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Application of Optical Methods in Standardization of Collagen-containing Hydrogen for 3D Bioprinting of Supporting and Connective Tissues

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Abstract. The paper presents the results of the application of optical methods in the standardization of collagen-containing allogeneic hydrogel produced at the Biotech Research Institute (Samara, Russia) from bioimplants of the Lioplast® trademark in comparison with the hydrogels available on the market from Rokit and Cellink companies. The Raman spectroscopy method was used as the main research method. An additional research method was the method of IR Fourier spectroscopy.

As a result of the conducted research using optical methods, it was found that the collagen structure is completely preserved in the composition of the allogeneic hydrogel produced at the Biotech Research Institute (Samara, Russia). Hydrogels of imported Rokit and Cellink companies also have a similar spectral composition.The obtained results can be further used as a rapid assessment and standardization of collagen-containing hydrogel with the addition of various components for personalized 3D bioprinting of human supporting and connective tissues.

As part of the current import substitution task, the developed collagen-containing allogeneic hydrogel may in the future represent a competitive analogue to foreign commercial products — hydrogels in bioprinting.

Keywords: Raman spectroscopy, infrared Fourier spectroscopy, collagen-containing hydrogel, supporting and connective tissues, 3D bioprinting.

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Introduction

To date, 3D bioprinting of tissues and organs is one of the promising methods of biofabrication and is of great interest in the direction of creating complex cellular and tissue constructs for tissue regeneration, which allows for the manufacture of personalized implants taking into account the anatomy, pathology and biomechanical properties of organs and body parts of the patient's body [1]. The bioprinting process is carried out using bioinks, which include two main components — cells and hydrogel. Hydrogels are three-dimensional polymer meshes that, due to The properties of hydrophilicity are capable of retaining large amounts of water. Various natural polymers are used to produce hydrogels, among them biopolymers of allogeneic origin have predominant characteristics, including good biocompatibility and biodegradability, as well as low antigenicity and high regenerative potential. Commercial bioinks presented on the market today are characterized by a fairly high cost, which makes it necessary to develop our own domestic competitive analogues of such bioinks within the framework of the direction import substitution [2, 3].

Among the large number of biogenic hydrogels available on the market today, the hydrogels of PureColo "Cellink" (Sweden) and hydrogel can be distinguished INVIVO-GEL-ESSENTIAL (Korea), which have already successfully proven themselves as products for bioprinting and perfectly combined with various types of cells. The presented products are mainly used in scientific research tasks. Today, the Russian Federation is actively working on the development of domestic hydrogels, the creation of which will solve the problem of import substitution of foreign bioinks.

An important step in the bioprinting process of various constructs is their standardization, both at the stage of obtaining the initial material for bioprinting - hydrogels, and the final printed product. Therefore, it is necessary to evaluate the quality of hydrogels and bioinks in order to obtain data on the structure of the components contained in them. Among the physical research methods, optical methods such as Raman spectroscopy and IR Fourier spectroscopy are widely used, which They are non-destructive and operational methods of analysis, as well as widely used for solving biomedical problems [4-9]. Thus, in the work of the authors [4-5], studies on the use of Raman spectroscopy to assess the composition of tissues with a detailed interpretation of the main lines of Raman are presented.

The authors of the work [6], using the method of Raman spectroscopy, investigated the composition of biomaterials and extracellular matrix, including bone marrow. In the work of the authors [7] it is shown that using this method it is possible to determine the composition of the cell. So in The work of the authors [8] shows that non-destructive analysis of various biomaterials can be carried out using IR spectroscopy (FTIR).

Therefore, the aim of the work was to evaluate the possibility of using optical methods in the standardization of collagen-containing hydrogel for 3D bioprinting of human supporting and connective tissues.

Materials and methods of research

The objects of the study were: group 1 — allogeneic collagen-containing hydrogel (obtained from human bone tissue), Samara, Russia, Biotech Research Institute, Lioplast ©; group 2 — PureCol hydrogel © "Cellink" (Sweden); group 3 — INVIVO-GEL-ESSENTIAL hydrogel (Korea); group 4 — type I collagen sample "Cellink" (Sweden). The collagen-containing hydrogel was

obtained from an allogeneic material of demineralized bone tissue, pretreated using the original technology for obtaining bioimplants of the trademark "Lyoplast"© (TU- 9398-001 -01963143-2004, patent R+ No. 2366173 dated 05/15/2008; Certificate of conformity ISO 13485:2016, reg. No. RU CMS-RU.PT02.00115; ISO 9001:2015 certificate, per TIC 15 100 159171) [9].

Raman spectroscopy and IR Fourier spectroscopy were used as research methods. The Raman spectroscopy (Raman) method was implemented using an experimental stand consisting of a semiconductor laser (LML-785.ORB-04.450 MW), a spectrograph (Andor Sharmrock SR-303i) with an integrated digital camera cooled to –60°C, an optical raman module (PBL785) and a computer. The use of this spectrograph provided a resolution of 0.15 nm in wavelength at a low level of intrinsic noise. In this work, the Raman spectra were analyzed in the range 700–1800 cm^{-1}. The Raman spectra were recorded using an optical probe, which was located above the object at a distance of 7 mm. Further processing of the Raman spectra consisted in filtering autofluorescence in the Raman spectra using the method of subtracting the fluorescent component by polynomial approximation I-ModPoly with polynomial degree 11 [10].

Normalization and smoothing of Raman spectra were performed using the SNV and Maximum Likely good Estimation Savitzky–Golay filter (ME-SG) (=4) methods. Infrared spectra were obtained using a Fourier spectrometer FT modification 801 (factory number 465; manufacturer Limited Liability Company Scientific and production company "SIMEX" (LLC NPF "SIMEX"), Novosibirsk). Transmission spectra were recorded using the prefix of multiple disturbed total internal reflection (hereinafter referred to as the prefix MNPVO) at the following parameter values:

- spectrum resolution of 8 cm^{-1};
- the number of scans (accumulations) for obtaining spectra is 36.

The results of the study

Fig. 1 shows the averaged Raman spectra of the samples.

Fig. 1. Research materials

It can be seen from Fig. 1 that lines are present in all three studied groups 1-3 of hydrogels CR, which are characteristic of type I collagen "Cellink": 1242-1265 cm^{-1} (AmideIII/a-helix), 1412 cm^{-1} (CH_2 bending and scissoring modes of collagen and phospholipids), 1560 cm^{-1} (Amid II Parallel/Antiparallel a-sheet structure). At the same time, it can be seen that in the hydrogel of Korean production in the Raman spectra there are lines 1003 - 1075 cm^{-1} (Phenylalanine,

Breathingmode (collagenassignment)), 1650 cm^{-1} (AmideI/a-helix), which also correspond to type I collagen "CELLINK" and determine the elasticity of tissues. Spectra of allogeneic collagen-containing hydrogel and hydrogel PureCol0 "Cellink" have similar spectral characteristics and, unlike the Korean-made hydrogel, have a Raman line at 832 cm^{-1} (C-C stretching, proline and hydroxyproline (collagen assignment)), which determines the properties of collagen fibrils such as elasticity and elasticity, which is important for tissue bioprinting. Further, a detailed analysis of the studied objects was carried out, the results of which are presented in Tab. 1 and in Figs. 2 and 3.

Table 1. Metric values for each sample group.

–	Precision	Recall	fl-score	support
Hydrogel	0.00	0.00	0.00	2
Purecol	0.00	0.00	0.00	1
ESSENTIAL	0.40	1.00	0.57	2

As can be seen from Tab. 1, the value of the Fl-Score model is at the level of 40 percent, the ROC AUC is within 60 percent, and the rest of the metrics have low indicators, which indicates that groups 1–3 are difficult to distinguish between each other, which indicates the spectral similarity of their composition.

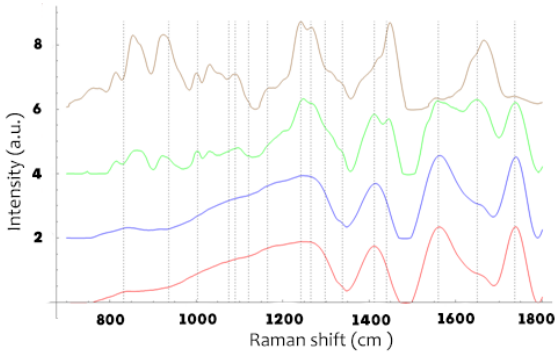

Fig. 2. Averaged Raman spectra of the samples: 1 (red line) — allogeneic collagen-containing hydrogel, 2(blue line) — PureCol° "Cellink" hydrogel, 3(green line) — INVIVO-GEL-ESSENTIAL hydrogel, 4(brown line) — type I collagen sample "Cellink")

According to the decision matrix, it is clear that all test spectra are correctly classified (5). According to the ROCAUC and Precision-Recall graphs, the low classifying ability of the resulting classifier is also visible.

The value of ROC AUC (Receiver Operating Characteristic Area Under Curve) equal to 0.5 indicates that the model is not able to identify differences between the studied groups, which also indicates their spectral similarity.

Thus, the results obtained using Raman spectroscopy showed spectral similarities of the three studied hydrogels.

Fig. 4 shows the Fourier-infrared spectra of the three types of hydrogels studied from different manufacturers, as well as type I collagen "Cellink".

In the infrared spectra of all samples in the range of 3310–3291 cm^{-1}, a wide peak of high intensity is observed, characteristic of valence vibrations of O-H, N-H ("Amide A") bound by intermolecular interaction (hydrogen bonds) . In the region of 3078–3074 cm^{-1}, both valence vibrations of the C-H (sp3) bond of heterocyclic fragments of amino acids that make up collagen (proline, oxyproline) and the "Amide B" band (stretching vibrations of C-N bonds) can

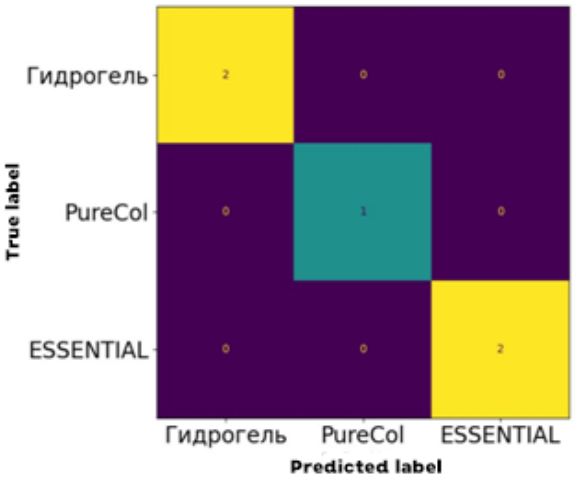

Fig. 3. Results matrix

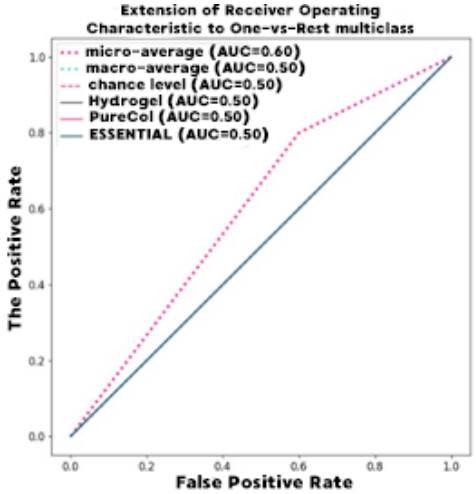

Fig. 4. Obtaining operating characteristics

manifest themselves. Absorption bands in the region of wave numbers 2958–2932 cm^{-1} and 2875–2874 cm^{-1} correspond to valence bands fluctuations in the C-H (sp3) bond of alkyl groups. The most significant and widely used band for the characterization of biological materials is the peak "Amide 1", located near the range of 1700–1580 cm^{-1}. It corresponds to the valence fluctuations of the C=O bond of the peptide group. In this case, high intensity bands at 1657–1631 cm^{-1} are present on the infrared spectra of all samples See^{-1}. These bands are characteristic of valence vibrations of C=0 and N-H bonds (peak "Amide 1"). In the region of 1555–1536 cm^{-1} on the infrared spectra of the standard collagen type 1 "Cellink" and samples of all bands of high intensity corresponding to stretching vibrations of C-N and planar vibrations of N-H bonds (peak "Amide 2") are observed in the analyzed hydrogels.
In the region of 1453–1447 cm^{-1} and 1405–1404 cm^{-1}, bands of medium and low intensity are present on all spectra , characteristic of both deformation vibrations of C-H and N-H bonds, and valence vibrations of C-N, C-C bonds. Further, in the region of 1340–1332 cm^{-1}, maxima are observed, most likely associated with deformation fluctuations of the CH_2 groups of the proline

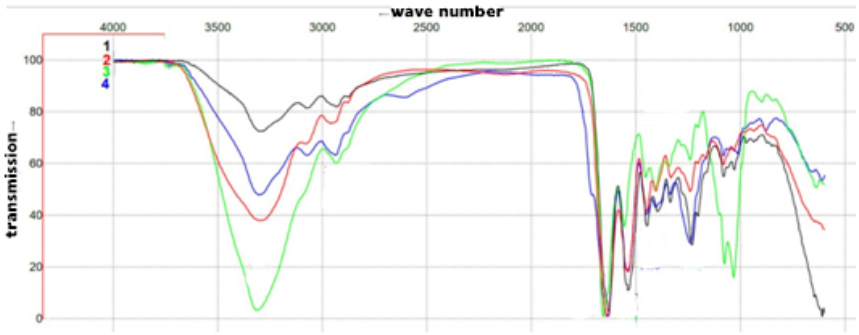

Fig. 5. Averaged IR spectra of the samples: 1 (black line) — type I collagen sample "Cellink"; 2(red line) — INVIVO-GEL-ESSENTIAL hydrogel; 3(green line) — PureCol° "Cellink" hydrogel; 4(blue line) — allogeneic collagen-containing hydrogel

residue in the collagen polypeptide chain.
Absorption bands in the area of 1240–1232 cm^{-1} are caused by characteristic fluctuations of the amide group, deformation fluctuations of the carbon skeleton of amino acids that are part of the polypeptide chain of the material under study ("Amide 3"). Based on the spectral analysis carried out, it can be concluded that the main absorption bands in the infrared spectrum of the allogeneic hydrogel developed at the Biotech Research Institute are associated with characteristic fluctuations of specific groups in polypeptides. The IR spectrum of the allogeneic hydrogel is identical to the IR spectrum of the standard type 1 collagen "Cellink", which reflects their qualitative composition. The transmission spectrum of the Biotech Research Institute hydrogel has similar absorption bands with commercial hydrogels from "Rokit" and "Cellink" companies.

Taking into account the spectral similarities of the hydrogel developed by Vinni Biotech and the PureCol Cellink hydrogel, it can be concluded that the allogeneic hydrogel developed at the Biotech Research Institute can subsequently be applied to 3D bioprinting of human supporting and connective tissues using commercially available 3D bioprinters in the framework of import substitution.

Results

As a result of the conducted studies using the Raman spectroscopy method, it was found that the composition of the studied hydrogels of imported production, as well as the manufactured collagen-containing hydrogel, revealed AMIDII (Raman Spectroscopy lines 1200–1300 cm^{-1}), Amide II (Raman Spectroscopy line 1554 cm^{-1}), CH_2 bending and scissoring modes of collagen and phospholipids 1450 cm^{-1} and Amide I (KP line 1650–1665 cm^{-1}. These CD lines indicate the presence and preservation of the collagen structure in the composition of the studied hydrogels. The spectral composition of the developed at the Research Institute Biotech (Russia, Samara, Biotech Research Institute, Lioplast8) of allogeneic hydrogel has a similar composition to hydrogels of imported production. Additionally, using IR Furie spectroscopy, it was found that the main absorption bands on the infrared spectrum of the allogeneic collagen hydrogel of the Biotech Research Institute are associated with characteristic fluctuations of specific groups in They are identical to the IR spectrum of the human type 1 collagen standard "Cellink", which reflects their qualitative composition and confirms the preservation of the collagen structure, and also has similar absorption bands with commercial hydrogels of Rokit and Cellink companies, which indicates the possibility of using the proposed allogeneic hydrogel of the Biotech Research

Institute in the future as an alternative to analogues available on the market as part of the import substitution program. The obtained research results can be further used as an express assessment and standardization of collagen-containing hydrogel with addition for personalized 3D bioprinting in the restoration of human supporting and connective tissues.

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Применение оптических методов в стандартизации коллагенсодержащего гидрогеля для 3D-биопечати опорных и соединительных тканей

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Аннотация. В работе представлены результаты применения оптических методов в стандартизации коллагенсодержащего аллогенного гидрогеля, произведенного в НИИ "Биотех" (г. Самара, Россия) из биоимплантатов торговой марки Lioplast® в сравнении с имеющимися на рынке гидрогелями компаний "Rokit" и "Cellink". В качестве основного метода исследования был использован метод спектроскопии комбинационного рассеяния. Дополнительным методом исследования являлся метод ИК-Фурье-спектроскопии.

В результате проведенных исследований с помощью оптических методов установлено, что в составе аллогенного гидрогеля, произведенного в НИИ "Биотех" (г. Самара, Россия), полностью сохранена коллагеновая структура. Подобный спектральный состав также имеют гидрогели импортного производства компаний Rokit и Cellink.Полученные результаты могут быть в дальнейшем использованы в качестве экспресс-оценки и стандартизации коллагенсодержащего гидрогеля с добавлением различных компонентов для персонализированного 3D-биопринтинга опорных и соединительных тканей человека.

В рамках актуальной на сегодняшний день задачи импортозамещения разработанный коллагенсодержащий аллогенный гидрогель в дальнейшем может представлять собой конкурентоспособный аналог зарубежным коммерческим продуктам — гидрогелям в биопечати.

Ключевые слова: инфракрасная Фурье-спектроскопия, метод спектроскопии комбинационного рассеяния, коллагенсодержащий гидрогель, опорные и соединительные ткани, 3D-биопечать.