Polylactide film deposition onto titanium surface from different solutions

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Abstract. The dependence of the porosity, roughness and contact angle with water of polylactide films, produced by dip-coating, on the solution composition and concentration, the drawing rate, as well as the influence of the nitrogen and air atmosphere are investigated. Films with low porosity and roughness from tetrahydrofuran (THF) and dichloromethane (DM) solutions were obtained on the titanium covered glass surface. Technique of a residual solvent detection was proposed, and optimal parameters of films drying were determined.

1. Introduction
Coatings based on biodegradable polymers on titanium can significantly improve growth and attachment of cells [1], and can be used to significantly reduce the integration time of titanium implants, which are currently widely used in prosthetics, and for development of new bioactive materials for prosthetics and tissue engineering purposes. Aliphatic polyesters, in particular, polylactide (PLA), are the most used biopolymers, their biodegradation rate can be varied over a wide range by altering the degree of crystallinity [2] and the composition of copolymers based on them [3], thus it was chosen as a model polymer. Surface morphology plays an important role in the formation of coatings from biodegradable materials because it determines the effectiveness of attachment and the proliferation of cultured cells. Moreover, at the initial stages of tissue repair the formation of the submicron roughness is important [4]. In addition, functional groups contained on the surface, even in the case of its low roughness, are important factor for cell growth [5]. Thus, to investigate the adhesion and proliferation of cells, it is necessary to study the methods of formation of PLA films with controlled porosity, roughness and to investigate the influence of composition of the material and especially its near-surface layers on it. Films morphology according to theoretical and experimental studies depends on solvent concentration [6], its evaporation rate, water content in solution and in atmosphere [7]. However, there is not enough data about PLA films formation with nanoscale roughness and pore absence. Our work is devoted to solving this problem. In view of complex form of implants, it requires to use dip-coating or spray deposition methods.

2. Materials and methods
PLA polymer (4043D, NatureWorks LLC, Minnetonka, MN, USA) was dissolved in tetrahydrofuran (THF), dichloromethane (DM) and benzyl alcohol (BA) (with purity ≥99.8%, Komponent-Reaktiv Ltd, Moscow, Russia), the last one, known as non-solvent of PLA, was added in selected experiments [8]. Dissolution of the polymer was performed using stirring and ultrasonic treatment. The films were deposited on cover glasses, with a 15 nm layer of magnetron sputtered titanium on one side by dip-coating method at low drawing rate (~5 mm/min) under normal conditions and nitrogen
atmosphere. Drying of thin films was carried out in atmosphere at temperature about 85 °C for 7–10 minutes, and for the study of thick films samples to determine the solvent evaporation parameters were additionally dried in vacuum at 55 °C for 48 hours. Drying temperatures were below 100 °C to prevent possible unwanted formation of crystallites of the polymer which can be formed even at the temperature about 90–120 °C [9], and have a substantially longer biodegradation time due to additional barriers for the diffusion of molecules and other mechanisms of biodegradation [2]. The surface morphology and thickness of films were studied by the atomic force microscopy (AFM) (Solver PRO microscope, NT-MDT Ltd, Moscow, Russia) using cantilever with resonance frequency in the range of 90–150 kHz. The presence of the residual solvent and the degree of crystallinity in the films were studied by Raman spectroscopy (Centaur U HR spectrometer, Nano Scan Technologies Ltd, Dolgoprudny, Russia). The water contact angle \( \theta_w \) was measured with a deionized water droplet (volume 5 µl) produced by a micropipette. The edges of droplet were captured on camera of a horizontal optical microscope and processed in specialized software for measuring the value \( \theta_w \).

3. Results and discussions

PLA films, deposited from THF and DM solution (~4.5 wt %) by dip-coating technique, showed less porosity and pore size compared to the results of solution deposition with though higher concentration (8 wt %) reported in the literature [1]. Average pore size decreases with increase of PLA concentration (see fig. 1a and 1b), which is consistent with other studies [6].

![AFM-images of PLA films obtained by the dip-coating from solutions with different concentration and conditions: (a) 25 mg/ml in THF; (b) 45 mg/ml in THF; (c) 45 mg/ml in THF in a nitrogen atmosphere; (d) 45 mg/ml from mixture of 3.5 wt % BA and THF, (e) 45 mg/ml in DM, (f) 45 mg/ml from mixture of 1 wt % BA and DM. (d-f) was formed in nitrogen atmosphere.](image)

In the case of anhydrous atmosphere, a significant reduction of microparticles number on the surface, as well as an additional decrease in the average pore size were observed (see fig. 1b with 1c). Also, the presence of residual water content in the solvent and in the film, deposited in air, has a significant effect on the pores formation. Higher evaporation rate of DM solution compared to THF as expected resulted in lower film porosity (see fig. 1c and 1e) that is correlated with literature results [7]. To prevent pores formation, we suggested to add a low volatile component (BA) to the solution. As a result, the decrease of films porosity was occurred for both THF and DM solutions (fig. 1c, 1d and 1e, 1f). The BA, as we suppose, interfered the process of phase separation of the solution into a polymer
and a solvent leading to the formation of pores, by realizing the presence of a slowly evaporating component that interacts sufficiently with both the polymer and the solvent. That role can be played by a low-molecular PLA used as a component [10] of composition of film or other organic molecules corresponding to the foregoing properties. But in our work the BA was chosen as such component. However, its excessive content (3.5 %) caused the appearance of nanoparticles on the films surface (fig. 1c and 1d). The optimal concentration of BA for minimization of pores and particles number is expected to be less than 1%. Initially dip-coating were carried out in a draft chamber with airflow. The average films thickness deposited from THF solution with PLA concentration of 8, 25 and 45 mg/ml were about 10, 130 and 180 nm respectively with significant porosity for 10 nm film, it was also weakly depended on drawing rate in a range of 1–10 mm/min. Increasing of the surface roughness was observed in the case of an ultra-slow drawing rates (1 mm/min). Observed complexity of film thickness dependence on solution concentration was probably caused by predominant role of solvent evaporation in a film deposition process and pore formation mechanism [6], prevailing in the case of low concentrations and low drawing rates realized in our experiments. Our result is contrary to power law thickness dependence on drawing rate and concentrations reported in the literature for processes where wetting of the substrate, drawing rate, viscosity of the solution are critical [11].

To reduce the effect of water vapour present in the air, the formation of films was carried out in a glovebox in a nitrogen atmosphere. The average thickness of the films deposited in nitrogen atmosphere from solutions with various concentrations is shown in fig. 2. The obtained results revealed a different dependence of the thickness of the formed films on the concentration of solutions, from experimental results in the air, but it is in agreement with known models in which the viscosity of the solution has a predominant effect on the thickness of the deposited film [12], which is expressed in the fact that the experimental points are acceptably approximated by power function. In this case, in contrast to the air atmosphere, the influence of the solvent evaporation rate factor was significantly reduced due to the absence of a gas flow near the surface of the film being formed. In addition, due to the substantial difference in the mechanism of film formation in air, the pore size significantly increases in comparison with films formed in a nitrogen atmosphere. It was also shown that it is possible to proportionally increase the thickness of the film by iterative coating cycles without changing the solution and special drying.

Figure 2. The results of measuring the thickness of PLA films obtained from THF and DM solutions at different concentrations at drawing rate 5 mm/min, approximated by a power function.

It was found by comparing the AFM data before and after drying that even thin films should be dried to remove the residual solvent at temperatures below 100 °C, which leads to the partial formation of open pores from microcavities formed during the deposition of films [6] and observed on AFM after
drying. However, the addition of a low-volatility component to the solution can lead to its only partial removal from the film. To determine the presence of residual solvent, thicker films were formed, followed by drying in vacuum and analyzed by Raman spectroscopy. The optimum drying parameters were determined by analyzing films with 5–15 μm thickness because of the greater accuracy of determining the absence of a fluorescent background and relative intensity of PLA Raman peaks in the region of 2800–3100 cm\(^{-1}\). It has been shown (see fig. 3) that when THF is used as the main solvent, the presence of a noticeable amount of BA in the film after drying, detected by the presence of peaks near 3060 cm\(^{-1}\) and 1000 cm\(^{-1}\) [13], is not detected, but when DM is used as the main solvent, the spectra shows the presence of BA.

![Figure 3(a, b). Raman spectra of PLA films: (a/b): I – reference spectra of pure PLA; II, III – PLA from THF(a) / DM(b) solution before and after vacuum drying; IV, V – PLA from THF(a) / DM(b) and 3.5 wt % (a) / 1 wt % (b) BA solution before and after vacuum drying.](image)

This fact indicates a significant difference in the mechanism of BA evaporation using different solvents. Presumably, DM, which is characterized by a lower boiling point and a higher vapor pressure compared to THF, evaporated from the film too quickly during film drying, apparently without forming the conditions for complete removal of the BA that remains in the film and presumably interacts with PLA replacing the evaporated DM, which in turn creates an additional deterioration in the conditions for its removal from the film upon drying, in view of its substantial interaction with PLA, the prerequisite of which are the known results on the substantial swelling of PLA in BA [8]. In addition, the reason for such behavior probably is difference in the energy of interaction between DM and BA compared with THF with BA, in combination with the difference in the interaction energies of the used molecules with PLA. The latter is evident in view of the better solubility of PLA in DM in comparison with THF [8]. Thus, based on the obtained spectral data, in the case of DM, it is impossible to effectively evaporate the BA, whose presence is detected on the spectra after prolonged vacuum drying. On the other hand, the usage of THF as the main solvent allows completely (at the level of the absence of the peak near 3060 cm\(^{-1}\) in the spectrum) to remove solvents from the film during drying.

Also, decrease in the relative intensity of 2880 cm\(^{-1}\) to 2950 cm\(^{-1}\) peak, indicates an increase in the material crystallinity [14] and microscale crystallites formation, which is undesirable for developing
coatings, and shows the lowest degree crystallinity in the case of THF films and maximal in the case of DM, especially with BA (the peak of 2880 cm\(^{-1}\) is almost zero and coincides with the intensity in the region of 2900–2920 cm\(^{-1}\) ), and the intermediate value in the case of THF with BA. A low degree of crystallinity can also improve the solvent removal conditions of the film, which is an additional factor for removing the BA. And additionally the presence of a significant fluorescent background indicates the presence of a residual solvent, as well as the presence of a noticeable peak near 2850 cm\(^{-1}\) (only for THF). In the case of DM, in view of the absence of preliminary drying of the films in air, were observed peaks near 700 cm\(^{-1}\) [15] and 3060 cm\(^{-1}\) that are comparable in intensity with a peak near 2880 cm\(^{-1}\), but there was no fluorescent background, characteristic as we assume for a small number of residual solvent molecules interacting with the polymer.

These results of Raman spectroscopy correlate well with the measurement data of water contact angle and film surface (\(\theta_w\)), which revealed a significant effect of the solvent composition on the value of \(\theta_w\) (see fig. 4).

![Figure 4. Results of water contact angle measurements.](image)

The addition of BA to the PLA solution in DM led to a significant increase of the water contact angle of film from 80° to 93°, that in combination with obtained spectroscopy data from thick films, indicates that the probable cause of the observed effect is the presence of residual BA molecules near the surface of thin PLA film, the presence of which presumably forms a smaller number of polar groups on the film surface. Possibility of participation of such a mechanism in this case agrees with the data known from the literature on the effect of functional groups formed on the surface by plasma treatment on the wetting angle, as well as on the relaxation of their influence during annealing of films above the glass transition temperature [5]. The absence of this kind of change in the wetting angle in the case of films obtained from THF solution also correlates with the Raman data demonstrating the best results of the BA releasing from the formed films, and also indicates that there is no need for long drying of the films thinner than 200 nm to remove the residual solvent. The observed slight difference in the wetting angle of films obtained from pure DM and THF (see fig. 4) is apparently caused by a greater porosity in the case of THF, that is consistent with the known Cassie-Baxter model, which describes an increase in the wetting angle in view of the presence of pores that create microregions where liquid-gas boundaries are formed [16], which are not specific for a smooth surface or a surface with sparsely lying nanoparticles. And known Wenzel model for surfaces with nanoparticles or roughness, which are found on the films deposited from BA-containing solution, does not explain increasing or non-changing water contact angle on them. However, the detected surface hydrophobicity is a negative factor for cells adhesion and growth, which proves the necessity of
careful selection of solution composition to ensure controllable porosity and at the same time suitability for cell culturing.

4. Conclusion
The comparison of the results of the formation of films in different atmospheres and solutions with different concentrations of THF and DM revealed significant differences in the mechanism of formation, as well as in the thickness and degree of porosity of the films, and films formed in the nitrogen atmosphere have fewer defects and smaller size of pores. It was found that the sample with a low volatile BA, added to the solution, differs by low porosity and a slightly higher degree of crystallinity at the same amount of residue solvents compared to pure THF sample so it is possible to completely remove it from the film after drying in the case of using THF as the main solvent. Maximum degree of crystallinity was detected in films deposited from DM solution, in which BA can not be fully evaporated by drying, that additionally appears also at a significant increase in the wetting angle with water. Thus, in the absence of longtime vacuum drying, main peaks of residual solvents in thick film was observed on the Raman spectra, which makes such process and films unacceptable for using as a biodegradable coating. A technique for estimating the presence of a residual solvent in a PLA film was proposed, based on observing the characteristic peaks of the solvents themselves in the case of a large amount of them in film, as well as on detecting the fluorescent background of the films, even in the case of a small amount of a residual solvent. Optimal vacuum drying time for films with thickness more than 500 nm was determined as more than 48 hours, and the drying of the films to remove the residual solvent at thicknesses less than 200 nm can be carried out at a temperature of up to 100 °C for 10 minutes. Despite the possibility of a significant change in the porosity of the film with the addition of BA, it is necessary to completely remove the residual solvent, which can also be detected by the absence of an increase in hydrophobicity with respect to the samples with a completely removed solvent. Thus, careful selection of the solution composition and quality control are required to form films suitable for cell cultivating and for use as coatings for implant.

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