INFLUENCE OF SURFACE (NANO)ROUGHNESS ON CELL BEHAVIOUR

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Abstract

The influence of surface (nano)roughness of soft elastomeric materials containing nanocrystalline TiO2 on tissue cell behaviour after implantation tests was investigated. Addition of small amount (0.2wt.%) of TiO_2 into polymer matrix changed surface roughness giving material of well developed lamellar morphology and slightly diminished contact angle (wettability). This lamellar (fibrilar) morphology had strong influence on cell response after implantation into soft tissue indicating the absence of eosynophiles in tissue around implant.

***key words**: nanocomposites; AFM, cantact angle, cell/tissue response*

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Introduction

Biomaterial characterization in term of its biofunctionality must include such properties as mechanical stability, chemical structure, morphology, degradation profile and chemical character of the surface [1]. All these parameters and their interactions with biological system decide about the functionality and possibility of such materials to be used in specific applications. From the biological point of view, surface properties such as hydrophobicity/hydrophilicity, wettability, roughness and topography plays crucial role in cells and tissues response [2].

Information about surface properties can be obtained using microscopic, spectroscopic or thermodynamic methods, depending from the requirements [3]. The goal of this study was to characterize the surface properties of polymeric nanocomposite films. Their contact angles, roughness and morphology and has been measured and analyzed with respect to cellular response after implantation test.

Experimental

Materials

PET/DLA (poly(ethylene terephthalate)/dilinoleic acid) multiblock copolymers was prepared as the neat material at hard/soft segments weight ratio as 30/70wt%. Then, by adding 0.2wt% nanocrystalline TiO_2 during the synthesis step, PET/DLA-based nanocomposite was prepared as described in the authors' previous work [4]. Thin films (60-160nm thickness) were obtained from polymers solution in chloroform by spin-coating on glass substrates (cover glasses φ=18mm).

Morphology

AFM measurements were performed on the Nanoscope IV A (Veeco/Digital Instruments) AFM in tapping mode. The AFM was equipped with a dimension scanner - maximum scan size of 150x150μm.

Contact angle

Contact angles measurements were carried out on spin-coated samples using drop technique on a DataPhysisc, Contact Angle System OCA. During each measurement on the instrument, 15 points were collected from each polymer (three samples from each composition at 5 points). The contact angle was measured with ultra pure distilled water.

Implantation test

Implantation test was performed according procedure described in details in [5]. Briefly, the PET/DLA and PET/DLA–0.2%TiO_2 used were small polymer rods, 10 to 12mm long, 3 to 5mm wide and 0.6 to 0.8mm thick. Polymer rods were implanted into the muscles of 30 Wistar rats weighing 200–220g. Animal observations were performed for 12 weeks and sacrificed with sodium pentobarbital in the amount 200mg (kg b.w.). The structure of histological slides was analyzed following the preparation of tissues with implanted polymers.

Results and discussion

Addition of nanoparticles into polymer matrix is a simple method for modification of surface as well as bulk properties of polymeric materials. Depending from the particles character, they can act as osteoinductive or antimicrobial agents, or controlled drug delivery systems [6-8]. Song et al. [9] showed that new type of nanocomposite material, prepared by blending TiO_2 nanoparticles with PNIPAM-co-PS electrospinned fibers may find some new applications in field of bioanalysis or as directed drug carriers. El Fray and Piegat [4,10] showed that addition of small amount of TiO_2 into thermoplastic elastomer matrix is easy way to control mechanical behavior and susceptibility to degradation of this type of materials.

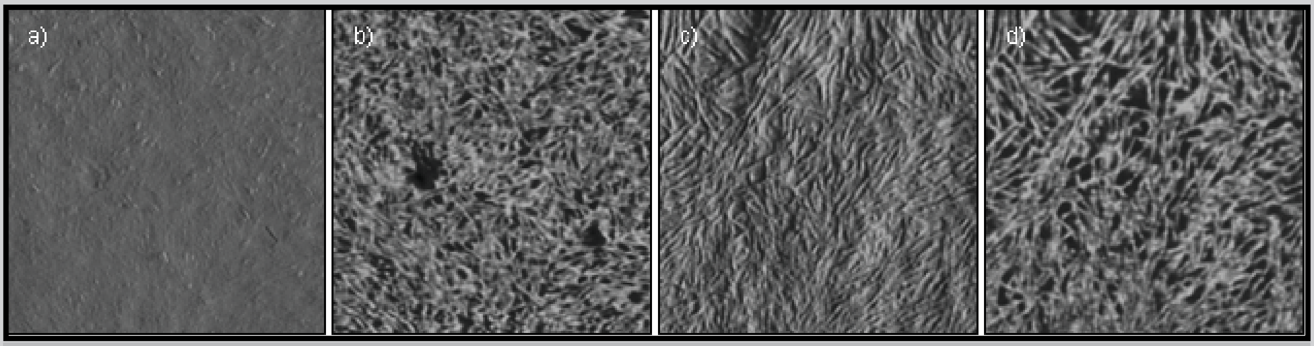

FIG.1. Morphology by AFM at 1x1μm^2 for a spin-coated PET/DLA sample (a,b): a) height image, b) phase image; c,d) PET/DLA 0,2wt% TiO_2 : c) height image, d) phase image.

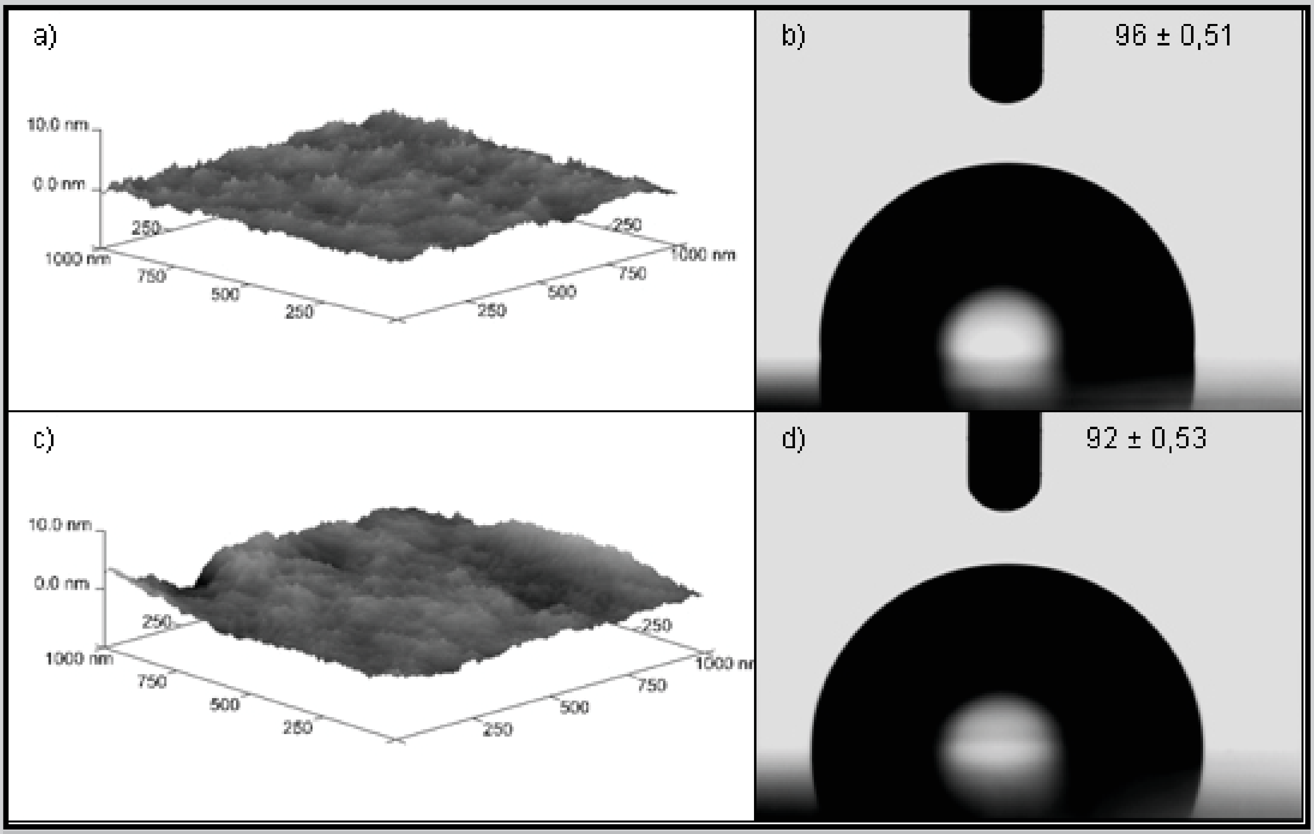

FIG.2. AFM 3D images for neat PET/DLA copolymer (a) and for nanocomposite containing 0,2wt% TiO_2 (c) and corresponding contact angles PET/DLA (b), nanocomposite (d).

The morphology of the neat PET/DLA and nanocomposite containing TiO_2 was studied by AFM. In FIG.1, morphology (a) and phase images (b) of the neat PET/DLA and the same images for PET/DLA 0,2 wt% TiO_2 (FIG.1 c,d) are presented. The AFM pictures (especially phase images) showed well defined morphology (lamellar structure) of hard PET segments embedded in a soft amorphous matrix (DLA). The spin-coated samples showed lamellar morphology for both polymeric systems, however neat PET/DLA copolymer showed finer and shorter lamella compared to material containing TiO_2. Addition of nanoparticles led to formation of longer and thicker lamella (FIG.1d) and some spherulitic structures were also observed.

Differences in height images correspond to 3D surface images for the same samples are presented in FIG.2. Surface of the nanocomposite is rougher than for the neat copolymer, what can be related to presence of TiO_2 particles. Similar observations were described by the authors in their previous work [11] in case of melt-pressed samples, where rms parameter was significantly higher for PET/DLA with 0,2wt% nanocrystalline TiO_2.

Despite of apparent differences in morphology of spin-coated polymers, measurements of contact angles showed rather similar values for both systems, with slightly lower values for TiO_2 containing material. More distinct differences are not observed probably due to the fact that TiO_2 nanoparticles are covered by thin polymer layer and therefore surface hydrophilic/hydrophobic characteristic is so similar despite of strongly hydrophilic character of TiO_2.

Well developed surface morphology for TiO_2 containing material as well as slightly diminished hydrophobicity was correlated with cellular tissue response after implantation test [5]. It is important to notice that no eosynophiles were present in a capsule around elastomer containing TiO_2 nanoparticles thus indicating that surface nano-roughness play an important role in cell/tissue response.

Conclusions

We demonstrated that well developed surface nano-topography in thermoplastic elastomer-based nanocomposites plays an important role in cellular response after soft tissue implantation test. It can be concluded that presence of lamellar structures in TiO_2 containing materials contributed to formation of thin fibrillar tissue capsule where no eosinophiles were detected.

Acknowledgements

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