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Plasma activated polymers grafted with cysteamine improving surfaces cytocompatibility

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ABSTRACT

Cysteamine was grafted on polymer foils (poly L-lactic acid, polystyrene, low and high density polyethylenes, polyethyleneterephthalate, polytetrafluoroethylene, polyvinylfluoride and polyvinylidenefluoride) previously treated (activated) in plasma discharge. Grafting of cysteamine on polymer surface is expected to provide a new material applicable in medicine treatment. Properties of the sample surfaces change significantly before and after plasma treatment and cysteamine grafting and they were studied using various methods to characterize changes in surface chemistry, polarity, wetability, etc. Surface chemistry was studied by X-ray photoelectron spectroscopy, chemistry and polarity by electrokinetic analysis and by goniometry, roughness and morphology by atomic force microscopy. Representatives of unmodified and modified polymers were used for *in vitro* study of adhesion and proliferation of vascular smooth muscle cells. Plasma treatment and cysteamine grafting improve dramatically surface cytocompatibility. Electrokinetic analysis and X-ray photoelectron spectroscopy confirmed the cysteamine bonds to polymer surfaces via opposite functional groups (−SH or $-NH_2$) depending on chemistry and polarity of polymers under study. This preferential grafting influence an adhesion and proliferation of vascular smooth muscle cells significantly. Even cytocompatibility of plasma treated polytetrafluoroethylene and subsequently grafted with cysteamine is "better" in comparison with tissue culture polystyrene.

1. Introduction

Polymers are of general interest for their excellent bulk properties (e.g., low density, flexibility, mechanical and electrical strength, chemical resistance). They are frequently employed in industry, biotechnology, tissue engineering studies, medicine, etc. However, inert nature of most polymer surfaces may limit their use. Surface chemistry, polarity, roughness and morphology are important for many potential applications of polymers in medicine and related fields since they strongly influence cell adhesion, proliferation and functionality [1,2].

Many modification techniques (e.g. plasma treatment and/or grafting of new functional groups on surface) have been developed to improve surface properties (e.g. wettability, metal adhesion, chemistry and biocompatibility of polymer surfaces) [3]. One of the techniques combines plasma treatment and subsequent grafting of polymer surface with cysteamine [4]. Cysteamine is the simplest stable aminothiol and it is a degradation product of the amino acid cysteine finding quite wide usage in medicine [5].

Grafting polymers with cysteamine is expected to provide new materials with excellent properties and potential usage in industry, biomedicine and tissue engineering. Cysteamine grafted on polymer was used e.g. to synthesize "new materials" with high cross-linking degree, high thermal resistance and different glass transition temperatures [6]. By cysteamine grafting of polymer membranes substrates were prepared materials for subsequent coating with metal nanoparticles and preparation of systems with different applications (e.g. Ag nanoparticles coated membranes with anti-biofouling and antimicrobial properties [7], Au nanoparticles coated polymers with modified polarity, wettability and biocompatibility [4,8]).

In our previous work we have studied grafting of polyethyleneterephthalate (PET) and polytetrafluoroethylene (PTFE) with 2-mercaptoethanol, 4,4′-biphenyldithiol and cysteamine and

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subsequently with gold nanoparticles [4]. This research provided extraordinary results for grafting of these two polymers of different polarity with cysteamine. While on PTFE surface the cysteamine is bonded preferentially via −SH group with "free" $-NH_2$ group, on PET the binding is probably without preferency with "free" both of $-NH_2$ and −SH, the second one available for subsequent bonding of Au nanoparticles. These results may be of interest for the application of these materials and processes in nanotechnology and in biomedicine [8].

In this work different 8 polymers previously treated in argon plasma were grafted with cysteamine from water solution and all samples were characterized by different analytical methods to obtain complex information on their surface properties. Due to presence of different functional groups on surface after modification we expect different behavior, adhesion and proliferation of cell. Therefore an interaction of tested polymers with vascular smooth muscle cells (VSMC) was studied *in vitro* in this study.

2. Materials and methods

2.1. Materials

The following polymers in the form of foils supplied by Goodfellow Ltd., UK were used in this study: polyethyleneterephthalate (PET, 23 μm thick foils, density 1.30 g cm^{-3}), polytetrafluoroethylene (PTFE, 25 μm, 2.20 g cm^{-3}), polyvinyl fluoride (PVF, 50 μm, 1.37 g cm^{-3}), polyvinylidene fluoride (PVDF, 50 μm, 1.76 g cm^{-3}), polystyrene (PS, 30 μm, 1.05 g cm^{-3}), poly L-lactic acid (PLLA, 50 μm, 1.25 g cm^{-3}). The high density polyethylene (HDPE, 40 μm, 0.96 g cm^{-3}) and low density polyethylene (LDPE, 30 μm, 0.92 g cm^{-3}) were supplied by Granitol Ltd., CR. Tissue culture polystyrene (TCPS, TPP Switzerland) substrate was used as a control for cytocompatibility test of VSMCs.

2.2. Plasma treatment

The samples were first treated in DC Ar plasma in Balzers SCD 050 at room temperature (RT) under the following conditions: gas purity 99.997%, flow rate 0.3 l s^{-1}, pressure 10 Pa, electrode distance 50 mm, its area 48 cm^2, chamber volume approx. 1000 cm^3, plasma volume 240 cm^3, discharge power 8.3 W, treatment times were 120, 240 and 480 s [9].

2.3. Cysteamine grafting

Immediately after the plasma treatment the samples were inserted into water solution (2 wt. %) of cysteamine (2-aminoethanethiol, $HS-(CH_2)_2-NH_2$, CYST) for 24 h. Then the samples were rinsed by methanol and dried by inert nitrogen and then at Petry dishes for 48 h [4].

2.4. Used analytical methods

Properties of all samples, pristine polymers, polymers treated by the plasma and polymers treated by the plasma and then grafted with cysteamine were characterized using different methods. Surface chemistry was characterized by X-ray photoelectron spectroscopy, chemistry and polarity by electrokinetic analysis and by goniometry, roughness and morphology by atomic force microscopy.

Elektrokinetic analysis is quite known and spread methods for dispersions analyses but we use it as a new method for characterization of flat samples [10]. Combination of electrokinetic analysis and XPS measurement give complex information about functional groups on surface important for surfaces biocompatibility. Surface wettability, roughness and morphology are also important for cell adhesion and proliferation. Electrokinetic analysis (determination of zeta potential) of all samples (pristine, plasma treated for 120 s and plasma treated for 120 s and grafted with cysteamine) was accomplished on SurPASS Instrument (Anton Paar, Austria). Samples were studied inside the adjustable gap cell in contact with the electrolyte (0.001 mol dm^{-3} KCl) at RT. For each measurement a pair of polymer foils 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). All samples were measured four times at constant pH (pH = 6.0) with a relative error of 10%. For determination of the zeta potential the streaming current method was used and the Helmholtz–Smoluchowski equation was applied to calculate zeta potential [10].

Surface concentrations of elements on pristine polymers, polymers treated by plasma for 120 s and treated by plasma for 120 s and grafted with cysteamine were measured by X-ray photoelectron spectroscopy (XPS) 3 days after cysteamine grafting. Omicron Nanotechnology ESCAProbeP spectrometer was used to measure photoelectron spectra (typical error of 10%). Exposed and analyzed area had dimension 2 × 3 mm^2. X-ray source was monochromated at 1486.7 eV with step size 0.05 eV. The spectra were measured stepwise with a step in the binding energy of 0.05 eV at each of the six different sample positions with respect to the detector axis, which translated into different angles (0° – perpendicularly to sample, and 81° – a very surface). The spectra evaluations were carried out by CasaXPS program. The concentrations of the elements (with only exception of hydrogen which cannot be observed by XPS) are given in at. % [11].

Surface wettability was determined by measurement of contact angle of samples (pristine, plasma treated for 120, 240 and 480 s and plasma treated for 120, 240 and 480 s and grafted with cysteamine). Surface contact angle of distilled water was measured by goniometry, i.e. the static (sessile) water drop was measured at RT at ten positions on two samples of each polymer using a Surface Energy Evolution System (SEES, Masaryk University, Czech Republic). Drops 8.0 ± 0.2 μl in volume were deposited using automatic pipette (Transferpette Electronic Brand, Germany) and their images were taken with 5 s delays. Then, the contact angles were evaluated using SEES code. Plasma treated samples were studied 5 min after the treatment, samples grafted with cysteamine 48 h after the grafting process. Experimental error is 5%.

Surface roughness and morphology of samples (pristine, treated by plasma for 120 s and plasma treated for 120 s and grafted with cysteamine) were examined by AFM method using VEECO CP II setup (tapping mode). Si probe RTESPA-CP with the spring constant 20–80 N m^{-1} was used. It was proved by repeated measurements of the same region (2 × 2 μm^2 or 10 × 10 μm^2 in area) that the surface morphology did not change after five consecutive scans [12].

2.5. Cell adhesion, proliferation and viability

The adhesion and proliferation of vascular smooth muscle cells (VSMCs) on selected polymers (PET, PTFE, HDPE, PLLA) – pristine, plasma treated for 120 s and plasma treated and grafted with cysteamine were studied by *in vitro* method. Three samples were used for analysis by randomly chosen fields and one sample was used for determination of viability of the adhered or proliferated VSMCs. The samples were sterilized for 1 h in ethanol (70%), air-dried and fixed to the well bottom of the polystyrene 12-well plates (TPP, Switzerland, diameter 2.14 cm) with plastic rings. On each sample was seeded 50 000 cells (i.e. 16 000 cells cm^{-2}) into 3 ml of Dulbecco's Modified Eagle Minimum Essential Medium (DMEM, Sigma, USA, Cat. No. D5648) containing 10% fetal bovine