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Carbon coatings on polymers and their biocompatibility

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ABSTRACT

In this paper we modified the surface properties of polymer foils (polyethyleneterephthalate (PET) and polytetrafluoroethylene (PTFE)) by flash evaporation of carbon layers (C-layers). Adhesion and proliferation of vascular smooth muscle cells (VSMC) on carbon coated PTFE and PET were studied *in vitro*. Chemical composition of deposited C-layers was determined by Raman spectroscopy, surface contact angle was measured by goniometry. Surface morphology of carbon coated samples was studied using atomic force microscopy. Electrical properties of deposited C-layers were determined by measuring its sheet resistance. It was found that the carbon deposition leads to a decrease of surface roughness of PTFE and PET and to a significant increase of sample wettability. Electrical resistance and wettability of deposited C-layers depends significantly on both the thickness of C-layer and the type of polymeric substrate used. It was found that maximal stimulation of the VSMC (adhesion and proliferation) on carbon coated polymers depends on the surface roughness and contact angle of cell carriers used.

1. Introduction

Nowadays, modern medicine is able to partially substitute a variety of damaged or completely functionless organs by the means of synthetic materials. Those materials are generally known as biomaterials. Besides of widely used metals and ceramics, essential role in this issue plays also polymers. Polymers are characterized by favorable mechanical and chemical properties of which the most desired are good elasticity, mechanical resistance and inertness toward corrosive environment [1]. Polymers can be used simultaneously as a material inert to surrounding tissue [2], as well as resorbable materials [3]. Inertness of polymers, however, may not always be desirable. When integrated into living tissues ingrowth complication may occur as a consequence of limited interaction of artificial material with surrounding environment. By the means of appropriate techniques, however, polymer biocompatibility may be positively affected [4]. Different techniques are applied for increasing of tissue affinity toward polymeric carriers such as plasma discharge [5], carbon deposition [6] or deposition of interlayers with specific chemical composition [7,8]. Other promising methods for polymer biocompatibility enhancement are base on deposition of ultra-thin carbon layers. Those layers are predominately composed of biogenic elements and owing to large variety of carbon compounds a large variety of surface properties can be achieved [9]. Diamond like carbon (DLC) layers currently represents the most widespread carbon-based coatings. Due to specific sp^2/sp^3 carbon hybridization ratio these layers exhibit unique properties, *e.g.* enormous hardness, low friction coefficient, high thermal stability, and chemical inertness [10]. By appropriate combination of these properties attractive biomaterial suitable for coronary stents, heart valves or intraocular lenses may be produced [11,12]. Moreover, DLC layers with a minimal amount of hydrogen are suitable for orthopedic devices and dental protheses [13,14].

Carbon layers can be synthesized by several different methods, *e.g.* plasma-enhanced chemical vapor deposition [15], magnetron sputtering [16], ion beam deposition [17] and flash evaporation [18]. By flash evaporation, extremely thin layers may be produced while maintaining their continuity. Due to a very short deposition time no layer contamination by the residual gases occurs. Deposition rate also positively influences the stability of chemical composition of the layer [9]. Homogeneity and stability of the chemical composition are prerequisites for successful growth of cellular tissues and related good biocompatibility of the layers. For the chemical composition of the material promoting cellular activity the amount of functional groups is critical, providing the possibility of anchoring cells to the surface of the tissue carrier [19]. Chemical groups, having free electron pairs capable of forming the non-bonding interactions with anchoring amino acids, appear to be the most important feature in whole process. This interaction allows much easier adhesion and cell growth on the substrate. Among the most important representative of groups providing free electron pairs to non-bonding interaction are C—O and C—N groups [19].

Biocompatibility is known to be dependent not only on the chemical composition but also on the surface properties such as wettability, polarity and roughness [20]. Surface morphology

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is one of the fundamental factors affecting cell-material interaction. Increase of the material hydrophility causes better adhesion of binding proteins stimulating resultant cell adhesion [21]. The increase in hydrophilicity may be due to both the change of surface roughness and surface microstructure [22]. Moreover, the presence of polar groups may significantly contribute to increase the wettability as well. As mentioned above, the presence of polar groups leads to an increased ability to absorb surface anchor amino acids and, thus, to stimulate the cell growth. Each cell type requires optimal value of surface roughness (different for different types of substrates) at which the cell growth is most effective. Thus, for extremely smooth surfaces the poor capture of anchoring amino acids is characteristic, causing reduction in cell-substrate adhesion [23].

In this work we study physico-chemical properties of carbon films deposited by the flash evaporation technique on polymer substrates (polytetrafluoroethylene (PTFE) and polyethylenterephthalate (PET)). PTFE is a representative of hydrophobic polymers with a water-repellent surface and is therefore unsuitable for the growth of cell cultures. PET was chosen as a counterpart since it exhibits favorable wettability for cell tissue culturing. Evaporation of C-layers dramatically changes the surface properties of substrates. Parameters of deposited C-layers were investigated with respect to maximal stimulation of vascular smooth muscle cells (VSMC) during the process of adhesion and proliferation (*in vitro*) on such substrates.

2. Experimental

2.1. Materials and carbon layer deposition

Oriented polyethylenterephthalate (PET, $[—OOC—C_6H_4—COO—(CH_2)_2—]_n$, density 1.41 g cm^{-3}, supplied by Goodfellow Ltd., Cambridge, UK) and polytetrafluoroethylene (PTFE, $[—CF_2—CF_2—]_n$, density 2.2 g cm^{-3}, supplied by Goodfellow Ltd., Cambridge, UK) in the form of 50 μm thick foils was used in the present experiments. Carbon layers were prepared using the SCD 050 Carbon Thread Evaporation Device by flash evaporation process from carbon filaments (BAL-TEC, 15 mm in length). The deposition comprises two operations: filament degassing and flash evaporation. The filament degassing was performed for 10 s at the pressure of 4 Pa and room temperature and with the current of 2.5 A. During degassing process the substrate was shielded by a shutter to avoid substrate heating. Subsequent flash evaporation (after shutter removal) was performed with the current of about 16 A at the pressure of 4 Pa.

2.2. Layer characterization

Contact angles of distilled water, characterizing the surface wettability, were measured at RT at 6 positions (3 rows each 2 measuring positions situated at a 5 mm distance) using a Surface Energy Evaluation System (SEES, Masaryk University, Czech Republic). The 'static' contact angle was measured for pristine polymers and for samples with carbon evaporated structures immediately after deposition (with <10 min delay). Drops of 8.0 ± 0.2 μl volume were deposited and measured after 15 s delay. The values of contact angles were evaluated using SEES software.

The thickness of the deposited layer was determined on a microscopy glass exposed under the same conditions by scratch which can not be used on polymer. We examined the surface morphology of pristine and carbonized samples by AFM using VEECO CP II setup (contact mode). Si probe CONT20A-CP with the spring constant 0.9 N m^{-1} was used. By repeated measurements of the same region (1 μm × 1 μm) we certified that the surface morphology did not change after five consecutive scans. The mean roughness value (R_a) represents the arithmetic average of the deviations from the center plane of the sample.

The continuity of the carbon layer was examined by measuring electrical sheet resistance (R_s). R_s was determined by a two-point method using KEITHLEY 487 pico-ampermeter. For the measurement two Au contacts about 50 nm thick were deposited on the layer surface by sputtering (Balzers SCD 050 device). The measurements were performed at a pressure of about 10 kPa. Typical error of the sheet resistance measurement was ± 5%.

The deposited carbon layers were chemically characterized by near-infrared excited surface-enhanced Raman scattering (NIR SERS). The spectra of the layers were measured on SERS-active gilded platinum substrate. NIR Raman spectra were collected using a Fourier-transform near-infrared (FT-NIR) spectrometer Equinox 55/S (Bruker, Germany). The defocused laser beam of a Nd:YAG laser (wavelength 1064 nm, actual power 50 mW, Coherent, USA) was used for excitation of Raman scattering. The 1024 interferograms were obtained using a quartz beam splitter and a Ge detector (liquid N_2 cooled) and were processed by Fourier transformation using Blackman–Harris 4-term apodization and a zero filling factor of 1 to obtain individual FT Raman spectra with 2 cm^{-1} resolution. Averaged spectra and standard deviation records were calculated.

2.3. Cell culture, adhesion and proliferation

For cell culture experiments, the samples were sterilized for 1 h in ethanol (70%), air-dried, inserted into 24-well plates (TPP, Switzerland; well diameter 1.5 cm) and seeded with (VSMC). Cell cultures were seeded on the samples with the density of 17 000 cells cm^{-2} into 1.5 ml of Dulbecco's modified Eagle's Minimum Essential Medium (DMEM; Sigma, USA, Cat. No. D5648), containing 10% fetal bovine serum (FBS; Sebak GmbH, Aidenbach, Germany) and gentamicin (40 g/ml, LEK, Ljubljana, Slovenia). Cells were cultivated at 37 °C in a humidified air atmosphere containing 5% of CO_2. The number and morphology of initially adhered cells was evaluated 24 h after seeding. The cell proliferation activity was estimated from the increase in cell numbers achieved on the 3rd, and 7th days after seeding. The cells were rinsed in phosphate-buffered saline (PBS) and fixed in 70% ethanol. The cell membranes and cytoplasmic proteins were stained with Texas Red C2-maleimide (Molecular Probes, Invitrogen, Cat. No. T6008; 20 ng/ml), and the cell nuclei were visualized by the dye Hoechst #33342 (Sigma, USA; 5 μg/ml). The number and morphology of cells on the sample surface were then evaluated on microphotographs taken under an Olympus IX 51 microscope (obj. 20×; visualized area of 0.136 mm^2), equipped with an Olympus DP 70 digital camera. The number of cells was determined using the image analysis software LUCIA. For each experimental group and time interval, 20 independent measurements were performed.

3. Results and discussion

For determination of the effect of C-layers on the biocompatibility of polymers it was necessary to characterize their surface properties. Wettability, electrical sheet resistance, surface morphology and roughness was determined by means of contact angle measurements, resistivity measurements and atomic force microscopy (AFM), respectively.

It was shown (Fig. 1) that deposition of C-layer on PTFE causes dramatic decrease of contact angle (increase of wettability), reflecting significant masking effect of C-layer toward original PTFE surface. This change being most pronounced in case of thickest C-layer [18]. With decreasing thickness of C-layer the masking effect gradually disappears and the non-polar character of PTFE is fully manifested. In case of PET, however, the masking effect of deposited