DOI: 10.2478/9788367405805-009

COLLAGEN EXTRACTED FROM PERCH SKIN: RHEOLOGICAL CHARACTERIZATION AND *IN VIVO* ANIMAL STUDIES

ALINA ELENA COMAN[1], MĂDĂLINA GEORGIANA ALBU KAYA[1], ELENA-EMILIA TUDOROIU[2,3*], DENISA IOANA UDEANU[4], MIHAELA VIOLETA GHICA[2,3]

[1]*The National Research and Development Institute for Textiles and Leather - Division: Leather and Footwear Research Institute (INCDTP-ICPI), 93 Ion Minulescu St., Bucharest, Romania*

[2]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Physical and Colloidal Chemistry, 6 Traian Vuia Str., 020956, Bucharest, Romania, *Corresponding author: elena-emilia.tudoroiu@drd.umfcd.ro*

[3]*Innovative Therapeutic Structures R&D Center (InnoTher), "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*

[4]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Clinical Laboratory and Food Safety, 6 Traian Vuia Str., 020956, Bucharest, Romania*

To recover as much collagen as feasible to meet market demand, more efficient and sustainable sources should be exploited instead of mammalian collagen. One of the most effective potential sources of high-quality collagen is fish wastes, which represent over 75% of a fish's weight and is produced in huge quantities all over the world, during fish processing. This waste includes skin, bones, scales, viscera, ligaments and fins. Consequently, collagen extracted from fish wastes is environmentally friendly, sustainable, and especially profitable when using fish industry by-products as possible sources of the material. Furthermore, different socio-political groups consider collagen extracted from marine resources as religiously acceptable, making it more valuable in all industry sectors. The purpose of this paper was to analyse the rheological behaviour of collagen gel extracted from perch (*Perca fluviatilis*) skin and to evaluate the therapeutic effect of spongious matrix (obtained through freeze-drying process of the gel extracted from perch skin) in the healing process of an induced burn in an animal model (Wistar rats).

Keywords: perch skin collagen, rheological analysis, *in vivo* animal analysis.

INTRODUCTION

Studies on the extraction of collagen from fish waste were initiated over two decades ago. One of the most prevalent proteins in mammals is collagen, which is present in the extracellular matrix of connective tissues such as blood vessels, skin, bones, tendons, ligaments, cartilage, and intervertebral discs (Nimni & Harkness, 2018). Collagens play regulatory roles (i.e., through mechano-chemical transduction processes) during tissue growth and repair in addition to their involvement in the preservation and strength of tissue architecture (Mahmood *et al*., 2022). Collagens are inherently bioactive, biocompatible, and biodegradable due to their nature. Collagens considered as the most widely needed and used biomaterials across a wide range of industries, including the food, pharmaceutical, medical, cosmetic, and nutraceutical sectors. Collagens can be found in injectable solutions, thin substrates, porous sponges, nanofibrous matrices, and micro- and nano-spheres.

Despite the fact that fish collagen normally has a lower molecular weight and denaturation temperature than mammalian collagen, recent studies have shown that the molecular structure and biochemical properties of collagen derived from fish and mammalian sources are very similar (El Blidi *et al*., 2021; Xu *et al*., 2021). Multiple ways of extracting fish collagen have been established based on the type of tissue and fish species that are being exploited. Currently,

https://doi.org/10.2478/9788367405805-009

in the literature, there are not so many studies, as we know, regarding marine collagen extracted from perch (*Perca fluviatilis*) skin used for biomaterials synthesis, such as wound dressings.

In this respect, understanding the flow properties of perch collagen gels represents a key factor, due to the influence of these properties upon manufacturing technology, quality control, stability during storage, type of use, and therapeutic activity (Ghica *et al*., 2012).

The objective of this paper is represented by the rheological evaluation of collagen extracted from perch (*Perca fluviatilis*) skin, and pre-clinical tests of corresponding collagen spongious matrix (*in vivo* animal analysis) used for biomaterials synthesis, such as wound dressings.

MATERIALS AND METHODS

Materials

The fresh perch skin was bought from a local fishing company. The fresh skin was washed with cold distilled water in the first step. After washing, several treatments (acidic and alkaline) followed in order to extract collagen gel. The acid used for acidic treatment was ascorbic acid (Scharlau, Sentmenat, Spain).

Collagen gel extracted from perch skin through various treatments was rheological analysed. The extracted perch gel was poured in a glass Petri dish and freeze-dried for 48 hours using the Martin Christ 24 Delta LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) (Albu *et al*., 2010) to obtain the collagen spongious matrix. The collagen sponge resulted from lyophilization was subsequently *in vivo* animal tested. The animals were obtained from the biobase of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania.

Methods

Rheological Evaluation of Perch Collagen Gel

The rheological determination of the perch collagen gel was carried out with the RM100 PLUS rheometer (Lamy Rheology Instruments, France), using the MS-DIN11 coaxial cylinder measurement system. The temperature of the rheological analysis was kept constant at 24°±0.1°C (conditioning temperature of semisolid formulations), using a circulating water bath (Julabo Corio CD). During the experiment, the gel must be kept at a constant temperature because the rheological properties are strongly dependent on temperature. The experimental program was set-up from the RheoTex software. To determine the flow properties of the collagen gel, the limits for the shear rate were chosen between 0.4 and 78 s^{-1}, corresponding to rotational speeds in the range of 0.3-60 rpm.

Evaluation of the Spongious Matrix Therapeutic Effect in Healing Process on Animal Model

The *in vivo* study of therapeutic effect of the collagen sponge, extracted from perch skin, in the healing process of an induced burn to an animal model (Wistar rats), was carried out in accordance with Directive 2010/63/EU (Council of the European Union) and Law no. 43 (Romanian Parliament - April 11, 2014).

This study was performed on 12 Wistar male rats, weighing about 180 ± 10 g, kept under regular laboratory conditions (they received water *ad libitum* and food twice a day). The experiment was carried out in "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania with the agreement of the Ethics Committee no. 21226/ 26.07.2023. The animals were distributed into two groups of 6 animals each: group 1 – collagen perch matrix (COLL_A) and

group 2 – control (the wounds were covered with sterile gauze). The *in vivo* experiment was carried out as previously described (Ghica *et al*., 2017; Udeanu *et al*., 2018; Marin *et al*., 2018). Shortly, in order to properly perform the experiment, the animals were anesthetized with ethyl ether, and the hair in the dorsal area was removed. On this shaved surface, the experimental lesion was induced using a metallic accessory with a diameter of 1 cm, previously heated in a physiological solution, at the point of boiling. The metallic accessory was applied to the skin for 10-15 seconds. Afterwards, the collagen spongious matrix obtained from perch gel and the sterile gauze were applied on the experimentally induced injuries, being fixed with a silk bandage. The evolution monitoring of healing process was observed with a digital camera and the diameter of the lesion was measured at different intervals of time for a period of 16 days.

RESULTS AND DISCUSSION

Rheological Evaluation of Perch Collagen Gel

The results of the rheological study obtained for collagen gel from perch skin (GEL_COLL_A), tested at a temperature of 24°C, led to flow profile plotted as viscosity versus shear rate as shown in Figure 1.

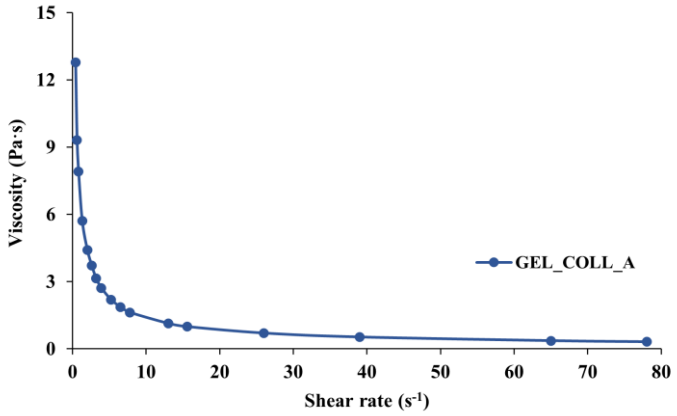

Figure 1. Flow profile – viscosity versus shear rate of GEL_COLL_A, recorded at 24°C

According to the rheogram illustrated in Figure 1, it can be seen that the viscosity of the tested gel decreases when the shear rate increases (Popa *et al*., 2021), suggesting a non-Newtonian pseudoplastic (shear-thinning) behaviour at 24°C. This rheological feature can be explained by the deformation or orientation of the gel molecular network that are induced by the shear succeeding the flow direction. This effect can become more detectable when the shear rate increases, inducing a decline of the internal friction considering the less interaction between the molecules (Tudoroiu *et al*., 2023).

The mathematical model used to quantify the shear-thinning behaviour of the perch collagen gel was the Power Law model that describes two principal parameters: m - consistency index factor, and n - flow behaviour index. The constitutive equation of Power Law model is presented by Equation (1) as follows:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (1)$$

The m parameter can be attributed to a Newtonian material viscosity for a shear rate value of $1 \cdot s^{-1}$. On the other side, n parameter indicates the pseudoplasticity degree of a semisolid formulation. Its values range between 0 and 1, and it is dimensionless; accordingly, a material is more pseudoplastic when its n value is lower than 1 (Tudoroiu *et al*., 2023). Both rheological

parameters are evaluated through the linearization of the previous equation by double logarithm. The Power Law model parameters values for the perch collagen gel tested at 24°C are listed in Table 1.

Table 1. Values of Power Law model parameters for the perch collagen gel, analyzed at 24°C

Gel	m	n	R^2
GEL_COLL_A	6.795	0.68	0.9990

According to Table 1, the determination coefficient R^2 value higher than 0.99 illustrates that the previously described mathematical model is adequate to properly fit the experimental data. Considering that the pseudoplasticity degree is greatly influenced by the value of the flow index, it can be noticed that the GEL_COLL_A sample has a n parameter value closer of 0.68, indicating a moderate pseudoplasticity character. The pseudoplastic behaviour represents a fundamental requirement for a semisolid formulation that is addressed for topical use, because this property allows a proper flow of the gel when it is expelled from the conditioning container, and when it is administered on skin surface.

An important rheological feature of certain dispersed pharmaceutical formulations is the dependence of the shear stress - shear rate relation on shearing time, but also on their earlier exposure to a shear stress. Accordingly, the formulation viscosity suffers modifications with the shear rate, but if the pharmaceutical system is maintained at rest long enough, its viscosity can restore to the initial value, the modification being reversible. Therefore, this behaviour named thixotropy represents the progressive decrease of a pharmaceutical system viscosity and consequently of its shear stress as a consequence of the agitation caused to the system through shear. After a more or less extended rest period, the pharmaceutical system recovers the rheological characteristics and restores its initial structure, which was impaired during the continuous shear measurements. The thixotropy is a characteristic of dispersed formulations with plastic and pseudoplastic flow (Ghica *et al.*, 2012).

To study this type of flow, the rheogram shear stress as a function of shear rate is plotted, which corresponds to the ascending, S_{asc} (forward) curve for increasing shear rate values, and to descending, S_{desc} (backward) curve for decreasing shear rate values. For the same shear rate, the points on the backward curve are correlated with smaller shear stress values compared to those on the forward curve. Thus, the thixotropy hysteresis loop is obtained.

The rheological profiles corresponding to the ascending and descending curves were built in order to assess the thixotropic behaviour of the perch collagen gel, tested at 24°C (Figure 2).

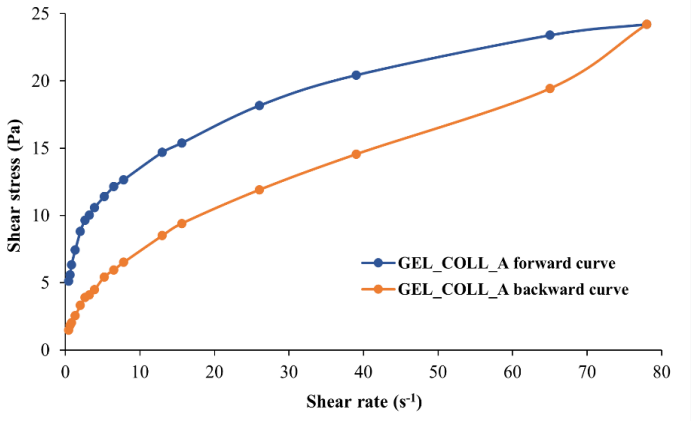

Figure 2. Ascending and descending shear rate versus shear stress curves of perch collagen gel, recorded at 24°C

Figure 2 shows that the perch collagen gel exhibits a thixotropic behaviour at 24°C, because the descending curve is placed below the ascending one, suggesting that the shear stress corresponding to the backward curve is lower at the same shear rate value.

The thixotropy phenomenon can be described by two main descriptors:

- thixotropy area (S_{thix}) represents the area between the ascending and descending curve;
- thixotropy index ($T_{hyst\%}$) defines the thixotropy relative area, which can be determined as percentage of the by the stirring at maximum rotational speed depending on the ascending area. Consequently, a system is more thixotropic if the thixotropy rheoimpaired area index value is greater than 5% (Tudoroiu *et al*., 2022).

The corresponding values of the thixotropic descriptors for GEL_COLL_A sample, analyzed at 24°C, are shown in Table 2.

Table 2. Values of thixotropic descriptors for perch collagen gel, tested at 24°C

Gel	S_{asc} (Pa· s^{-1})	S_{desc} (Pa· s^{-1})	S_{thix} (Pa· s^{-1})	$T_{hyst\%}$ (%)
GEL_COLL_A	1490.685	1104.387	386.298	25.92

According to Table 2, the value of the thixotropy index is much higher than 5% for GEL_COLL_A sample, which means that the tested semisolid system presents a strong degree of thixotropy at 24°C.

Evaluation of the Spongious Matrix Therapeutic Effect in Healing Process on Animal Model

In Figure 3 are illustrated the macroscopic images regarding the evolution of the healing process, recorded for each group for 16 days.

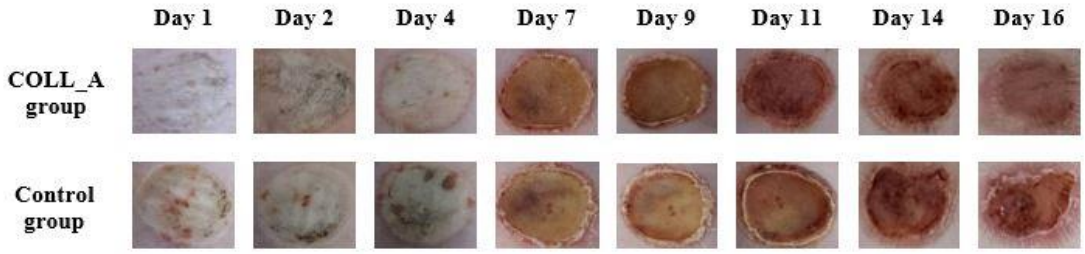

Figure 3. Evolution of the healing process of Wistar rats' lesions without treatment (Control group), and after treatment with spongious matrix from perch gel, at different time intervals

As it can be noticed in Figure 3, on the first day when the burn was induced, the injury can be described as a white eschar, with epidermal and dermal tissue destruction, and a hyperemic area that highlights the presence of an inflammatory process (Marin *et al*., 2018). According to the above images, the application of collagen spongious matrix, based on collagen extracted from perch skin, led to an acceleration of the healing process compared to the control group.

On the second day of investigation, it was found that the application of collagen spongious matrix conduced to the absorption of the exudate from the wounds. Consequently, on 4th day of animal observation, the initiation of the healing process at the edges of the lesions can be observed. Thereby, seven days after the induction of the burns, the complete formation of the scab for both groups can be noticed.

The evaluation of the healing process was carried out by measuring the dimensions of the wound and was calculated according to Equation (2):

$$\text{Healing process } (\%) = \frac{W_i - W_t}{W_i} \times 100 \quad (2)$$

where W (wound size) generally represents the average of the largest and smallest lesion size, W_i - initial size of the wound, and W_t - wound size at different intervals of time. The healing process was calculated according to Equation (2) and its evolution is shown in Figure 4.

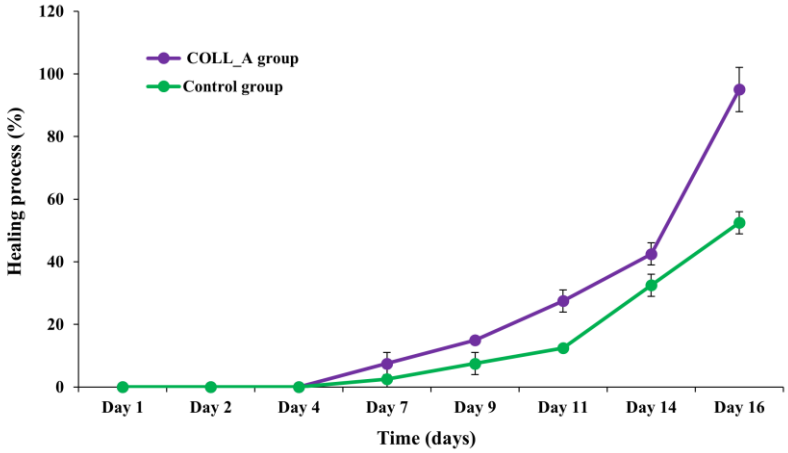

Figure 4. Evolution of the wound healing process after treatment with perch collagen spongious matrix and for untreated animals (control group)

According to Figure 4, after 11 days of observation, the lesions size reduced by 27.5% for the animals treated with perch collagen spongious matrix (COLL_A group) compared to the control group, where the wounds size reduced by only 12.5%. On 14th day of observation, the wounds size decreased by more than 40% for the COLL_A sample, while for the control group, the lesions dimension decrease was by 32.5%. On the last day of observation, the injuries size reduced by 95% for animals treated with the COLL_A sample, the animals being almost healed compared to the control group where the reduction of the animals' injuries was only by 52.5%.

CONCLUSIONS

Concluding, the rheological analysis of collagen gel extracted from perch (*Perca fluviatilis*) skin evaluated the flow properties by investigating the pseudoplastic behaviour through the analysis of the viscosity - shear rate dependence and by analysing the thixotropic character using the specific descriptors (thixotropy area and thixotropic index). Therefore, perch collagen gel exhibited a non-Newtonian pseudoplastic and thixotropic behaviour at working temperature of 24°C, which are fundamental features for a semisolid system in terms of improving its conditioning and spreadability on epidermal surface.

In vivo animal studies demonstrated that the animals treated with collagen spongious matrix from perch skin presented a faster healing, without any secondary effect compared to the control group. Thus, this perch collagen matrix can represent a potential scaffold to treat burns, with valuable effects on the cutaneous tissue's restoration.

Acknowledgement

This study was financially supported by a grant of the Ministry of Research, Innovation and Digitalization, project number PN 23 26 01 01/2023 & M-ERA-NET Project no.73/2024, and by the Carol Davila University of Medicine and Pharmacy Bucharest, Romania, through Contract No. CNFIS-FDI-2024-F-0570.

REFERENCES

Albu, M.G., Ficai, A. & Lungu, A. (2010). Preparation and Characterization of Collagen Matrices Obtained at Different Freezing Temperatures. *Leather and Footwear Journal*, *10*(3), 39-50.

El Blidi, O., El Omari, N., Balahbib, A., Ghchime, R., El Menyiy, N., Ibrahimi, A., Ben Kaddour, K., Bouyahya, A., Chokairi, O. & Barkiyou, M. (2021). Extraction Methods, Characterization and Biomedical Applications of Collagen: A Review. *Biointerface Research in Applied Chemistry*, *11*(5), 13587–13613. https://doi.org/10.33263/-BRIAC115.1358713613

Ghica, M.V., Albu Kaya, M.G., Dinu-Pirvu, C.E., Lupuleasa, D. & Udeanu, D.I. (2017). Development, Optimization and *In Vitro*/*In Vivo* Characterization of Collagen-Dextran Spongious Wound Dressings Loaded with Flufenamic Acid. *Molecules*, *22*(9), 1552. https://doi.org/10.3390/molecules22091552

Ghica, M.V., Albu, M.G., Dinu-Pirvu, C. & Moisescu, S. (2012). *In vitro* Kinetic Release and Flow Behaviour of Some Collagen-Minocycline Topical Hydrogels. *Revista de Chimie*, *63*(9), 929-935.

Mahmood, A., Patel, D., Hickson, B., DesRochers, J. & Hu, X. (2022). Recent Progress in Biopolymer-Based Hydrogel Materials for Biomedical Applications. *International Journal of Molecular Sciences*, *23*(3), 1415, https://doi.org/10.3390/ijms23031415

Marin, Ș., Albu Kaya, M.G., Ghica, M.V., Dinu-Pîrvu C.E., Popa, L., Udeanu, D.I., Mihai, G. & Enăchescu, M. (2018). Collagen-Polyvinyl Alcohol-Indomethacin Biohybrid Matrices as Wound Dressings. *Pharmaceutics*, *10*(4), 224. https://doi.org/10.3390/pharmaceutics10040224

Nimni, M.E. & Harkness, R.D. (2018). *Collagen*, *Chapter 1: Molecular Structure and Functions of Collagen*. CRC Press: Boca Raton, FL. https://doi.org/10.1201/9781351070799

Popa, L., Ghica, M.V., Popescu, R., Irimia, T. & Dinu-Pîrvu, C.E. (2021). Development and Optimization of Chitosan-Hydroxypropyl Methylcellulose *in situ* Gelling Systems for Ophthalmic Delivery of Bupivacaine Hydrochloride. *Processes*, *9*(10), 1694. https://doi.org/10.3390/pr9101694

Tudoroiu, E.-E., Albu Kaya, M.G., Titorencu, I., Dinu-Pîrvu, C.E., Marin, M.M., Roșca, A.-M., Popa, L., Anuța, V., Antoniac, A., Chelaru, C., Kaya, D.M., Prisada, R.M. & Ghica M.V. (2023). Design and Evaluation of New Wound Dressings Based on Collagen-Cellulose Derivatives. *Materials & Design*, *236*, 112469. https://doi.org/10.1016/j.matdes.2023.112469

Tudoroiu, E.-E., Ghica M.V., Albu Kaya, M.G., Dinu-Pîrvu, C.E., Popa, L., Anuța, V., Vlescu, B.Ș., Kaya, D.A., Marin, M.M. & Prisada, R.M. (2022). Rheological Characterization of Some Cellulose Derivatives-based Hydrogels. *Proceedings of the 8th International Conference on Advanced Materials and Systems (ICAMS 2020)*, 139-144. https://doi.org/10.24264/icams-2020.II.6

Udeanu, D.I., Albu Kaya, M.G., Ghica, M.V., Marin, Ș, Marin, Kaya, D.A., Popa, L. & Dinu-Pîrvu, C. (2018). Anti-Inflammatory Drug-Loaded Biopolymeric Spongious Matrices with Therapeutic Perspectives in Burns Treatment. *Farmacia*, *66*(5), 783-790. https://doi.org/10.31925/farmacia.2018.5.7

Xu, Q., Torres, J.E., Hakim, M., Babiak, P.M., Pal, P., Battistoni, C.M., Nguyen, M., Panitch, A., Solorio, L. & Liu, J.C. (2021). Collagen- and Hyaluronic Acid-based Hydrogels and Their Biomedical Applications. *Materials Science and Engineering: R: Reports*, *146*, 100641. https://doi.org/10.1016/j.mser.2021.100641