collagen specimens

Parameter	Collagen	
	Aurelia aurita	*Rhopilema hispidum*
Water absorption, mmol/mL	7.03±0.57	13.25±1.1
Degradation rate, mmol/mL	0.015±0.003	0.057±0.005
Wetting angle (outer surface), gradians	58.51±1.75	39.58±1.18
Wetting angle (inner surface), gradians	50.81±1.52	40.43±1.21
Oxygen permeability×10^{-14} $sm^3/(m{\cdot}s{\cdot}Pa)$	26.0±0.4	42.0±0.9

Moreover, when comparing membranes derived from the two types of collagens, it is evident that collagen from the jellyfish *R. hispidum* exhibits better mechanical properties, which are significantly reduced in a wet state. For instance, the tensile strength of the *R. hispidum* collagen membrane in the dry state was 1.17 MPa, which is 2.2 times higher than the breaking point of the membrane made from the *A. aurita* collagen. This difference may be associated with a higher degree of collagen cross-linking, as indicated by its lower solubility and higher molecular weight.

The Young's modulus of the *A. aurita* collagen membrane in the dry state was 3.92 MPa, while that of the *R. hispidum* collagen membrane was 10.03 MPa. The elongation for these samples was 33.48 ± 1.23 and 30.76 ± 1.80%, respectively. For comparison, the tensile strength, elongation, and Young's modulus of collagens isolated from bovine Achilles tendon were 59 ± 4 MPa, 48 ± 3%, and 87 ± 8 MPa, respectively, which are considerably higher than those of the jellyfish-derived collagen.[32] Thus, for potential applications of jellyfish-derived collagen in regenerative medicine, additional research is needed to improve its mechanical properties.

As biomaterials are used in wound dressing and artificial skin, they must meet certain requirements for biocompatibility and bioactivity,[7] which were also investigated in this study. To assess cytotoxicity, collagen samples were ground and added to the culture medium in the wells of a 24-well plate containing HUVEC cells. Cytotoxicity was evaluated by detaching the cells from the surface using trypsin and Versen solutions, staining the resulting suspension with trypan blue, and counting the number of living cells. The results of this viability assessment using an automatic cell counter and the trypan blue exclusion method are presented in **Table 3**.

It was shown that vascular endothelial cells proliferated similarly in the presence of collagen from both jellyfish during the first 24 h. Cell viability analysis after trypan blue staining revealed that in all conditions, more than 70% of the cells were viable. After three days, a decrease in cell viability was observed in the presence of *R. hispidum* collagen samples. This decline may be attributed to the lower solubility of jellyfish collagen, resulting in reduced availability to the cells during cultivation.

In addition, to assess changes in the number of active living cells growing in the culture wells in the presence of collagen samples, a test was performed using resorufin, a water-soluble dye that easily penetrates cell membranes without damaging

Table 2. Results of mechanical tests of collagen membranes

Sample	Tensile strength (MPa)	Young's modulus (MPa)	Elongation (%)
Aurelia aurita collagen membrane (thickness~0.73 mm), in NaCl solution	0.11±0.02	0.60±0.03	39.57±2.21
A. aurita collagen membrane (thickness~0.50 mm), in dry condition	0.54±0.09	3.92±0.25	33.48±1.23
Rhopilema hispidum collagen membrane (thickness 0.75 mm), in NaCl solution	0.22±0.03	0.46±0.03	65.17±5.61
R. hispidum collagen membrane (thickness 0.51 mm), in dry condition	1.17±0.08	10.03±0.10	30.76±1.80

Abbreviation: NaCl: Sodium chloride.

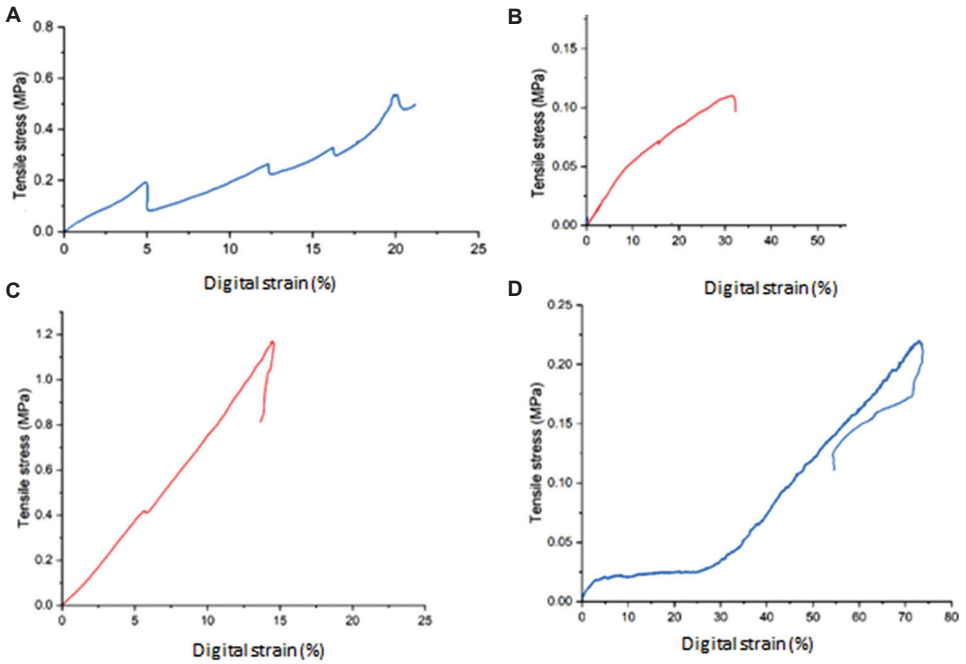

Figure 6. Stress–strain curves for collagen membranes. (A) Dry *Aurelia aurita* collagen, (B) *A. aurita* collagen in sodium chloride solution, (C) dry *Rhopilema hispidum* collagen, and (D) *R. hispidum* collagen in sodium chloride solution.

them. The test results showed that after 24 h of cell cultivation with collagen samples, the optical density of resorufin was significantly higher in both variants compared to the control, while no significant differences were observed after 72 h (**Figure** 7).

The results of evaluating the regenerative activity of collagen using the scratch test indicated that during the first 2 days, cell migration into the wound occurred in all collagen variants (**Figures 8** and **9**). After 5 days of the experiment, the cells formed a dense monolayer. Throughout the experiment, no cell death was observed, indirectly indicating the absence of toxic effects from collagen isolated from *A. aurita* and *R. hispidum* jellyfish.

The mechanism by which collagen influences cell migration is not fully understood. According to several studies,[33-35] the topological properties of collagen, such as fiber thickness and arrangement, provide directional signals for cell migration. Fu *et al.*[36] demonstrated that cell pseudopodia plays a key role in this process, the formation of which is activated by actin polymerization.[37-40] Cell migration is a multifactorial process that depends on the coordinated function of adhesion molecules and signaling pathways,[41] and thus requires further detailed studies.

The initial *in vivo* study of biocompatibility was conducted by introducing collagen sponge samples under the skin of laboratory animals. After the procedure, the animals were examined at least 3 times a week, with observations made on their behavior and the condition of the wounds, in comparison with the control groups. On Day 1 after the operation, mice from all groups behaved normally and did not refuse food or water. The wounds on their backs were dry and showed no signs of suppuration. Seven days after the operation, the wounds were completely closed in all groups, and no signs of swelling or suppuration were detected (**Figure 10**).

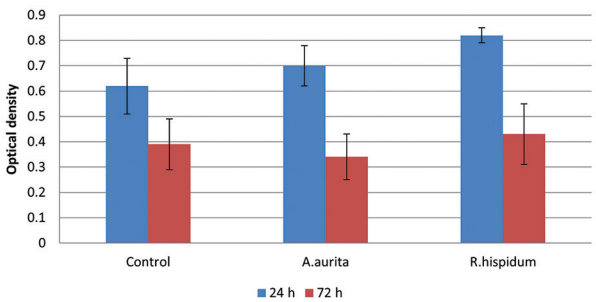

Figure 7. Evaluation of cell activity after 24 and 72 h of cultivation in the presence of collagen sponge samples introduced into the culture medium as a suspension with dilution factors of 1:5, using the resazurin test method.

Table 3. Percentage of living cells after one and three days of growth in the presence of collagen

Sample	24 h	72 h
Collagen *Aurelia aurita*	77.67±2.52	82.11±1.65
Collagen *Rhopilema hispidum*	80.61±1.40	76.83±1.04
Control (from rat tails)	80.44±0.58	84.33±0.58

Morphological studies of tissues after implantation of the collagen sponge were performed on histological samples. A description of the biological responses is provided below, corresponding to the images presented.

As shown in **Figure 11**, after 3 days, tissue in the control experiment (without collagen sponge) displayed a defect area filled with fibrin (homogeneously pink) masses and moderate lymphohistiocytic infiltration, and dermal edema was observed in the intact area of skin. After 7 days, the proliferation of granulation tissue was observed, with newly formed, dilated, blood-filled vessels (**Figure 11B**, item #3) and moderate

lymphohistiocytic infiltration. After 14 days, the proliferation of loose connective tissue was noted (**Figure 11C**), with no lymphohistiocytic infiltration. By Day 21 (**Figure 11D**), continued proliferation of loose connective tissue was observed in the control wounds, and lymphohistiocytic infiltration remained absent.

On the 3rd day, when applying a collagen sponge from *A. aurita*, cavities partially filled with collagen (yellow in color, **Figure 12**) were identified. Around the areas of tissue defects, fibrinous (homogeneously pink) masses with moderate lymphohistiocytic infiltration were observed. By Day 7, wounds treated with this sponge showed signs of regenerative granulation. The collagen sponge samples appeared defragmented and located within clearly defined cavities, which were surrounded by newly formed fibers of loose connective tissue. On Day 14, cavities filled with mesh-like connective tissue structures were identified, along with surrounding loose fibrous connective tissue growth. The boundaries between the wound healing zones and the preserved tissue were clearly defined. On Day 21, a cavity containing individual fragments of connective tissue structures was observed, surrounded by loose fibrous connective tissue.

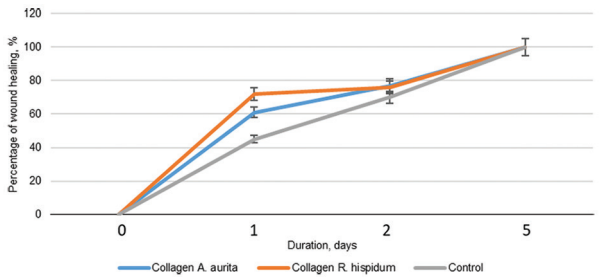

Figure 8. Dynamics of model wound closure in human umbilical vein endothelial cell culture over 5 days.

On Day 3, tissue samples from wounds treated with collagen sponges from *R. hispidum* also showed cavities partially filled with collagen (yellow). Fibrinous (homogeneously pink) masses with moderate lymphohistiocytic infiltration were noted around the tissue defect zones. By Day 7, cavities filled with reticular connective tissue structures, loosely arranged with individual cell clusters, were observed. The proliferation of loose fibrous connective tissue surrounded the cavities, although the boundaries between the wound healing zones and the preserved tissue were not clearly defined. On Day 14, the growth of loose fibrous connective tissue was seen at the edge of the transplant area, without clear boundaries from the preserved skin. By Day 21, giant multinucleated foreign body cells were detected in the experimental wounds, indicating a mild inflammatory response.

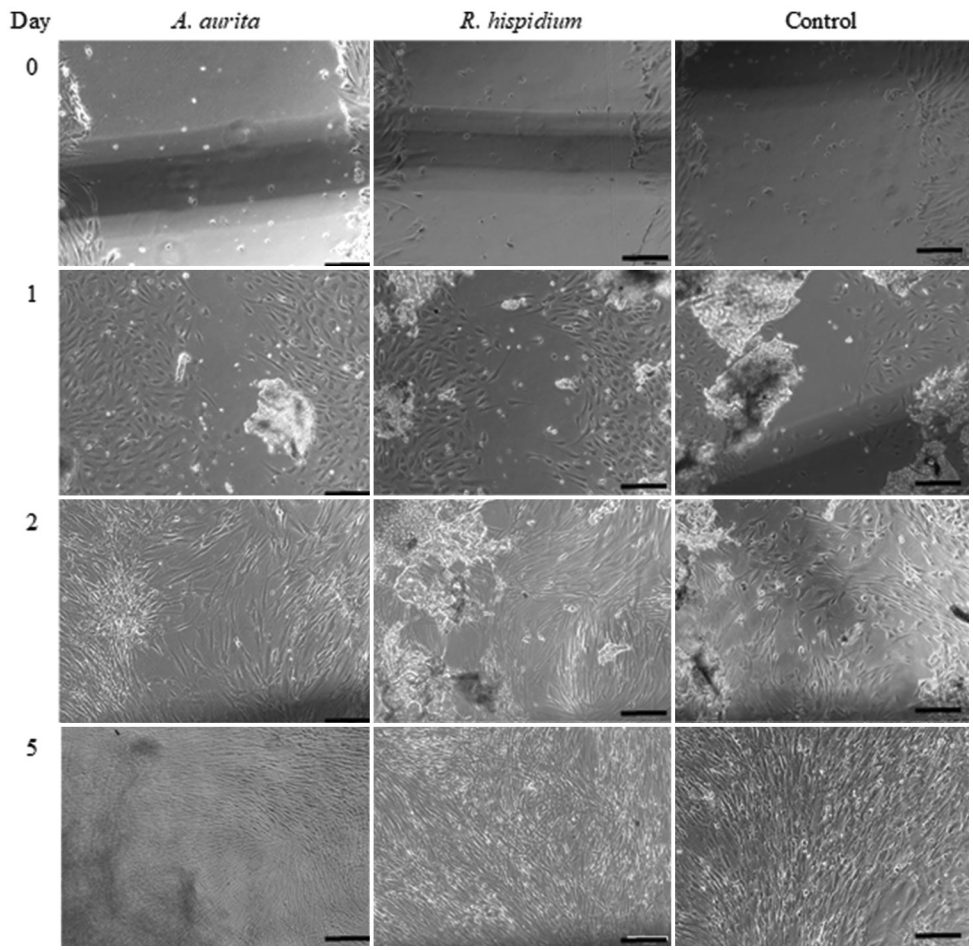

Figure 9. Phase contrast images of a scratch in a human umbilical vein endothelial cell layer at Days 0, 2, 1, and 5 after collagen addition (live imaging). Magnification = ×100; scale bar = 200 μm.

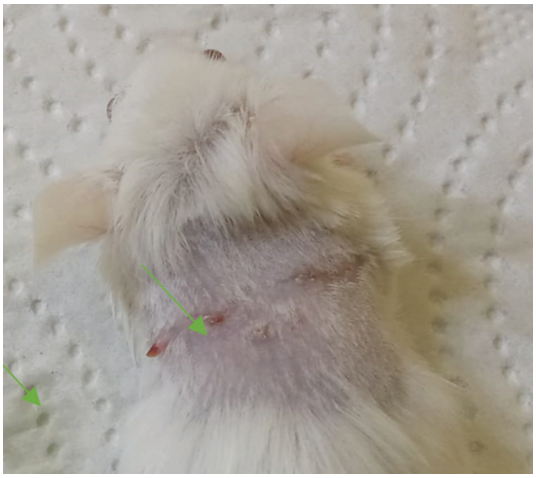

Figure 10. Appearance of wounds seven days after subcutaneous administration of collagen sponge samples.

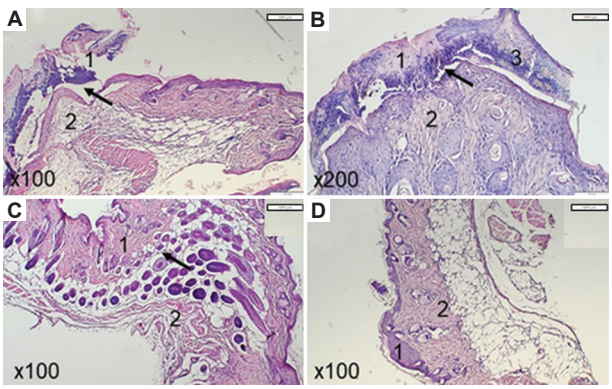

Figure 11. Histological sections of control wounds on (A) Day 3 (×100 magnification), (B) Day 7 (×200 magnification), (C) Day 14 (×100 magnification), and (D) Day 21 (×100 magnification) after surgery. Notes: The border between the wound (1) and intact skin (2) is indicated by an arrow, 3 refers to the vessel. Scale bar: 300 μm (A, C, D); 200 μm (B).

Thus, by Day 21, the control wounds and those treated with collagen showed a proliferation of loose connective tissue. In the experimental wounds, signs of ongoing assimilation of the remaining collagen were evident. More complete assimilation was observed with collagen derived from the jellyfish *R. hispidum*. However, slight inflammation was noted when using this type of collagen.

4. Discussion

In the present work, a comprehensive analysis of the properties of collagens obtained from *A. aurita* and *R. hispidum* was performed, along with a comparative analysis of the physicochemical and mechanical properties of collagens from marine and mammalian species. Based on electrophoretic studies,[22] it was demonstrated that the collagen of *A. aurita* consists solely of α-chains, while the collagen of *R. hispidum* is composed of α and β chains (**Figure 1**). The molecular mass of the tested collagen ranged from 105 to 240 kDa. Specifically, the molecular mass of the α chain was 105 kDa for *A. aurita* collagen and 135 kDa for *R. hispidum* collagen. The β chain of *R. hispidum* collagen exhibited a molecular mass of 240 kDa. According to the literature, the molecular mass of a collagen polypeptide chain is about 100 kDa.[16] Thus, the empirical data of our study are consistent with the results of other authors.[16,22] Electrophoretic analysis also confirmed that the collagen from *Chrysaora* spp. (ribbon jellyfish) consists of α chains (α1) and dimeric β chains (with molecular masses of ~137 and ~241 kDa, respectively), resembling the pattern of type II collagen.[22] Felician *et al.*[16] demonstrated that collagen extracted from *Rhopilema esculentum* consisted of two α chains between 100 and 150 kDa, a β chain (dimer), and a γ chain (trimer) located in the high molecular weight region (above 150 kDa). The α chains were the main component of jellyfish collagen, with α1 and α2 chains exhibiting different electrophoretic mobilities, indicating distinct molecular masses.[16] However, our data differ from studies reporting that collagen from the mesoglea of the jellyfish *Rhopilema asamushi* contains both α1 and α2 chains,[14] and that collagen from the jellyfish *Stomolophus nomurai* contains three α chains.[15] These differences are likely due to species-specific variations in collagen composition.[22]

Our study found that the molecular weight of jellyfish collagen is comparable to that of mammalian collagen. For example, collagen isolated from pork contains a β-chain (~205 kDa) and two α-chains: α1 (~115 kDa) and α2 (~97.4 kDa).[42] Acid-soluble collagen isolated from bovine Achilles tendon consists of trimers with a molecular mass of 300 kDa.[32] Mass spectroscopic analysis revealed that chicken bone collagen (type I) includes β and γ bands with molecular masses of 245 kDa, corresponding to dimers and trimers.[43] Similar results were obtained when collagen was extracted from chicken feet using acetic acid.[44]

Analysis of the isoelectric point of collagen from the jellyfish *A. aurita* and R. hispidum showed that this parameter is highly dependent on the collagen source (**Figure 2**). It was found that the isoelectric point of *R. hispidum* collagen is 5.19, while the isoelectric point of *A. aurita* collagen is significantly lower, at 4.90. Earlier studies reported that the isoelectric point of *Chrysaora* spp. collagen is 6.64;[22] for bamboo shark collagen, it is 6.56;[45] for blacktip shark, 6.96; and for brownbanded bamboo shark, 7.26.[27] The isoelectric points of collagen from pork, beef, and chicken were determined by measuring zeta potential across a pH range. Pork collagen typically shows an isoelectric point of 5.5,[46] bovine collagen ranges from 5.0 to 6.9,[46,47] and chicken collagen from 5.09 to 6.54.[43,46]

A comparative analysis of empirical data with these published data suggests that differences in isoelectric points of collagen from different sources correlate with their amino acid compositions. Variations in charged side chains affect the surface charge of proteins.[22,26] Khong *et al.*[48] also found that the isoelectric point can vary depending on the method of collagen extraction. For example, *Acromitus hardenbergi* collagen had isoelectric points of 4.92 and 4.46 (acid and pepsin extraction from bells) and 5.40 and 4.93 (acid and pepsin extraction from tentacles). Acid extraction of *A. aurita* collagen in one study yielded an isoelectric point of 4.46, slightly lower than our results.[49] Jellyfish collagen holds advantages over animal- and avian-derived collagens due to its low isoelectric point, which is particularly beneficial for applications in regenerative

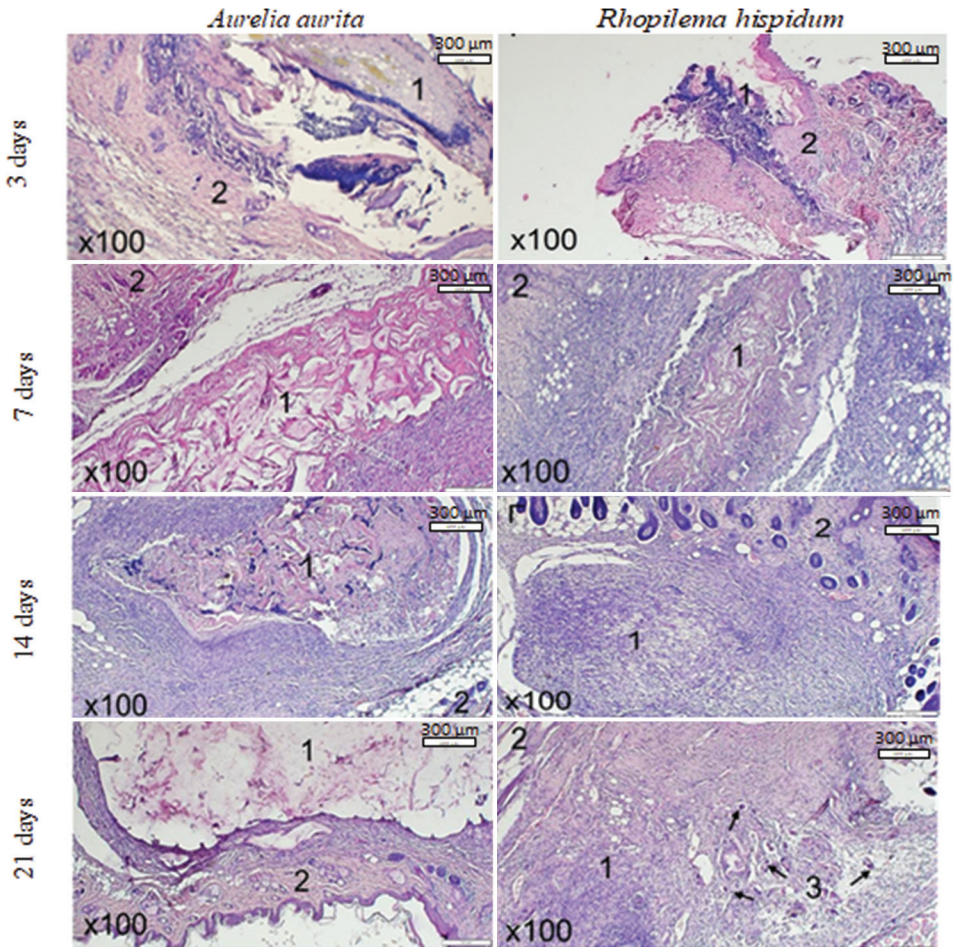

Figure 12. Histological sections of experimental wounds on Days 3, 7, 14, and 21 after implantation of collagen samples.
Notes: 1 = Transplantation area, 2 = Preserved skin, and 3 = Area of giant multinucleated cells accumulation. Scale bar: 300 µm, magnification: ×100.

medicine. A low isoelectric point at physiological ionic strength can improve the linear viscoelastic properties and transparency of biomaterials.[50] Collagen with an isoelectric point between 4.0 and 5.5 has been shown to exhibit better biocompatibility at physiologic pH.[51,52] A low isoelectric point may also indicate a strong negative surface charge, potentially affecting interactions with cell membranes. However, our previous studies on human keratinocyte adhesion did not support this hypothesis, as collagen from *A. aurita* significantly improved cell proliferation and adhesion.[20] Similar results were obtained by Pugliano *et al.*[53] when culturing human stem cells with jellyfish type II collagen.

The difficulty in working with marine organism-derived collagens is their lower denaturation temperatures compared to mammalian collagens.[13,22,54] For collagen derived from *A. aurita*, we observed a denaturation temperature of 32.23°C, while *R. hispidum* collagen exhibited a slightly higher value of 33.15°C. Both collagen types degraded within the temperature range of 40 – 150°C. The low denaturation temperature of *A. aurita* collagen is likely linked to the temperature of its natural habitat. Barzideh *et al.*[22] showed that marine inhabitants from colder environments tend to have lower denaturation temperatures due to a lower number of cross-links. Sharks and other cold-water hydrobionts typically have lower glycine, proline, and hydroxyproline, contributing to reduced thermal stability.[22,42,54] For comparison, collagen extracted from silver carp skin with acetic acid has a denaturation temperature of 30.37°C.[55] Mammalian collagen typically denatures at significantly higher temperatures, ranging from 48.3°C to 79.31°C.[42,55] For example, collagen extracted from pig skin through the acid-enzyme binding method (acetic acid and pepsin) showed a denaturation temperature of 67.75°C,[56] while chicken leg tendon collagen extracted with 0.15 M citrate buffer (pH 3.8) had a denaturation temperature of 38°C.[57] These findings indicate that jellyfish collagen requires additional cross-linking to improve its thermal stability. Before it can be effectively used as a wound-healing agent, such modifications may be necessary to raise its denaturation temperature to levels compatible with physiological conditions.

It was found that the studied collagen samples were completely insoluble in acetic acid, methylene chloride, hexane, ethanol, methanol, ethyl acetate, 35% hydrochloric acid solution, sodium hydroxide, and distilled water. These results align with previously presented empirical data.[58] According to the study, the spongy collagen did not dissolve in any of the tested solvents. A slight dispersion was observed in solutions with pH 8 – 10, and hydrolysis of collagen fragments occurred in acidic media.[58] However, our results differ from solubility data reported for fish and mammalian collagens. For example, collagen isolated from bovine Achilles tendons exhibited

poor solubility in the pH range of 4.0 – 5.0.[59] Type I collagen from turkey tissues (supplied by Essentia Protein Solutions, Denmark) showed maximum solubility (80%) between pH 2.0 and 10.0, while its minimum solubility (around 20%) was observed between pH 5.0 and 7.0.[46] Similarly, fish collagen demonstrated high solubility (75 – 95%) across the entire studied pH range (2.0 – 10.0).[46] These differences in solubility can be attributed to variations in the degree of cross-linking and amino acid composition among collagen sources. The solubility of collagen has been found to decrease with an increasing number and degree of stable cross-links.[60] Fish skin collagen, which has a relatively low content of stable cross-links, is easily soluble, even in mature fish.[60]

The low solubility of collagen from *A. aurita* and *R. hispidum* has important implications for the production of medical devices. Prolonged acid pre-treatment during the extraction process can result in high collagen yield with minimal loss.[60]

Empirical data also indicated that the collagen samples studied were characterized by a moderate degree of hydrophilicity but high hydration capacity, which prevents them from being classified as super-wetting materials (**Table 1**). In a previous study,[61] the water-holding capacity of *A. aurita* collagen was measured at 4.194 g/g, consistent with our findings. This value exceeds that of medical gauze (2 g/g) but is lower than that of collagen sponges derived from *R. esculentum*.[62] Collagen extracted from porcine skin, with water absorption ranging from 18.9% to 27.4%,[56,63] and collagen from silver carp skin extracted using acetic acid (water absorption capacity of 31.13 g/g), also showed poor water absorption.[45] In contrast, acid-soluble collagen isolated from the bovine Achilles tendon exhibited high water retention, with water absorption reaching 99% and a swelling factor of 95%.[32] Type I collagen from turkey tissues (Essentia Protein Solutions, Denmark) had a swelling degree of 181%.[46] Despite its modest water-holding capacity, jellyfish collagen from *A. aurita* and *R. hispidum* exhibited a hydrophilic nature and molecular structure that makes it suitable for use as an absorbent, especially to stop bleeding.[63] A variety of commercially available hemostatic products utilize sponge-like structures that are highly absorbent and capable of retaining multiple times their weight in fluid. These collagen-based products can rapidly absorb blood and form an artificial clot-like matrix that effectively stops bleeding *in situ*.[64,65]

Collagen is one of the most versatile biomaterials used in biomedical applications, mainly due to its biomimetic properties and structural role in the extracellular matrix.[50] Tissue engineering provides promising developments in this area.[50,66] Ideally, a new biomaterial should exhibit natural strength and anisotropy. However, the biomaterial and their derived scaffolds tend to degrade over time, potentially causing undesirable tissue remodeling in response to applied strain.[66] In this regard, it is crucial to study the degradation profile of jellyfish-derived collagen, which is being considered as a basis for regenerative medicine products. As shown in **Table 1**, native jellyfish collagen undergoes near-complete degradation under the influence of collagenase, which is in agreement with the results reported by other researchers.[8] For example, Terzi *et al.*[8] demonstrated that unmodified collagen films were rapidly and completely digested by collagenase within 1 h, while cross-linked films (modified using double processing methods) remained relatively stable and were almost completely cleaved after 4 hours. Guan *et al.*[47] observed partial degradation (up to 50%) of bovine hide collagen when exposed to collagenase, pepsin, and trypsin. Bovine and porcine collagens were more resistant to collagenase than chicken and fish collagens, likely due to a higher content of glycosaminoglycans.[47] Therefore, native jellyfish collagen is unsuitable for direct application in regenerative medicine due to its rapid degradation in the presence of collagenase. To make it viable for medical use, collagen must be modified to improve its stability.[50]

For modification purposes, alkaline and/or heat treatment of proteins can be applied to induce inter- or intramolecular crosslinking.[67] Chemical crosslinking agents are often used to increase thermal stability, gelling properties, and resistance to collagenase. These agents form covalent bonds within polymer chains, thereby improving their stability and essential mechanical properties. Examples of commonly used cross-linkers include genipine,[68] glutaraldehyde,[69] formaldehyde,[70] methacrylic anhydride with a photoinitiator (phenyl-2,4,6-trimethylbenzoyl lithium phosphinate),[71] 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, and N-hydroxysuccinimide.[72,73] However, such crosslinking methods can lead to cytotoxic effects.

Enzymatic crosslinking, using enzymes such as transglutaminase, phosphopantetheine transferase, and lysyl oxidase, is considered a safer alternative. These enzymes facilitate crosslink formation in proteins without inducing toxicity in cells.[67,74] It is important to prioritize non-toxic crosslinking agents that preserve the integrity and shape of three-dimensional-printed matrices containing cells. In the next phase of this study, we will conduct experiments to select and evaluate the effectiveness of different crosslinking agents and assess the safety of the resulting materials.

Collagen is a well-studied biomaterial that has been researched since the beginning of the past century. The formation of collagen fibrils is also well-documented, especially in collagen-based porous scaffolds produced by freeze-drying.[13] However, there are very few studies on the microstructure of fibrillated marine organism-derived collagens dried in open air. Therefore, in this study, the microstructure of collagens from the jellyfish *A. aurita* and *R. hispidum* was investigated. SEM analysis revealed that the studied collagen samples were characterized by a highly porous structure. The pores were predominantly channel-shaped. In *A. aurita* collagen, pore sizes ranged from 57.1 μm to 256.7 μm, and wall thicknesses ranged from 58.2 μm to 241.7 μm (**Figure 4**). Collagen from *R. hispidum*, when dried in the open air, also exhibited a highly porous structure, with pore sizes ranging from 57.1 μm to 337.6 μm and wall thicknesses from 48.7 μm to 163.6 μm (**Figure 5**). The pore shape in *R. hispidum* collagen was predominantly spherical. The pores were formed by filaments and collagen sheets, composed of multiple collagen fibrils and fibers bound together into a network of fibrils and a dense, folded, sheet-like structure. It was also revealed that the collagen samples from *A. aurita* and *R. hispidum* were sufficiently homogeneous and free of extraneous inclusions or other

structures. Their microstructure corresponds to that of fish collagen,[75,76] differing from mammalian collagen.[43,47,55,77] SEM images confirmed that collagen extracted from Jack mackerel and meager bone exhibits a porous structure with pore sizes ranging from 70 μm to 140 μm.[76] In contrast, collagens derived from pig skin and bovine pericardium have less pronounced porosity.[47] Bovine collagen, in particular, forms a coarse matrix with large pores.[55] Electron microscopy revealed that collagen from bovine pericardium contains disorganized nanofibers in a spongy structure with unevenly distributed, interconnected pores.[77] Similarly, collagen from chicken bone forms a coarse matrix with large pores.[43] The difference in microstructure between mammalian and marine organism-derived collagens can be explained by the higher number of covalent bonds in mammalian skin and bone collagen compared to marine organism-derived collagen.[43]

A comparative evaluation of the mechanical properties of jellyfish collagen from *A. aurita* and *R. hispidum* revealed that their ultimate tensile strength in the dry state was 0.54 MPa and 0.60 MPa, respectively, and 0.11 MPa and 0.22 MPa, respectively, when immersed in physiological NaCl solution. In contrast, the tensile strength of collagen from bovine Achilles tendon is 59 MPa,[32] and that of collagen from bovine membrane waste is 58 N/mm^2.[56] The compressive strength of type I collagen from chicken foot skin ranges from 30 MPa to 70 MPa.[78] The strength values of animal and avian collagens are thus significantly higher than those of jellyfish collagen.[32,56,78] The high strength of bovine and avian collagens makes them suitable for bone tissue engineering applications.[77] This superior mechanical performance is attributed to the higher structural order found in mammalian and avian collagen fibrils compared to marine origin.[79,80]

A similar conclusion can be drawn from a comparative evaluation of the Young's modulus of collagen. The Young's modulus of jellyfish collagen was found to be 3.92 MPa for *A. aurita* (dry state) and 10.03 MPa for *R. hispidum* (dry state), which is significantly lower than that of animal and avian collagens. According to Chowdhury *et al.*,[80] the Young's modulus of collagen monomers isolated from bovine Achilles tendon is 6 – 7 GPa, while that of collagen from rat tail tendon is 11.0 GPa.

The relative elongation of collagen extracted from cattle processing waste (membrane) is 7%.[56] In comparison, our results showed that the elasticity index of *A. aurita* and *R. hispidum* collagens was 4.5 – 4.7 times higher than that of bovine collagen. Specifically, the elasticity index of *A. aurita* collagen in the dry state was 33.48%, and for *R. hispidum* collagen, it was 30.76%.

Thus, jellyfish collagen demonstrates superior elasticity compared to bovine collagen, though it is lower than that of porcine collagen.[56] For instance, collagen obtained from pig skin using acetic acid and pepsin exhibited a tensile strength of 2.3 MPa and an elongation of 52.5%.[56] However, native collagen used in medical devices is typically applied in solution form rather than in a dry state. Therefore, it is critical that the mechanical properties of collagen-based biomaterials are preserved or even enhanced under physiological conditions.

Our results (**Table 2**) indicate that collagen in physiological NaCl solution exhibits suboptimal mechanical performance in terms of Young's modulus, tensile strength, and elongation. To improve these mechanical properties, collagen must be modified through advanced crosslinking techniques or by incorporating a second, stiffer phase. These modifications could improve the suitability of the biomaterial for applications in regenerative medicine.[81]

While crosslinking methods effectively stabilize collagen and increase its mechanical strength and stability, some methods may reduce the biocompatibility of the material and lead to potential cytotoxicity.[10] Ahmed *et al.*[82] showed that combined treatments enhance the mechanical stiffness of collagen-based materials.

Collagen is known to play an important role in the wound healing process, as it promotes the proliferation and migration of keratinocytes and fibroblasts to the wound site, stimulates chemotactic factors, and enhances fibroblast production.[16,64] Therefore, in the final stage of this study, the potential of collagen isolated from the jellyfish *A. aurita* and *R. hispidum* in wound healing was investigated *in vitro* (**Figures 7** and **8**). It was found that in the presence of collagen, the migration of HUVEC cells was significantly enhanced on the 1st day compared to cells without collagen. Similar results were obtained when evaluating the wound-healing activity of collagen peptides derived from *R. esculentum* jellyfish.[16] Marine organism-derived collagen peptide may shorten the inflammatory period by reducing inflammatory cell aggregation, which leads to the expression of FGF and vascular endothelial growth factor, promotes the production of basic FGF, and enhances collagen deposition and maturation.[64,65] Bovine collagen peptides can also promote angiogenesis and epithelialization, effectively shortening the wound-healing cycle in mice, as shown in a skin perforation model.[50] This may be due to the chemotactic effect of collagen peptides, which enhance cell migration to the artificial wound area.[16]

In vivo test on Balb/C laboratory mice showed that by Day 21, both the control wounds and those treated with collagen exhibited proliferation of loose connective tissue. In the experimental wounds, there were signs of ongoing assimilation of the remaining collagen. More complete assimilation was observed with the sample from the jellyfish *R. hispidum*; however, when using this collagen type, slight inflammation was noted. This may be due to suboptimal sterilization, as sponges were sterilized by ultraviolet irradiation, which did not provide a sufficient level of sterility due to equipment limitations.

This *in vivo* study was preliminary. In the next stage, the biological activity of collagen samples will be re-evaluated using female Wistar rats. In addition to histological parameters, the study will assess blood markers of inflammation (interleukin-6, interleukin-10, and tumor necrosis factor-alpha), animal welfare (appearance, activity, facial signs of pain), wound healing dynamics, and conduct immunohistochemical analyses using the following markers: EGF, FGF, cluster of differentiation 31, and antitrypsin for tissue visualization.

5. Conclusions

A comparative analysis of the collagen properties of jellyfish *A. aurita* and *R. hispidum*, which inhabit different temperature conditions, along with those of animals and birds, reveals the prospects of marine organism-derived collagen for use in medical products for treating wounds and burns. It was found that the molecular weight of jellyfish collagen is similar to that of animal and avian collagen, but it has a lower isoelectric point and denaturation temperature, which enhances its biocompatibility at physiological pH. Jellyfish collagen was also found to be less soluble than collagen isolated from fish, animals, and birds. However, due to its low solubility, jellyfish collagen provides high extraction yields with negligible losses. Although jellyfish collagen is inferior to animal and avian collagen in terms of hydrophilicity, hydration capacity, and mechanical strength, it remains a viable candidate for the production of biomaterials in tissue engineering. To improve its mechanical properties, additional modifications are necessary through improved cross-linking mechanisms or by incorporating a second, stiffer phase, allowing the biomaterial to meet specific application requirements. Our study demonstrated that collagen significantly enhanced HUVEC cell migration on the 1st day, which may contribute to shortening the inflammatory period and accelerating the wound healing process.

Thus, *A. aurita* and *R. hispidum* represent promising marine sources of collagen for regenerative medicine. Jellyfish-derived collagen is characterized by high yield, low processing losses, improved biocompatibility at physiological pH, and a capacity to reduce the duration of inflammation and accelerate wound healing. In addition, jellyfish collagen does not carry the risk of transmitting infectious diseases. The results of the present study support the continued development of collagen-based biomaterials for use in regenerative medicine, particularly in wound and burn treatment. At present, the toxicity of collagen has been assessed indirectly through HUVEC migration experiments, indicating that the collagen is non-toxic. In future work, direct *in vitro* testing on immune cells and *in vivo* wound healing studies on rats should be performed to further validate its safety and efficacy.

Acknowledgement

The authors express their sincere gratitude to the I. Koltsov Institute of Biomedical Problems of the Russian Academy of Sciences team for their assistance in organizing and conducting the *in vivo* studies.

Financial support

The work was supported by the Ministry of Science and Higher Education of the Russian Federation, contract no. 075-15-2023-601 (extension no. 13.2251.21.0219).

Conflicts of interest statement

The authors declare that they have no competing interests.

Author contributions

Conceptualization: OB and SA; *Funding acquisition*: OB; *Investigation*: YK, AZ; *Methodology*: YK; *Project administration*: OB; *Resources*: OB; *Visualization*: SN; *Writing – original draft*: YK, SS, and AZ; *Writing – review & editing*: OB and YK. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The research was reviewed and approved by the Bioethics Commission at the I. Koltsov Institute of Biomedical Problems of the Russian Academy of Sciences (protocol no. 82, dated April 25, 2024).

Consent for publication

Not applicable.

Availability of data

The data obtained in this study are available from the corresponding author upon reasonable request.

References

1. Cadar E, Pesterau AM, Sirbu R, Negreanu-Pirjol BS, Tomescu CL. Jellyfishes-significant marine resources with potential in the wound-healing process: A review. *Mar Drugs.* 2023;21(4):201. doi: 10.3390/md21040201
2. Wang Y, Beekman J, Hew J, *et al.* Burn injury: Challenges and advances in burn wound healing, infection, pain and scarring. *Adv Drug Deliv Rev.* 2018;123:3-17. doi: 10.1016/j.addr.2017.09.018
3. Herndon DN, Tompkins RG. Support of the metabolic response to burn injury. *Lancet.* 2004;363:1895-1902. doi: 10.1016/S0140-6736(04)16360-5
4. Czapran A, Headdon W, Deane AM, Lange K, Chapman MJ, Heyland DK. International observational study of nutritional support in mechanically ventilated patients following burn injury. *Burns.* 2015;41(3):510-518. doi: 10.1016/j.burns.2014.09.013.4
5. Pozzolini M, Millo E, Oliveri C, *et al.* Elicited ROS scavenging activity, photoprotective, and wound-healing properties of collagen-derived peptides from the marine sponge Chondrosia reniformis. *Mar. Drugs.* 2018;16(12):465. doi: 10.3390/md16120465
6. Verde MEQL, Ferreira-Júnior AEC, De Barros-Silva PG, *et al.* Nile tilapia skin (*Oreochromis niloticus*) for burn treatment: Ultrastructural analysis and quantitative assessment of collagen. *Acta Histochem.* 2021;123(6):151762. doi: 10.1016/j.acthis.2021.151762
7. Li Z, Ruan C, Niu X. Collagen-based bioinks for regenerative medicine: Fabrication, application and prospective. *Med Nov Technol Devices.* 2023;17:100211. doi: 10.1016/j.medntd.2023.100211
8. Terzi A, Storelli E, Bettini S, *et al.* Effects of processing on structural, mechanical and biological properties of collagen-based substrates for regenerative medicine. *Sci Rep.* 2018;8(1):1429. doi: 10.1038/s41598-018-19786-0
9. Ben C, Liu X, Shen T, *et al.* A recombinant human collagen hydrogel for the treatment of partial-thickness burns: A prospective, self-controlled clinical study. *Burns.* 2021;47(3):634-642. doi: 10.1016/j.burns.2020.01.006
10. Yamada S, Yamamoto K, Ikeda T, Yanagiguchi K, Hayashi Y. Potency of fish collagen as a scaffold for regenerative medicine. *Biomed Res Int.* 2014;2014(1):302932. doi: 10.1155/2014/302932
11. Geahchan S, Baharlouei P, Rahman A. Marine collagen: A promising biomaterial for wound healing, skin anti-aging, and bone regeneration. *Mar Drugs.* 2022(20):61. doi: 10.3390/md20010061
12. Davison-Kotler E, Marshall WS, García-Gareta E. Sources of collagen for biomaterials in skin wound healing. *Bioengineering (Basel).* 2019;6(3):56. doi: 10.3390/bioengineering6030056
13. Hoyer B, Bernhardt A, Lode A, *et al.* Jellyfish collagen scaffolds for cartilage tissue engineering. *Acta Biomater.* 2014;10(2):883-892. doi: 10.1016/j.actbio.2013.10.022
14. Nagai T, Worawattanamateekul W, Suzuki N. Isolation and characterization of collagen from rhizostomous jellyfish (*Rhopilema asamushi*). *Food Chem.* 2000;70:205-208. doi: 10.1016/S0308-8146(00)00081-9
15. Nagai T, Ogawa T, Nakamura T. Collagen of edible jellyfish exumbrella. *J Sci Food Agric.* 1999;79:855-858. doi: 10.1002/(SICI)1097-0010(19990501)

16. Felician FF, Yu RH, Li MZ, *et al.* The wound healing potential of collagen peptides derived from the jellyfish *Rhopilema esculentum. Chin J Traumatol.* 2019;22(1):12-20.
doi: 10.1016/j.cjtee.2018.10.004
17. Lahmar A, Rjab M, Sioud F, *et al.* Design of 3D hybrid plant extract/marine and bovine collagen matrixes as potential dermal scaffolds for skin wound healing. *Sci World J.* 2022;30:8788061.
doi: 10.1155/2022/8788061
18. Abedi M, Shafiee M, Afshari F, Mohammadi H, Ghasemi, Y. Collagen-based medical devices for regenerative medicine and tissue engineering. *Appl Biochem Biotechnol.* 2024;196(8):5563-5603.
doi: 10.1007/s12010-023-04793-3
19. Wenger MP, Bozec L, Horton MA, Mesquida P. Mechanical properties of collagen fibrils. *Biophys J.* 2007;93(4):1255-1263.
doi: 10.1529/biophysj.106.103192
20. Barzkar N, Babich OO, Sukhikh SA, *et al. Aurelia aurita* jellyfish collagen: Recovery properties. *Food Raw Mater.* 2024;13(2):296-305.
doi: 10.21603/2308-4057-2025-2-648
21. Cooksey K. In: Robertson GL, editor. *Food Packaging, Principles and Practices.* 2nd ed. Boca Raton, FL: Taylor and Francis, CRC; 2006. p. 57-59.
doi: 10.1002/pts.777
22. Barzideh Z, Latiff AA, Gan CY, Benjakul S, Karim AA. Isolation and characterisation of collagen from the ribbon jellyfish (*Chrysaora sp.*). *Int J Food Sci Technol.* 2014;49:6-1490-1499.
doi: 10.1111/IJFS.12464
23. Montero P, Jimenez-Colmenero F, Borderias J. Effect of pH and the presence of NaCl on some hydration properties of collagenous material from trout (Salmo irideus Gibb) muscle and skin. *J Sci Food Agric.* 1991;54:137-146.
doi: 10.1002/jsfa.2740540115
24. Witono JR, Noordergraaf IW, Heeres HJ, Janssen LPBM. Water absorption, retention and the swelling characteristics of cassava starch grafted with polyacrylic acid. *Carbohydr Polym.* 2014;(103):325-332.
doi: 10.1016/j.carbpol.2013.12.056
25. Moore S, Stein WH. Chromatography of amino acids, colorimetric ninhydrin method for analysis of the effluent. *Fed Proc.* 1948;7(1):174.
26. Ahmad M, Benjakul S, Nalinanon S. Compositional and physicochemical characteristics of acid solubilized collagen extracted from the skin of unicorn leatherjacket (*Aluterus monoceros*). *Food Hydrocoll.* 2010;24:588-594.
doi: 10.1016/J.FOODHYD.2010.03.001
27. Kittiphattanabawon P, Benjakul S, Visessanguan W, Shahidi, F. Isolation and characterization of collagen from the cartilages of brownbanded bamboo shark (*Chiloscyllium punctatum*) and blacktip shark (*Carcharhinus limbatus*). *LWT Food Sci Technol.* 2010;43:792-800.
doi: 10.1016/J.LWT.2010.01.006
28. Chen X, Guo Z, Zhang J, Li Y, Duan R. A new method for determining the denaturation temperature of collagen. *Food Chem.* 2021;343:128393.
doi: 10.1016/j.foodchem.2020.128393
29. Pawelec KM, Best SM, Cameron R. Collagen: A network for regenerative medicine. *J Mater Chem.* 2016;4(40):6484-6496.
doi: 10.1039/c6tb00807k
30. Krki N. Chitosan-laminated collagen film. *Food Technol Biotechnol.* 2012;50(4):483-489.
31. Thomas V, Dean DR, Vohra YK. Nanostructured biomaterials for regenerative medicine. *Curr Nanosci.* 2006;2(3):155-177.
doi: 10.2174/1573413710602030155
32. Krishnamoorthy G, Selvakumar R, Sastry TP, Mandal AB, Doble M. Effect of D-amino acids on collagen fibrillar assembly and stability: Experimental and modelling studies. *Biochem Eng J.* 2013;75:92-100.
doi: 10.1016/j.bej.2013.04.002
33. Riching KM, Cox BL, Salick MR, *et al.* 3D collagen alignment limits protrusions to enhance breast cancer cell persistence. *Biophys J.* 2014;107(11):2546-2558.
doi: 10.1016/j.bpj.2014.10.035
34. Fraley SI, Wu PH, He L, *et al.* Three-dimensional matrix fiber alignment modulates cell migration and MT1-MMP utility by spatially and temporally directing protrusions. *Sci Rep.* 2015;5:14580.
doi: 10.1038/srep14580
35. Sapudom J, Rubner S, Martin S, *et al.* The phenotype of cancer cell invasion controlled by fibril diameter and pore size of 3D collagen networks. *Biomaterials.* 2015;52:367-37513.
doi: 10.1016/j.biomaterials.2015.02.022
36. Fu R, Zhu K, Li Z, *et al.* Type III collagen promotes pseudopodium-driven cell migration. *Chem Bio Eng.* 2024;2(2):97-109.
doi: 10.1021/cbe.4c00133
37. Condeelis J. Life at the leading edge: The formation of cell protrusions. *Annu Rev Cell Biol.* 1993;9:411-444.
doi: 10.1146/annurev.cb.09.110193.002211
38. Parent CA, Blacklock BJ, Froehlich WM, Murphy DB, Devreotes PN. G protein signaling events are activated at the leading edge of chemotactic cells. *Cell.* 1998;95(1):81-91.
doi: 10.1016/s0092-8674(00)81784-5
39. Chodniewicz D, Zhelev DV. Novel pathways of F-actin polymerization in the human neutrophil. *Blood.* 2003;102(6):2251-2258.
doi: 10.1182/blood-2002-09-2936
40. Zigmond SH. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. *J Cell Biol.* 1977;75(2):606-616.
doi: 10.1083/jcb.75.2.606
41. Holly SP, Larson MK, Parise LV. Multiple roles of integrins in cell motility. *Exp Cell Res.* 2000;261(1):69-74.
doi: 10.1006/excr.2000.5040
42. Lin YK, Liu DC. Comparison of physical-chemical properties of type I collagen from different species. *Food Chem.* 2006;99:244-251.
doi: 10.1016/j.foodchem.2005.06.053
43. Oechsle AM, Akgün D, Krause F. Microstructure and physical-chemical propertie sof chicken collagen. *Food Struct.* 2016;7:29-37.
doi: 10.1016/j.foostr.2016.02.001
44. Potti RB, Fahad MO. Extraction and characterization of collagen from broiler chicken feet (*Gallus gallus* domesticus) -biomolecules from poultry waste. *J Pure Appl Microbiol.* 2017;11(1):315-322.
doi: 10.22207/jpam.11.1.39
45. Kittiphattanabawon P, Benjakul S, Visessanguan W, Kishimura H, Shahidi F. Isolation and characterisation of collagen from the skin of brown banded bamboo shark (*Chiloscyllium punctatum*). *Food Chem.* 2010;119:1519-1526.
doi: 10.1016/j.foodchem.2009.09.037
46. Perez-Puyana VM, Jiménez-Rosado M, Romero A, Guerrero A. Highly porous protein-based 3D scaffolds with different collagen concentrates for potential application in tissue engineering. *J Appl Polym Sci.* 2019;136:47954.
doi: 10.1002/APP.47954
47. Guan Y, He J, Chen J, *et al.* Valorization of fish processing by-products: Microstructural, rheological, functional, and properties of silver carp skin type I collagen. *Foods.* 2022;11:2985.
doi: 10.3390/foods11192985
48. Khong NMH, Yusoff FM, Jamilah B, *et al.* Improved collagen extraction from jellyfish (Acromitus hardenbergi) with increased physical-induced solubilization processes. *Food Chem.* 2018;251:41-50.
doi: 10.1016/J.Foodchem.2017.12.083
49. Balikci E. Characterization of collagen from jellyfish *Aurelia aurita* and investigation of biomaterials potentials. *Appl Biochem Biotechnol.* 2024;196:6200-6221.
doi: 10.1007/s12010-023-04848-5
50. Lin K, Zhang D, Macedo MH, Cui W, Sarmento B, Shen G. Advanced collagen-based biomaterials for regenerative biomedicine. *Adv Funct Mater.* 2019;29(3):1804943.
doi: 10.1002/adfm.201804943
51. Van Vlierberghe S, Graulus GJ, Keshari Samal S, Van Nieuwenhove I, Dubruel P. 12 - Porous hydrogel biomedical foam scaffolds for tissue repair. In: Netti P, editor. *Biomedical Foams for Tissue Engineering Applications.* Sawston UK: Woodhead Publishing; 2014. p. 335-390.
doi: 10.1533/9780857097033.2.335
52. Perez-Puyana V, Jiménez-Rosado M, Romero A, Guerrero A. Fabrication and characterization of hydrogels based on gelatinised collagen with potential application in tissue engineering. *Polymers (Basel).* 2020;12(5):1146.
doi: 10.3390/polym12051146
53. Pugliano M, Vanbellinghen X, Schwinté P, Benkirane-Jessel N, Keller L. Combined jellyfish collagen type ii, human stem cells and

tgf-B3 as a therapeutic implant for cartilage repair. *J Stem Cell Res Ther.* 2017;7(2):382.
doi: 10.4172/2157-7633.1000382

54. Mathew-Steiner SS, Roy S, Sen CK. Collagen in wound healing. *Bioengineering.* 2021;8:5-63.
doi: 10.3390/bioengineering8050063
55. Zhang Z, Li G, Shi B. Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. *J Soc Leather Technol Chem.* 2005;90:23-28.
56. He L, Lan W, Zhao Y. Characterization of biocompatible pig skin collagen and application of collagen-based films for enzyme immobilization. *RSC Adv.* 2020;10:7170-7180.
doi: 10.1039/c9ra10794k
57. Sakai R, Ikeda S, Isemura T. Soluble collagen of chicken leg tendon, its denaturation temperature and hydrodynamic properties. *Bull Chem Soc Jpn.* 1967;40(12):2890-2894.
doi: 10.1246/bcsj.40.2890
58. Swatschek D, Schatton W, Kellermann J, Müller WE, Kreuter J. Marine sponge collagen: Isolation, characterization and effects on the skin parameters surface-pH, moisture and sebum. *Eur J Pharm Biopharm.* 2002;53(1):107-113.
doi: 10.1016/s0939-6411(01)00192-8
59. Manimegalai NP, Ramanathan G, Gunasekaran D, Jeyakumar GFS. Cardinal acuity on the extraction and characterization of soluble collagen from the underutilized abattoir junks for clinical demands. *Process Biochem.* 2022;122:29-37.
doi: 10.1016/j.procbio.2022.08.011
60. Muyonga JH, Cole CGB, Duodu KG. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). *Food Chem.* 2004;85:1-81-89.
doi: 10.1016/j.foodchem.2003.06.006
61. Neuffer MC, McDivitt J, Rose D, King K, Cloonan CC, Vayer JS. Hemostatic dressings for the first responder: A review. *Mil Med.* 2004;169:716-720.
doi: 10.7205/milmed.169.9.716
62. Cheng X, Shao Z, Li C, Yu L, Raja M, Liu C. Isolation, characterization and evaluation of collagen from jellyfish *Rhopilema esculentum* Kishinouye for use in hemostatic applications. *PLoS One.* 2017;12(1):e0169731.
doi: 10.1371/journal.pone.0169731
63. Shi Y, Zhang H, Zhang X, Chen Z, Zhao D, Ma J. Comparative study of two porous sponge scaffolds prepared by collagen derived from porcine skin and fish scales as burn wound dressings in a rabbit model. *Regen Biomater.* 2020;7(1):63-70.
doi: 10.1093/rb/rbz036
64. Coppola D, Oliviero M, Vitale GA, *et al.* Marine collagen from alternative and sustainable sources: Extraction, processing and applications. *Mar Drugs.* 2020;18(4):214.
doi: 10.3390/md18040214
65. Mahesh L, Kurtzman G, Shukla S. Regeneration in periodontics: Collagen-a review of its properties and applications in dentistry. *Compend Contin Educ Dent.* 2015;36(5):358-363.
66. De Jonge N, Foolen J, Brugmans MC, Söntjens SH, Baaijens FP, Bouten CV. Degree of scaffold degradation influences collagen (re) orientation in engineered tissues. *Tissue Eng Part A.* 2014;20(11-12):1747-1757.
doi: 10.1089/ten.TEA.2013.0517
67. Buchert J, Ercili Cura D, Ma H, *et al.* Crosslinking food proteins for improved functionality, *Annu Rev Food Sci Technol.* 2010;1:113-138.
doi: 10.1146/annurev.food.080708.100841
68. Nair M, Best SM, Cameron RE. Crosslinking collagen constructs: Achieving cellular selectivity through modifications of physical and chemical properties. *Appl Sci.* 2020;10(19):6911.
doi: 10.3390/app10196911
69. Alavarse AC, FrachiniE CG, Da Silva RLCG, Lima VH, Shavandi A, Petri DFS. Crosslinkers for polysaccharides and proteins: Synthesis conditions, mechanisms, and crosslinking efficiency, a review. *Int J Biol Macromol.* 2022;202:558.
doi: 10.1016/j.ijbiomac.2022.01.029
70. Leonard AR, Cumming MH, Ali MA, Cabral JD. Fish collagen cross-linking strategies to improve mechanical and bioactive capabilities for tissue engineering and regenerative medicine. *Adv Funct Mater.* 2024;2405335.
doi: 10.1002/adfm.202405335
71. O'Neill L, Dobbyn P, Kulkarni M, Pandit A. Wound healing using plasma modified collagen. *Clin Plasma Med.* 2018;12:23-32.
doi: 10.1016/j.cpme.2018.10.002
72. Lv X, Lai L, Xu Y, *et al.* Effects of riboflavin/ultraviolet-A scleral collagen cross-linking on regional scleral thickness and expression of MMP-2 and MT1-MMP in myopic guinea pigs, *PLoS One.* 2023;18(1):0279111.
doi: 10.1371/journal.pone.0279111
73. Ouyang L, Highley CB, Sun W, Burdick JA. A generalizable strategy for the 3D bioprinting of hydrogels from nonviscous photo-crosslinkable inks. *Adv Mater.* 2017;29(8):1604983.
doi: 10.1002/adma.201604983
74. Adamiak K, Sionkowska A. Current methods of collagen cross-linking. *Int J Biol Macromol.* 2020;161:550-560.
doi: 10.1016/j.ijbiomac.2020.06.075
75. Ab Aziz NA. Extraction, anti-tyrosinase, and antioxidant activities of the collagen hydrolysate derived from *Rhopilema hispidum. Prep Biochem Biotechnol.* 2021;51(1):44-53.
doi: 10.1080/10826068.2020.1789991
76. Sampath Kumar NS, Nazeer RA, Jaiganesh R. Wound healing properties of collagen from the bone of two marine fishes. *Int J Pept Res Ther.* 2012;18-185-192.
doi: 10.1007/s10989-012-9291-2
77. Santos MH, Silva RM, Dumont VC. Extraction and characterization of highly purified collagen from bovine pericardium for potential bioengineering applications. *Mater Sci Eng C Mater Biol Appl.* 2013;33(2):790-800.
doi: 10.1016/j.msec.2012.11.003
78. Zourazema S, Zendeh Z, Aksun ET, *et al.* Extraction, characterization, and biocompatibility evaluation of type i collagen from chicken feet skin. *Biomed Mater Devices.* 2025:1-15.
doi: 10.1007/s44174-024-00266-1
79. Buehler MJ. Nature designs tough collagen: Explaining the nanostructure of collagen fibrils. *Proc Natl Acad Sci USA.* 2006;103(33):12285-12290.
doi: 10.1073/pnas.0603216103
80. Chowdhury SR, Mh Busra MF, Lokanathan Y, *et al.* Collagen type I: A versatile biomaterial. *Adv Exp Med Biol.* 2018;1077:389-414.
doi: 10.1007/978-981-13-0947-2_21
81. Cunniffe GM, O'Brien FJ. Collagen scaffolds for orthopedic regenerative medicine. *JOM.* 2011;63:66-73.
doi: 10.1007/s11837-011-0061-y
82. Ahmed M, Verma AK, Patel R. Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: A review. *Sustain Chem Pharm.* 2020;18:100315.
doi: 10.1016/j.scp.2020.100315

Received: April 2, 2025
Revised: May 13, 2025
Accepted: May 15, 2025
Available online: June 20, 2025