PREPARATION OF FIBRIN NETWORKS ON POLYMERS CONTAINING NANO-FILLER

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Introduction

The modification of the biomaterial surface can be realized by different methods, e.g.: using of hydrophilic/ hydrophobic monomers, that influence the chemical character of the polymer surface, by an addition of micro- and nanofillers to differentiate micro- and nanoroughness, as well as by obtaining porous structure, that supports the cell adhesion and mimic the natural tissue architecture [1]. All these modifications influence other stage of implant-tissue interactions and different part of the compatibility process.

It is recognized, that wettability, the chemical composition and the surface roughness strongly influence the protein absorption process that affect biological response and determine the implant tolerance. These specific interactions occur at the atomic level, in a very thin interface of less than 1nm in thickness [2], but determine the cell behavior and the recognition of the polymer or other biomaterial surfaces.

In previous work, we presented the influence of titanium dioxide (TiO_2) in the poly(ethylene terephthalate)/dilinoleic acid (PET/DLA) copolymer matrix on selected surface properties. At very low concentration (0.2 and 0.4 wt%), the nanofiller strongly influenced roughness and micro- and nanotopography of the materials [3,4], what resulted in better adhesion and spreading of the cells in comparison to the unmodified material.

In this work we present the effect of two different nanofillers, namely TiO_2 and CeO_2 on the surface wettability and the results of preliminary studies on preparation of fibrin networks on the prepared surfaces. Both of these oxides are tested for biomedical applications and the examination of their influence on protein-surface interaction will be helpful in interpretation of in vitro and in vivo tests.

Experimental part

Nanocomposites were synthesized by in-situ polycondensation in the presence of TiO_2 or CeO_2 at two different concentrations, i.e. 0.2 and 0.4 wt%. The obtained copolymer of poly(ethylene terephthalate) as hard segments (30 wt%) and dilinoleic acid as a component of soft segments (FIG. 1) and the nanocomposites were investigated to evaluate the surface wettability and the protein adsorption.

The water/air contact angle was determined by a sessile drop under static conditions using the Contact Angle Measuring System OCA_20 (Dataphysics, Germany). The samples were prepared by spin-casting from 1% wt. solution of polymers in chloroform on silanized glass substrates in dust-free chamber at constant temperature 25°C. The measurements were performed for 3 samples with five measurement points.

The dynamic Wilhelmy plate method (Tensiometer K12, Kruss Germany) was carried on polymer foils (15x20 mm). This method is suitable for calculating the average advancing (θ_A) and receding (θ_R) contact angles (CA) on solids.

The surface plasmon resonance (SPR) instrument based on the Kretschmann geometry of the attenuated total reflection (ATR) method and spectral investigation of SPR conditions was manufactured in the Institute of Radio Engineering and Electronics, AS CR, Prague [6]. The thin PET/DLA film (without the nanofiller, thickness of 20 nm) was prepared by spin casting from 1% wt. solution of the polymer in chloroform on sensing chips (SF_2 glass slides coated by chromium layer (thickness of 2 nm) followed by a gold layer (thickness: 50 nm) prepared by deposition in vacuum). The chip with the polymer film was fixed in a four-channel flow cell and the protein solutions (blood plasma, human serum albumin (HSA), immunoglobulin G (IgG)) were pumped through the flow cell at a constant flow rate with a peristaltic pump. The observed shifts in the resonant wavelength, i.e. λ_{res}, reflected changes in refractive index of the medium at the sensor surface within penetration depth of the SPR evanescent wave due to the attachment of proteins to the chip surface (an increase or decrease in the mass of immobilized proteins increases or decreases kres, respectively) [7].

The polymer films for the protein modification were prepared by spin casting on the silanized round-shaped glass slides. The thin fibrin network on the thin polymer supports was prepared according to [7]. Briefly, the fibrinogen solution (20 mg/ml) was adsorbed on the surface and than treated with thrombin (2.5 U/ml). Finally, a new solution of fibrinogen (200 mg/ml) containing antithrombin (0.5 U/ml) and heparin (60 mg/ml) was added. After two hours of incubation, a well defined fibrin network was formed on the surface.

Results

As it was mentioned above, the presence of the nanofiller increases the polymer surface roughness in the case of the composites containing TiO_2 [3]. Despite of hydrophilic character of titanium dioxide, the changes in static contact angles are not significant (FIG. 2). This is probably connected with the fact that after solvent casting of films, titanium dioxide nanoparticles are covered by a thin layer of relatively hydrophobic polymer matrix. This behavior is in a line with our previous work [8], where we demonstrated that TiO_2 significantly affects the bulk properties of PET/DLA nanocomposites what was verified by increase in mechanical properties of TiO_2-containing materials.

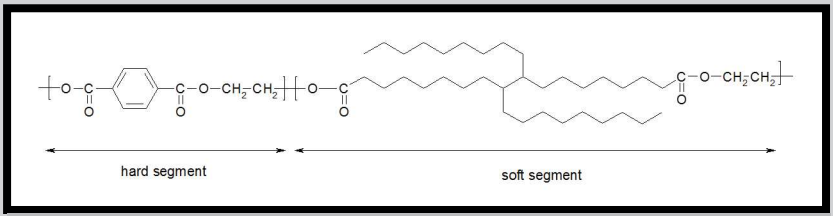

FIG. 1. The chemical structure of the PET/DLA copolymer.

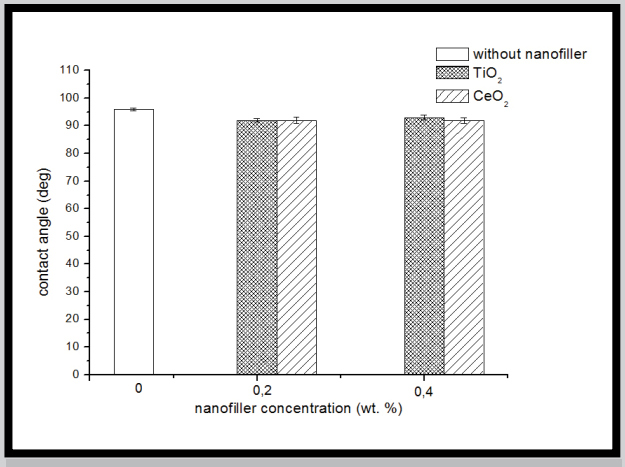

FIG. 2. Static contact angles of PET/DLA copolymer spin-cast films with a different concentration of TiO_2 and CeO_2 nanofillers.

A slight difference is also observed for the advancing and receding contact angles obtained by dynamic measurements (FIG. 3). The addition of TiO_2 or CeO_2 nanofillers results in negligible decrease in advancing and receding contact angles, what confirms observations from the static method. The most significant effect of the nanofiller on the increase in wettability (largest decrease in advanced and receding contact angles) is observed for the sample with 0.4 wt% of CeO_2 (FIG. 3b).

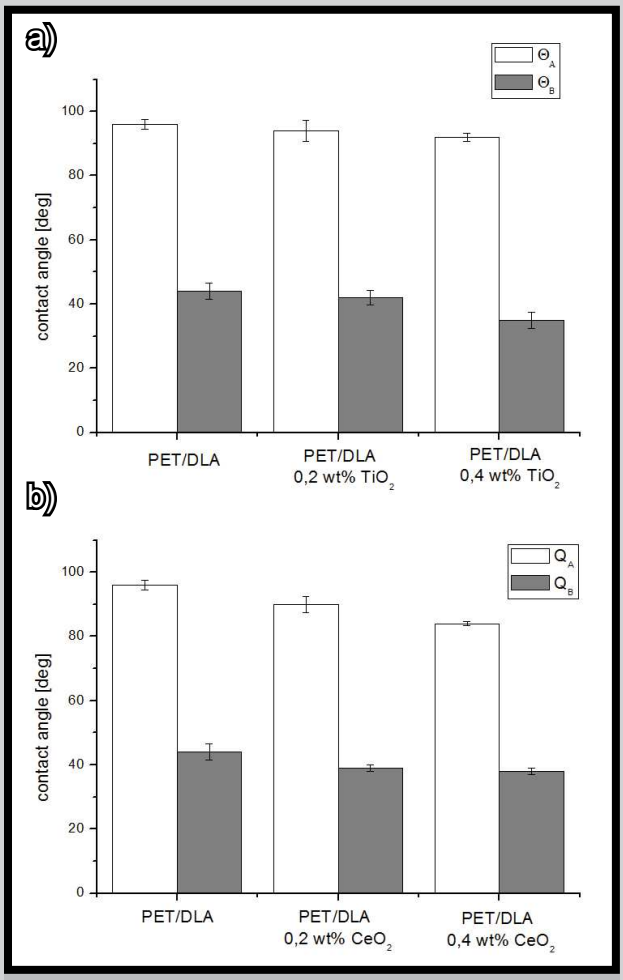

FIG. 3. The advancing (θ_A) and receding (θ_R) contact angles of PET/DLA copolymer follies with TiO_2 (a) and CeO_2 (b) nanofiller.

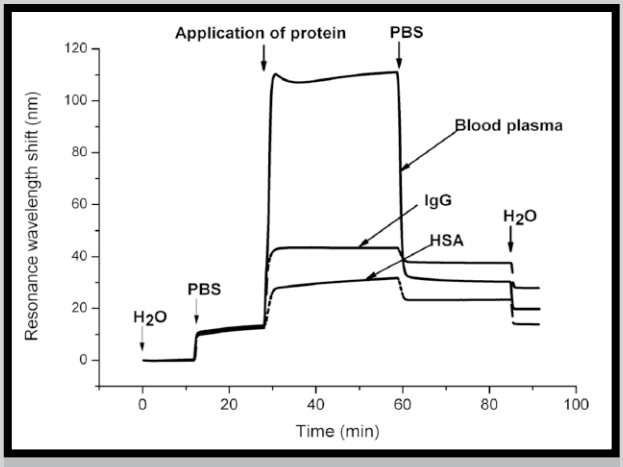

FIG. 4. The SPR monitoring of the growth of the protein layer (Blood plasma, human serum albumin (HSA), immunoglubulinG (IgG) on the PET/DLA copolymer film.

The results of SPR analysis (FIG. 4) showed that tested proteins (blood plasma, HSA, IgG) adsorbed very fast on the PET/DLA copolymer surface and that this adsorption was irreversible.

SEM micrographs presented in FIG. 5 a-c clearly show the fibrin network adhered on the PET/DLA polymer and their nanocomposite surfaces. It can be noticed that fibrin network is more dense and thicker for materials containing nanoparticles, i.e 0.4 wt% TiO_2 (FIG. 5b) and 0.4 wt% CeO_2 (FIG. 5c) as compared to the neat material (FIG. 5a).

Conclusions

Thin films of PET/DLA copolymer and their nanocomposites were prepared by spin coating and the surface wetteability was tested by static and dynamic methods. The results of water conatact angle measuremens did not reveal noticable differences in wettability for the neat copolymer and their nanocomposites what can be axplained that relatively hydrophilic titanium oxide and cerium oxide nanoparticles could be covered by a thin film of a hydrophobic polymer matrix during sample preparation. However, the presence of nanoparticles, and in consequences, higher nanoroughness, significantly affected the ability to fibrin network formation after short time.

Acknowledgements

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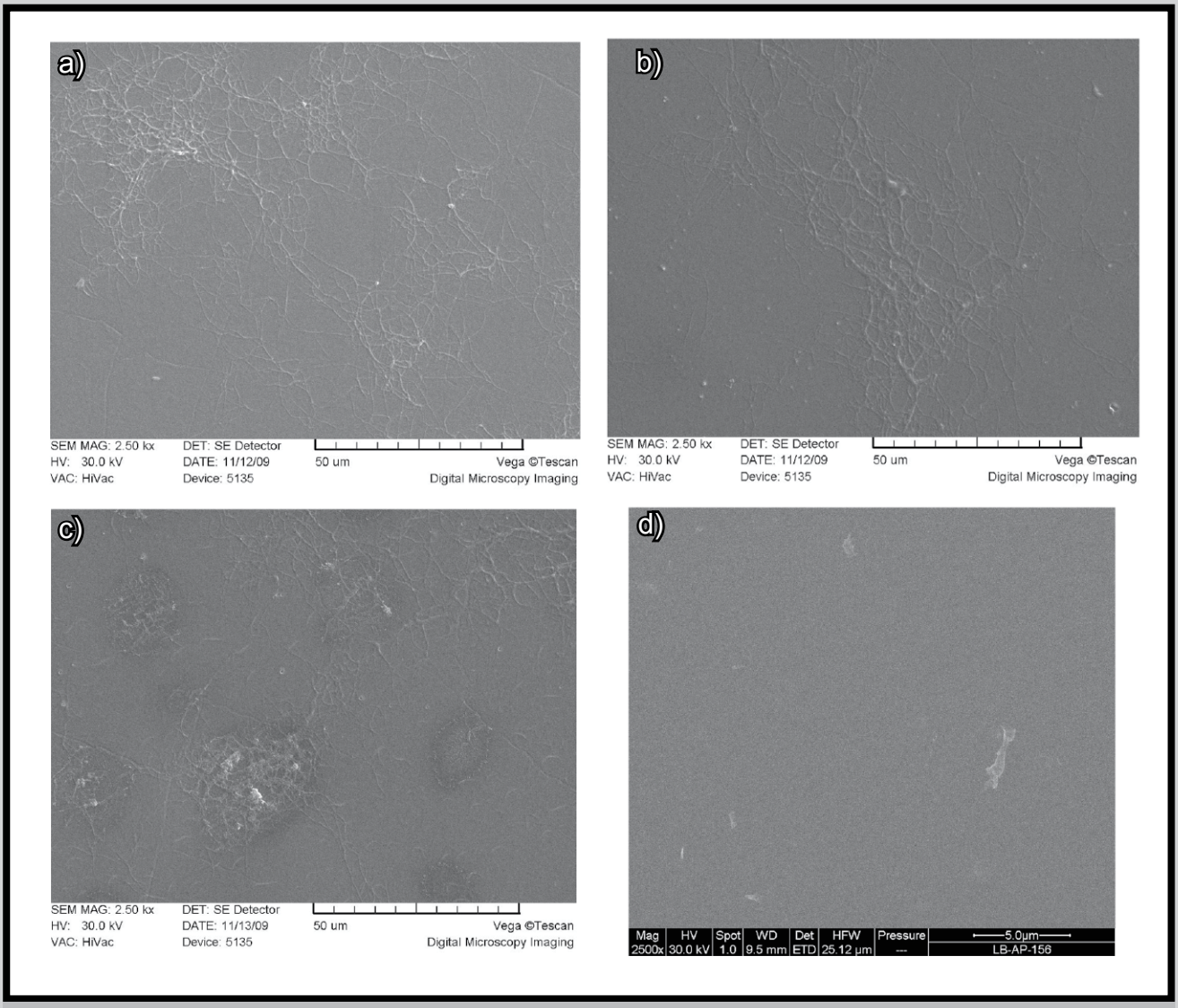

FIG. 5. SEM analysis of the fibrin network on: a) neat PET/DLA, b) PET/DLA 0.4 wt% TiO_2, c) PET/DLA 0.4 wt% CeO_2, d) PET/DLA 0.4 wt% TiO_2 without fibrin network.

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