Materials Science and Engineering C 52 (2015) 259–266

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Enhanced adherence of mouse fibroblast and vascular cells to plasma modified polyethylene

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 5 March 2015
Accepted 23 March 2015
Available online 25 March 2015

Keywords:
Ablation
Aging
Cell adhesion
Metabolic activity
Plasma treatment
Polyethylene (PE)

ABSTRACT

Since the last decade, tissue engineering has shown a sensational promise in providing more viable alternatives to surgical procedures for harvested tissues, implants and prostheses. Biomedical polymers, such as low-density polyethylene (LDPE), high-density polyethylene (HDPE) and ultra-high molecular weight polyethylene (UHMWPE), were activated by Ar plasma discharge. Degradation of polymer chains was examined by determination of the thickness of ablated layer. The amount of an ablated polymer layer was measured by gravimetry. Contact angle, measured by goniometry, was studied as a function of plasma exposure and post-exposure aging times. Chemical structure of modified polymers was characterized by angle resolved X-ray photoelectron spectroscopy. Surface chemistry and polarity of the samples were investigated by electrokinetic analysis. Changes in surface morphology were followed using atomic force microscopy. Cytocompatibility of plasma activated polyethylene foils was studied using two distinct model cell lines; VSMCs (vascular smooth muscle cells) as a model for vascular graft testing and connective tissue cells L929 (mouse fibroblasts) approved for standardized material cytotoxicity testing. Specifically, the cell number, morphology, and metabolic activity of the adhered and proliferated cells on the polyethylene matrices were studied in vitro. It was found that the plasma treatment caused ablation of the polymers, resulting in dramatic changes in their surface morphology and roughness. ARXPS and electrokinetic measurements revealed oxidation of the polymer surface. It was found that plasma activation has a positive effect on the adhesion and proliferation of VSMCs and L929 cells.

1. Introduction

Scaffolds used in tissue engineering applications should demonstrate compatible biological and physical properties which match with the physiological conditions in vitro and in vivo. The selection of appropriate materials for implant development requires fulfillment of some criteria, which keep a negative impact of an implant to the human as minimal as possible. Besides, the biodegradability and the ability of cells growing from the scaffolds are highly dependent to a large extent on their physical and structural properties, such as interconnectivity, porosity and surface morphology. In general, the design of scaffolds should allow the temporary support of body's structures or injured sites through the stress transfer from one part to another, and thereafter be degraded over time. To select an appropriate type of a material for scaffold design and manufacturing, several key factors have to be studied in detail [1].

Since cells adhere poorly on hydrophobic surfaces [2], cell detachment from biopolymer surfaces may occur upon restoring blood circulation [3]. To prevent thromboembolism, polymer surface treatment to enhance cell adhesion is essential prior to the device implantation [4,5]. Common surface modification techniques include treatment by flame, corona, plasma, photons, electron beams, ion beams, X-rays, and gamma-rays. In the past decades, surface treatment of polymers with non-thermal plasmas has been extensively studied [6–8], and it has become evident that this approach is promising also for biomedical polymers [9,10]. Surface modification by different plasma precursors including oxygen [6], ammonia [11], and air [12] can also promote cell adhesion on polymer surfaces because of the presence of polar surface functionalities. Argon plasma improves the surface hydrophilicity (through the formation of oxygen surface functionalities upon the exposure of the reactive polymer surface to the ambient conditions) without affecting the bulk characteristics [4,13,14]. Due to the versatility of this technology, it might be useful in many different applications [15,16].

For example, polyethylene (PE), which is very cheap and easy to process, has excellent mechanical properties and good resistance to weathering. It has been used in huge quantities to make films in the

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http://dx.doi.org/10.1016/j.msec.2015.03.052
0928-4931/

packaging industry. The key problems with polyethylene lie in its bad printability and bondability caused by its inertness and low surface energy. To overcome this difficulty, surface modifications, such as plasma treatment, corona discharge and flame treatment, are frequently employed to enhance printability and bondability of products made with PE. Actually, a simple way to modify the chemical and physical states of the polymer surface, without altering the bulk properties, is by plasma treatment. The plasma treatment of polymers leads to the creation of new chemical groups [17,18], cross-linking and branching of the macromolecules and formation of low molecular weight oxidized structures [19–21], the rates of these processes being a function of the plasma reactivity. Due to ablation surface topography of polymer is affected [22,23]. Further, ultra-high molecular weight polyethylene (UHMWPE) has been used for the acetabular cup in total hip replacement and for the components of other total joint replacements since the 1960s [8,24].

The objective of this study was to investigate adhesion and spreading of vascular smooth muscle cells (VSMCs) and mouse fibroblasts (L929) on low-density polyethylene (LDPE), high-density polyethylene (HDPE), and ultra-high molecular weight polyethylene (UHMWPE) surfaces exposed to Ar plasma and to evaluate the most suitable substrate for cell adhesion and spreading. Surface properties of three pristine PE types and their plasma-modified counterparts were studied by different experimental techniques: angle resolved X-ray photoelectron spectroscopy (ARXPS), electrokinetic analysis, and goniometry were used for chemical structure and polarity of surface characterization. Further, atomic force microscopy (AFM) was employed to study the surface morphology and roughness of the pristine and plasma treated PE samples. The biological response of the two model cell lines (VSMC and L929) incubated with untreated and plasma-treated PE matrices was quantified in terms of the cell density, metabolic activity, spreading, and morphology.

2. Experimental section

2.1. Materials and modification

Oriented high-density polyethylene (HDPE, 0.95 $g \cdot cm^{-3}$, $M_w = 4 \cdot 10^5$ $g \cdot mol^{-1}$) in the form of 40 μm thick foils (supplied by Granitol Ltd., Czech Republic), low-density polyethylene (LDPE, 0.92 $g \cdot cm^{-3}$, 35 μm foil, $M_w = 1 \cdot 10^5$ $g \cdot mol^{-1}$, Goodfellow Ltd., UK), and ultra-high molecular weight polyethylene (UHMWPE, 0.94 $g \cdot cm^{-3}$, 75 μm foil, $M_w = 3 \cdot 10^6$ $g \cdot mol^{-1}$, Goodfellow Ltd., UK) was used in this study. The crystallinity of individual samples was determined by DSC analysis taking into account theoretical heat of fusion of PE foil with 100% crystallinity ($\Delta H_f^0 = 290$ $J \cdot g^{-1}$) [25,26]. Crystallinity portion of studied PE foils was 30, 62 and 52% for LDPE, HDPE and UHMWPE, respectively. Values of crystallinity correspond with Ref [27].

The samples were modified in direct (glow, diode) Ar^+ plasma in Balzers SCD 050 device under the following conditions: gas purity was 99.997%, flow rate 0.3 $l \cdot s^{-1}$, pressure 10 Pa, electrode distance 50 mm and its area 48 cm^2, chamber volume approx. 1000 cm^3, and plasma volume 240 cm^3. Exposure times varied from 0 to 480 s, discharge power was 8.3 W and the treatment was accomplished at room temperature.

2.2. Measurement techniques

The thickness of the surface layer removed by the plasma ablation was studied using Mettler Toledo UMX2 microbalance. In order to enhance the sensitivity of the measurement, the samples were exposed from both sides. The thickness of the removed layers was calculated from the weight of three samples (ϕ 2 cm) before and after the plasma treatment.

Contact angles of distilled water, characterizing the structural and compositional changes induced by the plasma treatment, were measured at room temperature on two samples and at six positions using a Surface Energy Evolution System (SEES, Masaryk University, Czech Republic). The "static" contact angle was measured for pristine and plasma-treated polymers immediately after the plasma treatment (<10 min delay). Drops of 8.0 ± 0.2 μl volume were deposited using an automatic pipette (Transferpette Electronic Brand, Germany); their images were taken with a 5 s delay. Then, the contact angles were evaluated using SEES code. In this experiment, the contact angles on the plasma-modified samples were measured immediately after the modification and then after 15, 30 and 45 min, 1, 3 and 6 h, 1 day, and 3, 6, 10, 15, and 17 days after modification.

Omicron Nanotechnology ESCAProbeP spectrometer was used to measure angle resolved X-ray photoelectron spectra (ARXPS); relative error of 10%. X-ray source was monochromated at 1486.7 eV. Exposed and analyzed area had a dimension of 2 × 3 mm^2. Take off angles were 0 or 81°. Spectra were measured stepwise with step in binding energy 0.05 eV. The spectra evaluation was carried out by CasaXPS program.

The surface roughness and morphology of the samples were examined by AFM microscopy. The AFM images were taken under ambient conditions of Digital Instruments VEECO CP II set-up. "Tapping mode" was chosen in preference to "Contact mode" to minimize damage of the sample surfaces. Si probe RTESPA-CP with the spring constant 20–80 $N \cdot m^{-1}$ was used. By repeated measurements of the same region (2 × 2 μm^2) we certified that the surface morphology did not change after three consecutive scans. The mean roughness value (R_a) represents the arithmetic average of the deviations from the center plane of the sample.

Electrokinetic potential (zeta-potential, ζ-potential) of pristine and plasma treated polymers was determined by SurPASS Instrument (Anton Paar). Samples were studied inside an adjustable gap cell in contact with the electrolyte (0.001 $mol \cdot dm^{-3}$ KCl). For each measurement a pair of polymer films with the same top layer was fixed on two sample holders (with a cross section of 20 × 10 mm^2 and gap between 100 μm) [28]. All samples were measured three times at constant pH value of 6.1 with the relative error of 5%. For the determination of the zeta potential, the streaming current method was used and the Helmholtz–Smoluchowski equation was applied to calculate zeta potential [28].

2.3. Cell cultivation

The adhesion and proliferation of vascular smooth muscle cells (VSMCs) and mouse fibroblasts (L929) on pristine and modified PE samples were studied in vitro. The VSMCs were isolated by an explantation method from the intima–media complex of the rat thoracic aorta. L929 cells were purchased from Sigma, USA [29].

First, the PE samples were sterilized in 70% ethanol in Petri dish, inserted in 12-well plates (TPP, Switzerland, ϕ 2.14 cm), washed by PBS and mounted to the well bottom with hollow plastic cylinders.

VSMCs were seeded on the samples in the density of 17,000 cells $\cdot cm^{-2}$ into 3 ml of high glucose Dulbecco's Modified Eagle Minimum Essential Medium (DMEM; Sigma, USA) containing 10% fetal bovine serum (FBS, Sebak GmbH, Aidenbach, Germany) and 40 $\mu m \cdot cm^{-3}$ of gentamicin (LEK, Ljubljana, Slovenia). VSMCs were cultivated on the samples for 24, 72 and 144 h at 37 °C, humidity 95% and air atmosphere containing with 5% of CO_2.

L929 cells were seeded with a density of 14,019 cells $\cdot cm^{-2}$ in 1 ml of Minimal Essential Medium (MEM, Sigma, USA) supplemented with stable 2 $mmol \cdot l^{-1}$ L-Glutamine (Sigma, USA) and 10% of FBS.

Cells were fixed and stained similarly as described in Ref [30,31]. VSMCs and L929 cells were washed with PBS and fixed with 4% formaldehyde (Thermo Scientific, USA) in PBS (37 °C, 20 min). After PBS washing, cell cytoskeleton was labeled with phalloidin-Atto 565 (Sigma, USA) and cell nuclei with DAPI (4′,6-diaminido-2-phenylindole dihydrochloride; Sigma, USA) in PBS. After 20 min of staining, the cells