The physico-chemical properties of electrospun vascular PLLA scaffolds modified by the DC magnetron sputtering of a titanium target

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Abstract. In this study, we demonstrated the possibility of modification of the surface of PLLA scaffold fibers by reactive magnetron sputtering of a titanium target under a nitrogen atmosphere. The influence of exposure time and average fiber diameter on the morphological and physico-chemical properties of these materials was studied. During the research, it was found that the DC magnetron modification allows the formation of a coating whose penetration depth into the volume of the scaffold depends on the mean diameter of the fibers. The wettability studies of the obtained materials showed that the contact angle also depends on the fibers average diameter and concentration of titanium in the deposited coating.

1. Introduction
Cardiovascular diseases such as coronary heart disease, peripheral vascular disease and cerebrovascular disease are among the leading causes of death in the world. For the treatment of these diseases, auto-, allo-, and xenografts are now widely used [1, 2]. However, their use entails a number of shortcomings such as low availability of biological material, problems of compatibility with tissues and difficulties in preserving, storing and transporting transplant material. These limitations potentially reduce the effectiveness of grafts and the scope of their application [3].

Significant progress in solving this problem was achieved after the development of artificial vascular grafts based on synthetic polymeric materials. One of the most promising materials for applications in the field of tissue engineering is poly-L-lactic acid (PLLA). This is a biodegradable polymeric material with unique combinations of physical and chemical properties [4]. Currently, PLLA is used for the manufacture of resorbable coatings [5], artificial vascular grafts [6], and is also well processed by the method of electrospinning [7]. This method allows controlling the average fiber diameter in the range from 50 nm to 5 μm by changing the type of solvent, the concentration of the solution, the rotational speed of the collector, and the voltage at the power supply. It was previously shown that the morphology, in particular, the average fiber diameter, affects the orientation, proliferation and differentiation of cells [8]. Usually, electospun PLLA grafts have good mechanical properties, however their biocompatibility needs to be improved [9]. In order to improve the biological properties of vascular grafts produced by the electrospinning method, several strategies were applied, from which it is possible to distinguish plasma treatment [10].
It is known that thin titanium (Ti) coatings deposited by DC magnetron sputtering are used to increase the adhesion of endothelial colony-forming cells [11]. It is also known that such coatings have good adhesion to polymer substrates and have high elasticity [12].

Control of fibrous scaffolds morphology and modification of their fibers surface, makes it possible to very biocompatibility of the materials in the wide range.

Thus, the aim of this work was to study the effect of the fibers average diameter and time of modification by the DC magnetron sputtering of a titanium target in a nitrogen atmosphere, on a complex of physico-chemical properties of electrospun vascular PLLA grafts.

2. Materials and methods
The samples were fabricated from 5, 9 and 14 (wt.%) solutions of the poly-L-lactic acid (PLLA) (PL-38, Purac, The Netherlands) in trichloromethane (CHCl₃) (ECROS, Russia). The formation was carried out using NANON-01A electrospinning setup (MECC Co., Ltd., Fukuoka, Japan) under the following conditions: distance between nozzle and assembly manifold of 110 mm, feed rate of spinning solution of 2.5 ml/h, voltage of 30 kV.

The coating was formed using plasma of a magnetron discharge produced by sputtering a metal target made of chemically pure (99.99%) titanium (Ti) under nitrogen (N₂) atmosphere, the area of the target was 224 cm². Upgraded magnetron sputtering system, described earlier in [13], was used to form the coating. To reduce the degradation of the PLLA scaffold, the modification was performed in a cyclic mode: 1 minute of the plasma exposure, and 3 minutes of cooling the magnetron. The following technological parameters were used to form the coatings: the distance between the target and the sample was 33 mm, the preliminary pressure in the chamber was 3×10⁻³ Pa, the working pressure in the chamber was 0.4 Pa, and the working gas was nitrogen (N₂). Plasma treatment was carried out for 2, 4 and 8 minutes, with a fixed power value of 88 W. Ion purification was used to remove the adsorbate from the target surface with the following parameters: the current at the power source was 0.6 A, the gas was argon, and the ion cleaning time was 1 minute. Thus, three groups of samples were formed – PLLA 5%, PLLA 9% and PLLA 14%.

The morphology of the PLLA scaffolds was examined by scanning electron microscopy (SEM) QUANTA 200 3D (FEI, USA). To measure the diameter of the fibers, at least three images obtained from different sections of the PLLA scaffold were analyzed. Fibers average diameter was calculated from not less than 150 measurements. The images were processed using the Image J 1.38 software (National Institutes of Health, USA).

The chemical composition of PLLA scaffolds was studied using energy-dispersive spectroscopy (EDS, Genesis 4000, EDAX).

A study of the wettability of the modified PLLA scaffolds was conducted on the Easy Drop (Krüss) by the sessile drop method by measuring the wetting contact angle of a 2 μl liquid placed on the test surface. Measurements of the contact angle of wetting were carried out one minute after the liquid was placed on the sample surface. As a wetting liquid, glycerol was used.

The statistical analysis of the data was carried out using the Statistica software (StatSoft, Dell). The distribution normality was estimated using the Kolmogorov-Smirnov test. Differences were considered statistically significant at p <0.05. The data are presented as the mean ± standard deviation. Untreated PLLA scaffolds were used as control samples.

3. Results and discussion
Figures 1, 2 and 3 show images of PLLA 5%, PLLA 9% and PLLA 14% samples (at different magnifications), glycerol droplets on their surface, and titanium distribution as a function of plasma processing time. Table 1 shows the average diameter of the fibers as a function of the plasma exposure time.
Table 1. Average diameter of the sample fibers and their dependence of the plasma treatment time.

| Treatment time, min | PLLA 5% | PLLA 9% | PLLA 14% |
|---------------------|---------|---------|----------|
|                     | Average diameter of fibers, µm |         |          |
| 0                   | 1.13±0.48 | 2.24±0.28 | 4.85±1.42 |
| 2                   | 1.58±0.58 | 2.30±0.31 | 5.58±1.59 |
| 4                   | 1.35±0.53 | 2.23±0.30 | 5.64±1.20 |
| 8                   | 1.35±0.48 | 2.18±0.31 | 5.62±1.31 |

*(p < 0.05) relative to the control sample

Figure 1. SEM images of PLLA 5%, PLLA 9% and PLLA 14% scaffolds at different magnifications, glycerin droplets on their surface, and titanium distribution (cross-section) as a function of plasma treatment time: a, e, i – control; b, f, j – 2 minutes; c, g, k – 4 minutes; d, h, l – 8 minutes.

Increase of the polymer concentration from 5% to 14% (w/w) lead to significant rise of the fibers average diameter (table 1). For control samples, surface defects in the form of droplets, seals, melts were not observed. The scaffolds, regardless of the concentration of the polymer solution, were formed by cylindrically shaped fibers that were randomly intertwined with each other, had an irregular shape, with an average fiber diameter shown in table 1. An increase in the polydispersity of the fibers was observed, as evidenced by a rise of the standard deviation (figure 1, table 1).
The PLLA 5% scaffold fibers had a series of minor thickenings along the fiber (figure 1a-d). On the surface of PLLA 5% scaffold fibers treated for 2 minutes, no defects were found. However, with an increase of plasma exposure time cracks were observed. Average diameter of the fibers didn’t change significantly (figure 1a-d). The same was found for scaffolds from PLLA 9% group. In case of PLLA 14% group, cracks were found on the surface of scaffold fibers only after 8 minutes of plasma exposure. The crack formation on the fiber surfaces is probably due to the difference in their elasticity caused by the formation of a thin Ti coating on their surface.

The cross-section of PLLA 5%, PLLA 9% and PLLA 14% scaffolds (figure 1) shows that the distribution of titanium over the depth of the sample had an inhomogeneous profile. For the PLLA 5% scaffold, a maximum depth of Ti penetration into the volume of the material was ~20 μm, which corresponds to the data given in [13]. However, for PLLA 9% samples, the maximum penetration depth increases to ~40 μm, and the total concentration of the particles of the sputtered target in the volume of the material also rise. Increase in the polymer concentration in the solution to 14% (PLLA 14% sample) also leads to an increase in the penetration depth of plasma particles into the volume of the material (~100 μm) and rise in the total concentration of the particles of Ti. According to [14, 15], this mechanism is explained by the presence of a shadow effect.

Wettability of PLLA 5%, PLLA 9% and PLLA 14% scaffolds is shown on the left inserts on figure 1. The contact angles of wetting are given in table 2.

| Treatment time, min | PLLA 5% | PLLA 9% | PLLA 14% |
|---------------------|---------|---------|----------|
| 0                   | 131.0±5.3 | 138.4±0.4 | 107.3±1.7 |
| 2                   | 124.6±2.3 | 56.5±3.3 | 115.1±10.0 |
| 4                   | 112.8±4.9 | 84.3±29.2 | 111.9±4.4 |
| 8                   | 106.6±2.1 | 105.3±8.2 | 100.5±2.3 |

*(p < 0.05) relative to the control sample.

As can be seen from the table 2, plasma modification process influences on PLLA scaffolds with different fiber diameters in various manners. Thus, for PLLA 5%, a linear decrease in the wetting contact angle is characteristic (table 2). A similar effect was observed by the authors of [16], precipitating thin coatings, upon sputtering a titanium target, onto polymer substrates. For PLLA 9%, the wetting contact angle have a non-liner dependence. After 2 minutes of plasma exposure, a sharp decrease in contact angle from 138.4 ± 0.4° to 56.5 ± 3.3° occurs. A significant decrease in the contact angle of the PLLA 9% scaffold surface at a short exposure time is due to saturation of the fibers surface with nitrogen [17]. The gradual restoration of the wetting contact angle occurs at 4 minutes and reaches 105.3 ± 8.2 ° at 8 minutes of plasma treatment. This fact indicates that metal clusters are formed on the surface of the PLLA fibers of the scaffolds, which lead to a decrease in the contact angle of wetting [18]. It can be noted, that scaffolds with maximum fiber diameter (PLLA 14%) are characterized by the minimum wetting angle. There are no significant differences in the wetting contact angle between control and 8 minutes treatment samples.

4. Conclusion

In this paper, the possibility of modifying the surface of bioresorbable PLLA scaffolds by DC magnetron sputtering of a titanium target in a nitrogen atmosphere was demonstrated. The influence of exposure time and average fiber diameter on the morphological and physico-chemical properties of these materials were studied. It was found that the Ti penetration depth into the volume of the treated material and the wettability depend on the average diameter of the PLLA fibers.
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