Mendeleev Communications

Mendeleev Commun., 2025, **35**, 749–751

Polylactic acid-based scaffolds modified by hyaluronic acid

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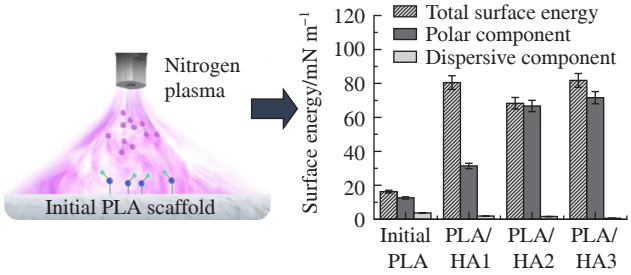
DOI: 10.71267/mencom.7761

The effect of two-stage surface modification of polylactic acid (PLA)-based scaffolds, including treatment with a low-temperature nitrogen plasma arc discharge and subsequent immobilization of biologically active molecules of hyaluronic acid, on their physicochemical and biological properties was investigated. It was found that the modification changes the chemical composition of the PLA surface, which contributes to an increase in surface energy and a decrease in the water contact angle. Modification of the PLA surface not only does not lead to the appearance of cytotoxic properties, but, on the contrary, helps to maintain the viability of immune cells on its surface.



Keywords: nonwoven fibrous materials, polylactic acid, electrospinning, low-temperature plasma, hyaluronic acid, surface properties, macrophages.

In recent years, there has been a need in medicine to develop effective methods for treating various skin diseases. This problem is becoming more and more urgent, which necessitates the use of innovative materials capable of providing suitable conditions for tissue regeneration. In this regard, nonwoven fibrous materials (scaffolds) based on polylactic acid (PLA) attract special attention due to their unique properties.[1] PLA is characterized by a high degree of safety for the body, as well as biodegradability, which makes this polymer suitable for medical use.[2,3] However, products made of plastics have low wettability, while medical materials must be hydrophilic and have optimal surface morphology to ensure cell adhesion.[4] This problem can be solved by improving the physicochemical and functional properties of the surface, combining methods of plasma treatment and immobilization of biologically active molecules, such as hyaluronic acid (HA).[5,6] In this work, nitrogen plasma is used as an activator of the polymer surface to form polar functional groups, which provides a stronger bond between the HA coating and the polymer substrate. The application of a coating of HA molecules on the surface of scaffolds has prospects in the field of tissue engineering, since HA is a natural component of the intercellular matrix and plays a key role in cell adhesion, migration and proliferation.[6] A number of studies have shown that the use of HA as a coating on the surface of PLA scaffolds significantly improves the biocompatibility and wettability of the surface, which contributes to more effective interaction with cells.[7,8] The study of the surface properties and the nature of the interaction of HA molecules with the plasma-modified PLA surface is an urgent task, since the quality and functionality of the created biomaterials directly depend on their interaction with the environment and cellular structures. The purpose of this work is to investigate the effect of two-stage modification on the physicochemical and biological properties of PLA-based scaffolds.[†]

The XPS spectra[‡] of all samples revealed a decrease in the oxygen atom content and an increase in the carbon atom content due to the two-stage modification, indicating parallel processes of destruction and cross-linking of polymer macromolecules. It should be noted that after modification, nitrogen atoms were detected on the surface of PLA-based scaffolds (Table 1) in an amount of 3.4 at% for PLA/HA1 and 8.7 at% for PLA/HA3, while the maximum value of 11.4 at% was achieved in the case of PLA/HA2. Atmospheric nitrogen is chemically inert and cannot interact with the activated surface layer of the sample after depressurization of the vacuum chamber. Consequently, nitrogen enters the sample directly during plasma modification. The presence of nitrogen

[†] Fiber materials based on PLA were obtained on a laboratory electrospinning setup (TSU, Tomsk, Russia). The obtained scaffolds were treated with nitrogen arc discharge plasma using a PINK plasma generator (IHCE SB RAS, Tomsk, Russia). The treatment conditions were as follows: discharge current 5 A, discharge power 250 W, working pressure in the chamber 0.3 MPa, distance between the sample and the plasma generator 150 mm and plasma concentration ~10^{10} cm^{-3}.[9] After surface activation, the samples were immersed in an aqueous solution of HA with concentrations of 0.1, 0.2 or 0.3 wt% for 24 h. The obtained materials were designated PLA/HA1, PLA/HA2 and PLA/HA3, respectively. Then the samples were washed with distilled water and dried at room temperature for 24 h.

[‡] The elemental and chemical composition of the surface was analyzed by X-ray photoelectron spectroscopy (XPS) at the RTU MIREA Nanocenter (Moscow, Russia) using an automated XPS microprobe. The CasaXPS software (Case Software Ltd.) was used to process the obtained spectra. According to the obtained data on the elemental composition of PLA, the initial substrate samples contain carbon and oxygen atoms.[10]

Table 1 The content of atoms of elements in the initial and modified PLA scaffolds, determined by XPS.

Sample	O 1*s* (at%)	C 1*s* (at%)	N 1*s* (at%)
Initial PLA	46.5 ± 2.3	53.5 ± 2.7	–
PLA/HA1	56.2 ± 2.8	40.4 ± 2.0	3.4 ± 0.2
PLA/HA2	30.2 ± 1.5	58.4 ± 2.9	11.4 ± 0.6
PLA/HA3	28.9 ± 1.4	62.4 ± 3.1	8.7 ± 0.4

atoms is also confirmed by the successful attachment of HA molecules to the PLA surface.

A more detailed study of the surface chemistry of the PLA/HA scaffolds was carried out using high-resolution XPS. The obtained results (Table 2)[‡,11] showed that after the two-stage modification, an increase in the content of carbon atoms of the CH_2–H/C–C bonds is observed for all samples, indicating the predominance of cross-linking of polymer macromolecules over their destruction, with the maximum increase of 1.4 times achieved for the PLA/HA2 sample.

After plasma treatment and HA immobilization, a decrease in the content of carbon atoms involved in –C–O bonds was observed. However, in addition to the destruction of polymer bonds, the addition of new atoms to macromolecules can also lead to the formation of new chemical bonds. The presence of nitrogen atoms in the samples allowed us to assume the formation of new bonds between carbon and nitrogen as a result of plasma modification and HA immobilization. To take this into account, a new elementary component was added to the analysis of the spectra of the modified materials, corresponding to the most probable chemical environment of the carbon atom and representing carbon bound to the amino group.[9] The two-stage modification resulted in the appearance in the spectra of all the studied samples of a peak corresponding to the binding energy of ~286.4 eV, which was attributed to the carbon atom in the –C–NH_x bond.

The formation of –C–NH_x bonds on the surface of all samples after plasma exposure was also confirmed by the N 1*s* spectra, in which a peak with a binding energy of 399.9–400.0 eV appeared, corresponding to nitrogen atoms in this bond (Figure 1). According to published data, nitrogen atoms in such chemical bonds as –C–NH_x and N–C=O have fairly close binding energies, which makes it difficult to decompose the N 1*s* spectrum into components.[10]

Thus, plasma treatment and HA immobilization change the chemical composition of the PLA surface, which in turn affects its wettability. The contact angle was estimated using the sessile drop method with subsequent calculation of the free surface energy.[§] Before surface modification, the contact angle was 123.0° and 142.1° when wetted with water and glycerol, respectively. As a result of the two-stage surface modification, the contact angle decreased for all samples and was less than 90.0°. Consequently, the surface of the samples became wettable. The surface energy of the samples

Table 2 Chemical speciation of carbon atoms in scaffold samples after the two-stage surface modification.

Bond with C atom	Binding energy/eV	Content of C atoms involved in certain bonds (at%)			
		Initial PLA	PLA/HA1	PLA/HA2	PLA/HA3
CH_2–H/C–C	285.00	35.2 ± 1.8	49.1 ± 2.5	50.6 ± 2.5	36.1 ± 1.8
–C–O	286.98	31.7 ± 1.6	21.7 ± 1.1	26.9 ± 1.3	33.5 ± 1.7
O–C=O	289.06	33.1 ± 1.6	14.9 ± 0.7	14.1 ± 0.7	13.5 ± 0.7
C=O	288.00	–	8.9 ± 0.4	1.9 ± 0.1	4.3 ± 0.2
–C–NH_x	286.40	–	5.4 ± 0.3	6.5 ± 0.3	12.6 ± 0.6

[§] The measurements were carried out at Tomsk State University (Tomsk, Russia) on a KRÜSS DSA25 drop shape analyzer using ADVANCE software (KRÜSS, Germany).

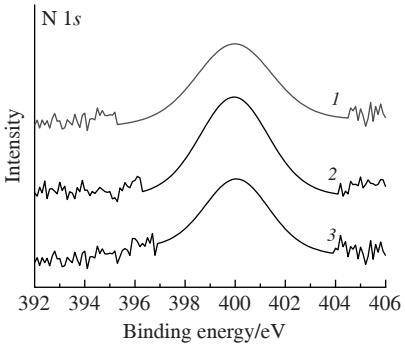


Figure 1 N 1*s* XPS spectra of samples (*1*) PLA/HA1, (*2*) PLA/HA2 and (*3*) PLA/HA3.

with HA immobilized on the surface increased compared to the initial samples [Figure 2(*a*)], and the increase in the total surface energy was accompanied by an increase in its polar component (strong interactions of surface atoms with adsorbed liquid molecules and hydrogen bonds) and a decrease in the dispersive component (van der Waals forces and other nonspecific interactions). The surface energy of PLA/HA samples tends to be higher with increasing content of carbon atoms involved in –C–NH_x bonds, from 81.8 mN m^{-1} for the PLA/HA1 sample with 5.4 ± 0.4 at% C to 83.2 mN m^{-1} for the PLA/HA3 sample with 12.6 ± 0.6 at% C [Figure 2(*b*)]. The increase in the total surface energy of the modified samples indicates an improvement in the adhesive properties of the materials.

An important morphological parameter affecting the attachment of a bioactive substance or drug, as well as cell adhesion, is the fiber diameter. The surface morphology of the experimental samples was examined using scanning electron microscopy (SEM) to determine whether the surface treatment causes a change in the fiber diameter.[¶] The fiber size distribution of the initial PLA is close to normal [Figure 3(*a*)]. As can be seen, plasma treatment followed by HA immobilization on the PLA surface leads to an insignificant increase in the average fiber diameter from 3.1 ± 0.8 μm in the initial PLA to 3.3 ± 0.8, 4.2 ± 1.1 and 4.2 ± 1.1 μm in PLA/HA1, PLA/HA2 and PLA/HA3, respectively, which is probably due to swelling of the fibers in solution and immobilization of HA molecules on their surface. It can be noted that with an increase in the HA solution concentration from 0.1 to 0.3 wt%, the average fiber diameter increases [Figure 3(*b*)–(*d*)].

The biocompatibility of the materials was assessed by culturing human macrophages on the surface of the samples for 6 days as described previously.[9] AlamarBlue reagent was used to analyze cell viability, and the proportion of viable cells was calculated

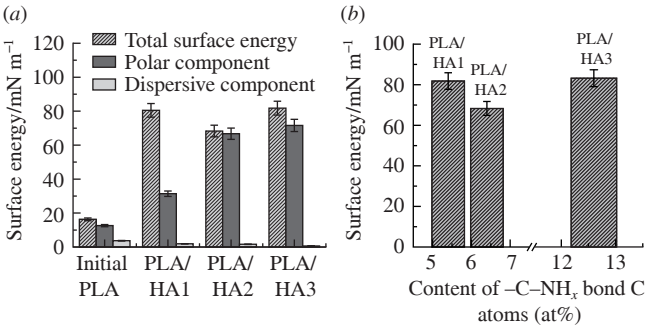


Figure 2 (*a*) Dependence of the total surface energy and its components on the HA concentration in solutions used in the synthesis of PLA/HA samples. (*b*) Dependence of the surface energy of PLA/HA samples on the content of carbon atoms involved in –C–NH_x bonds.

[¶] SEM images were obtained at TRCCU (Tomsk, Russia) using a Quanta 200 3D scanning electron microscope (FEI Company, USA).

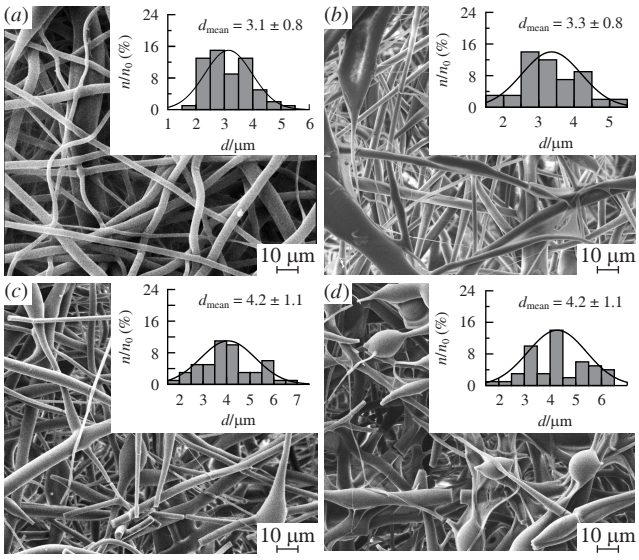


Figure 3 SEM images and fiber size distribution diagrams (insets) of (*a*) initial PLA, (*b*) PLA/HA1, (*c*) PLA/HA2 and (*d*) PLA/HA3 samples.

relative to the control (in the absence of the test sample). According to ISO 10993-5:2009, the test materials are not considered toxic to cells if the proportion of viable cells is more than 70%. It was found that in the case of the untreated PLA sample, the proportion of viable cells decreased to 60%, whereas in the presence of any of the modified samples, more than 80% of the cells remained viable (Figure 4).

Modification of the PLA surface with HA makes the material less 'foreign' since HA is widespread in the human body and is involved in cell recognition and signaling processes. The highest viability of macrophages (115.2%) was observed for the PLA/HA3 sample. Apparently, such a concentration of HA provides the best conditions for cell activity. The lower cell viability of 82.3% in the case of the PLA/HA2 sample can be due to both the unevenness of the HA layer on the surface and the individual reaction of the cells.

In conclusion, the nitrogen arc plasma was used for the first time in this work to modify the surface of PLA scaffolds to create active centers for HA immobilization. It was shown that the nitrogen arc plasma makes it possible to efficiently introduce amino groups onto the polymer surface without damaging its structure and ensuring a high density of HA immobilization. It was found that the two-stage modification of the PLA surface with plasma and HA molecules changes the chemical composition of the surface, increases the surface energy and surface wettability, which leads to improved biological compatibility of PLA scaffolds and expands the scope of their application in medicine.

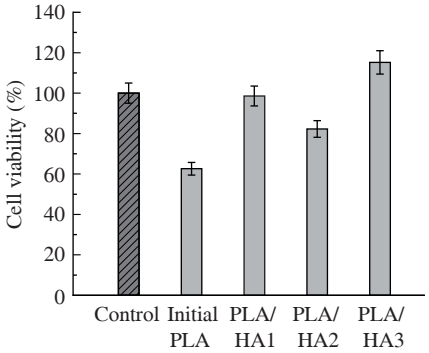


Figure 4 Assessment of human macrophage viability in the presence of initial and HA-modified PLA samples.

This work was supported by the Foundation for Scientific and Technological Development of Yugra (project no. 2024-536-05).

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Received: 11th March 2025; Com. 25/7761