STUDY OF BIOCOMPATIBILITY OF MODIFIED POLYETHYLENE

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Abstract

Polyethylene (PE) is a synthetic polymer used for biomedical applications and tissue engineering. Surface modification of this material relates to changes of its surface hydrophilicity, chemistry, morphology, microstructure, roughness, and topography, all influencing its biological response. This our research was focused on modification of PE by argon plasma discharge and then grafting with biologically active polyethylene glycol (PEG) with the aim to enhance its cytocompatibility of the cell lines L929 of mouse fibroblast. The surface properties of pristine PE and its grafted counterparts were studied by different experimental techniques: X-ray spectroscopy, goniometry, Atomic Force Microscopy and electrokinetic analysis (zeta potential). Our results show that grafting of PEG leads to higher wettability, surface roughness, and thus the adhesion and proliferation of cultured cells.

Keywords: Polyethylene, plasma treatment, polyethylene glycols grafting, surface properties, cell growth

1. INTRODUCTION

Polymers are often used successfully in field's tissue engineering for their excellent bulk physically and chemically properties. The polyethylene (PE) is a very cheap and unique synthetic polymer with outstanding mechanical properties [1], very low friction coefficient and high wear resistance [2]. Surface modifications of this material affect its hydrophilicity, morphology, energy, microstructure and roughness; all of these have a significant impact on its cytocompatibility [3].

The properties of polymer as cell carriers can be improved by the plasma modification, exposure to laser light or by surface grafting of their surface with suitable agents, which can enhance the cell adhesion and proliferation [4]. In biomedicine, plasma treatments have been used to modify biomaterials for various applications including devices and implants for therapy. The capability of plasma to alter only surface physico-chemical properties of a material without altering its bulk properties is advantageous in the design, development, and manufacturing of biocompatible polymers [5].

Nonspecific bioadhesion continues to hinder the use of biological materials [6]. Attempts at combating protein adsorption and cellular adhesion by surface modification include the use of polysaccharides [7,8], phospholipids [8], polyethylene glycol (PEG) [9] and many others. PEG is a simple molecule and its structure is similar to water and characterized by hydroxyl groups at either end of the molecule. Influence of PEG on adhesion and subsequent cell proliferation is however also strongly dependent on the chain length of PEG and its concentration on the surface of the substrate [10]

The presented research is focused on surface modification of ultra high molecular weight polyethylene for improvement of its biomedical properties. The aim of our work was to determine the effect of plasma treatment of samples in combination with grafting PEG and HS-PEG-SH on growth of cells seeded on these samples.

2. EXPERIMENTAL

2.1. Materials and methods

The ultra high molecular weight polyethylene, PE foil (the thickness 75 µm, density 0.94 g·cm^{-3}, M_w = 4x10^6, supplied by Goodfellow Ltd., UK) was plasma treated and grafted with PEG and HS-PEG-SH. The PE samples were modified in direct (glow, diode) Ar^+ plasma using Balzers SCD 050 device (BalTec AG, Pfäffikon, CH), conditions have been described in detail in [11]. The duration of the plasma treatment was 30, 90 and 240 s and the discharge power was 8.3 W. Immediately after plasma treatment samples were immersed into solution of PEG (polyethylene glycol, Sigma Aldrich), HS-PEG-SH (polyethylen glycol dithiol, Sigma Aldrich) or distilled water for 24 h. Subsequently were samples immersed into distilled water for 24 h and then dried in a Petri dish for 24 h. The prepared samples were stored at laboratory conditions (24°C, 40-60 % humidity).

2.2. Measurement Techniques

Wettability of the samples was determined by measuring surface water contact angles (WCA) by Drop Shape Analysis System DSA 100 (KRÜSS GmbH, DE) at room temperature (24°C, 40-60 % humidity). Water drops of 2.0 ± 0.2 µL were deposited on the tested samples using a stainless steel needle. Images of the drops were taken after a 2 s delay. At least 6 measurements of different positions on at least two replicates of each sample were performed and averaged to yield WCAs and their standard deviations. The measurement of WCA was performed on samples “aged” for 14 days.

The chemical composition of the prepared samples was determined from X-ray photoelectron spectra (XPS) measured by Omicron Nanotechnology ESCAProbeP spectrometer (supplied by the Omicron Nanotechnology GmbH, DE) with a relative error of 10 %. The measuring conditions were as follows: monochromated X-ray source at 1486.7 eV with the measuring step of 0.05 eV. Characteristic carbon (1 s), oxygen (1 s) and sulphur (2 p) peaks were searched. Measuring was performed in ultra-hight vacuum. The samples used for measurement were "aged" for 14 days.

The surface morphology of the samples was examined by atomic force microscopy (AFM) using VEECO CP II system. The surface roughness (R_a) was measured in a “tapping” mode using silicon P-doped probe RTESPA-CP with the spring constant of 20-80 N·m^{-1} (supplied by Bruker Corp., USA). By repeated measurements of the same region (3 × 3 µm^2), we verified that the surface morphology did not change after three consecutive scans. The samples used for the measurement were “aged” for 14 days.

Electrokinetic analysis (electrokinetic potential, zeta potential) of all samples was determined by SurPASS Instrument (Anton Paar). Samples were studied inside an adjustable gap cell in a contact with an electrolyte (0.001 mol·L^{-1} KCl). For each measurement, pair of polymer films with the same top layer were fixed on two sample holders (with a cross section of 20x10 mm^2 and a gap between them of 100 µm). All samples were measured three times at a constant pH = 7 with the relative error of 5 %. For the determination of zeta potential, the streaming current method was used and the Helmholtz-Smoluchowski equation was applied to calculate zeta potential [12]. All samples used for measurement of zeta potential were “aged” for 14 days.

2.3. Cell Culture

According to the international standard EN ISO 10993-5, cytotoxicity testing was performed *in vitro* using mouse fibroblast cell line - L929 (Sigma, USA) on pristine, plasma treated and PEGs grafted. The adhesion and proliferation process has been described in detail in [11]. Fluorescence microscopy was performed using an inverse fluorescence microscope Olympus IX-81 (Xcellence system). Parameters of the microscope have been described [11]. Cell adhesion was determined 6 h after inoculation, proliferation after 24 and 72 h of the cell growth. ImageJ 1.47 software (National Institute of Health, USA) was used for semi-automatic evaluation of the cell number (based on cell nuclei detection). All samples (“aged” for 14 days) were tested in triplicates.

3. RESULTS AND DISCUSSION

Between factors that significantly affect cytocompatibility belongs wettability [13,14]. The values of WCA measured in dependence on the plasma treatment and PEGs grafted are shown in **Table 1**. WCA of pristine PE was 97.5° [15]. The results show that plasma treatment did not cause any significant changes in WCA, on the other hand, PEG grafting resulted in a noticeable decrease in WCA. Grafting solution HS-PEG-SH caused hydrophilically for samples treated by plasma for shorter period.

Table 1 Dependence of PE's water contact angles (WCA, measured by goniometry) and concentration of carbon, oxygen and sulphur (determined by XPS) on Ar plasma treated (8.3 W for 30, 90 and 240 s) and grafted by PEG and HS-PEG-SH samples. The error of XPS measurement is ± 5 %

Sample	**WCA** (°)	**Element concentration** (at. %)		
		C (1 s)	**O** (1 s)	**S** (2 p)
PE	97.5 ± 3.1	100.0	0.0	-
PE / pl 30s	94.9 ± 3.2	79.2	20.8	-
PE / pl 90s	98.3 ± 2.8	75.8	24.2	-
PE / pl 240s	100.7 ± 2.2	72.5	27.5	-
PE / pl 30s / PEG	73.1 ± 3.3	81.0	19.0	-
PE / pl 90s / PEG	83.3 ± 3.1	75.8	24.2	-
PE / pl 240s / PEG	83.3 ± 8.3	67.4	32.6	-
PE / pl 30s / HS-PEG-SH	82.3 ± 3.0	77.1	22.3	0.6
PE / pl 90s / HS-PEG-SH	87.7 ± 4.6	79.0	20.6	0.4
PE / pl 240s / HS-PEG-SH	97.0 ± 2.9	71.2	28.8	-

The chemical composition of the pristine and modified PE surface was measured by XPS method. The observed element concentrations of C, O, and S in pristine, plasma treated, and PEGs grafted samples are summarized in **Table 1**. The carbon concentration decreased in the plasma treated samples from 100 % (pristine PE) to 72.5 % (after 240 s). This was caused by oxygen binding, when its content increased in the plasma treated samples from 0 (pristine PE) to 27.5 % after the plasma treatment. The increase of oxygen concentration is caused by newly created polar oxygen groups such as carbonyl, carboxyl, and hydroxyl [16]. The sample surface after 240 s of plasma treatment was ablated and disrupted to larger extent than that of 30 s, therefore the oxygen concentration was higher for samples treated with plasma for longer periods. The layer of PEGs is not continuous. PEG For samples immersed in HS-PEG-SH solution after plasma treatment, XPS showed decreasing concentration of sulphur with increasing ablation time. Surface treated by plasma for longer period allows HS-PEG-SH to anchor to the surface by both SH groups which are as result masked under bulk of the molecule resulting in decrease of sulphur in layer observed by XPS.

The surface morphology and roughness (**Figure 1**) of pristine, plasma treated, and PEGs grafted samples were studied by AFM. After plasma treatment the surface roughness of (pristine PE has R_a = 7.0 nm) increased to 11.7 nm. We observed a difference between the surface roughness of samples treated by plasma and grafted by solution, while samples of plasma treated and grafted by PEG formed sharper hills and protrusions; samples modified for 240 s had more rounded surface structures. This effect was probably caused by enhanced resistance of a crystalline phase to the plasma treatment [17] therefore; shorter plasma treatment caused only uncovering of crystals from the amorphous phase, while longer treatment caused also their ablation. Bigger differences were apparent for the grafted samples of plasma treated for 30 s. The roughness for PE/pl 30 s/HS-PEG-SH is 21.5 nm. This significant increase in surface roughness is caused mainly due to

formation of ripples on the surface of sample. These ripples were probably caused by interaction of HS-PEG-SH with plasma activated sample surface.

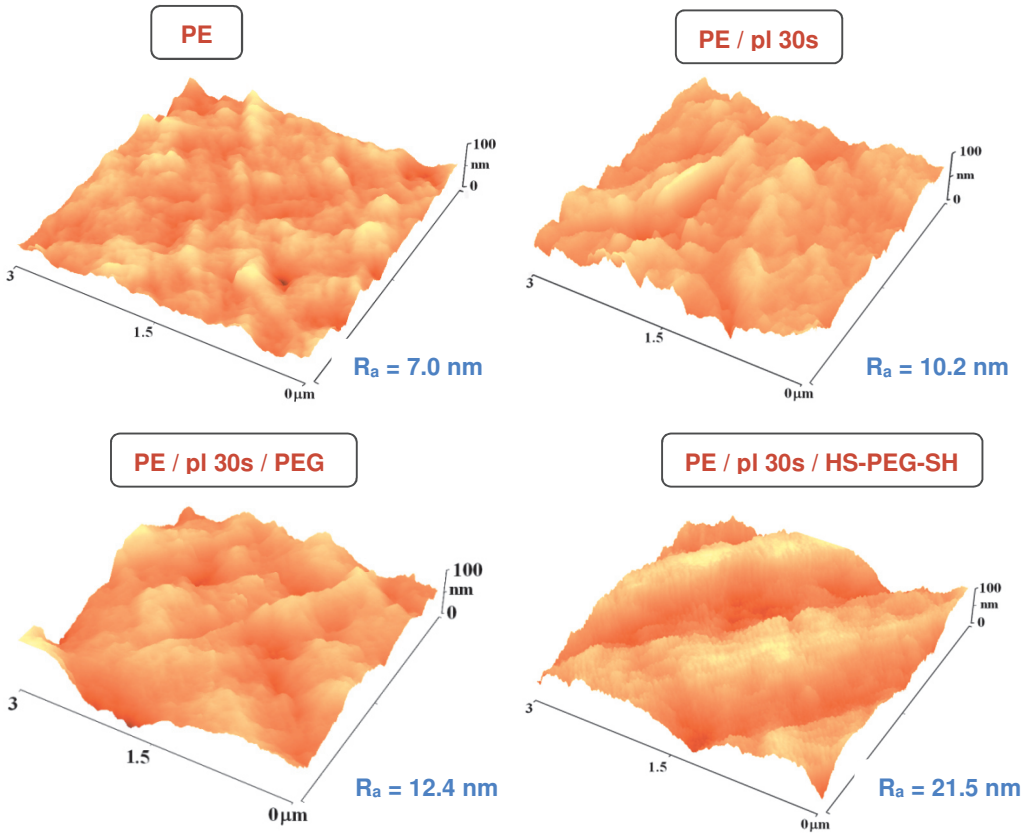

Figure 1 AFM images and the surface roughness of pristine PE, PE treated by plasma for 30 s and grafted by PEG and HS-PEG-SH

Results of electrokinetic analysis are presented in **Figure 2a** and provide information about surface chemistry and surface charge of samples. Both of these are important factors for a primary cell adhesion and proliferation [12]. From **Figure 2a** it is clear, that the value for pristine PE (-70 mV) corresponds to the strongly hydrophobic surface [18]. The zeta potential is known to depend on the surface chemistry, polarity, charge and the surface morphology and roughness [18]. Zeta potential of grafted samples dramatically changed to the less negative values due to increasing polarity of samples. These results correspond well with goniometry. PEG has chain long enough to bend and anchor on the both ends to the surface. SH groups are also preferably bound to the surface and thus chain is also immobilized.

We studied of the presence of grafted solutions PEG and HS-PEG-SH on the cell adhesion (6 h) and proliferation (24 and 72 h) of L929, it is shown in **Figure 2b**. Results of cell growth from these solutions were compared with TCPS (tissue culture polystyrene), pristine PE and plasma treated PE for 30 s. We used H_2O as "grafted" control solution for determining the physical and chemical bond. After 72 h of growth, the largest number was detected on samples with PEG and then HS-PEG-SH. We used PEG with Mw of 20.000 $g \cdot mol^{-1}$. It is likely, that PEG chain was anchored at multiple locations which immobilized the chain and created a structure supporting cell adhesion. We observed from the data that SH-group also supports cell adhesion [19]. Deviations of the number of cells are negligible for biological measurements [20]. Cell proliferation on the plasma treated surface and surface subsequently immersed in H_2O is almost the same. The measurement corresponds with results from zeta potential.

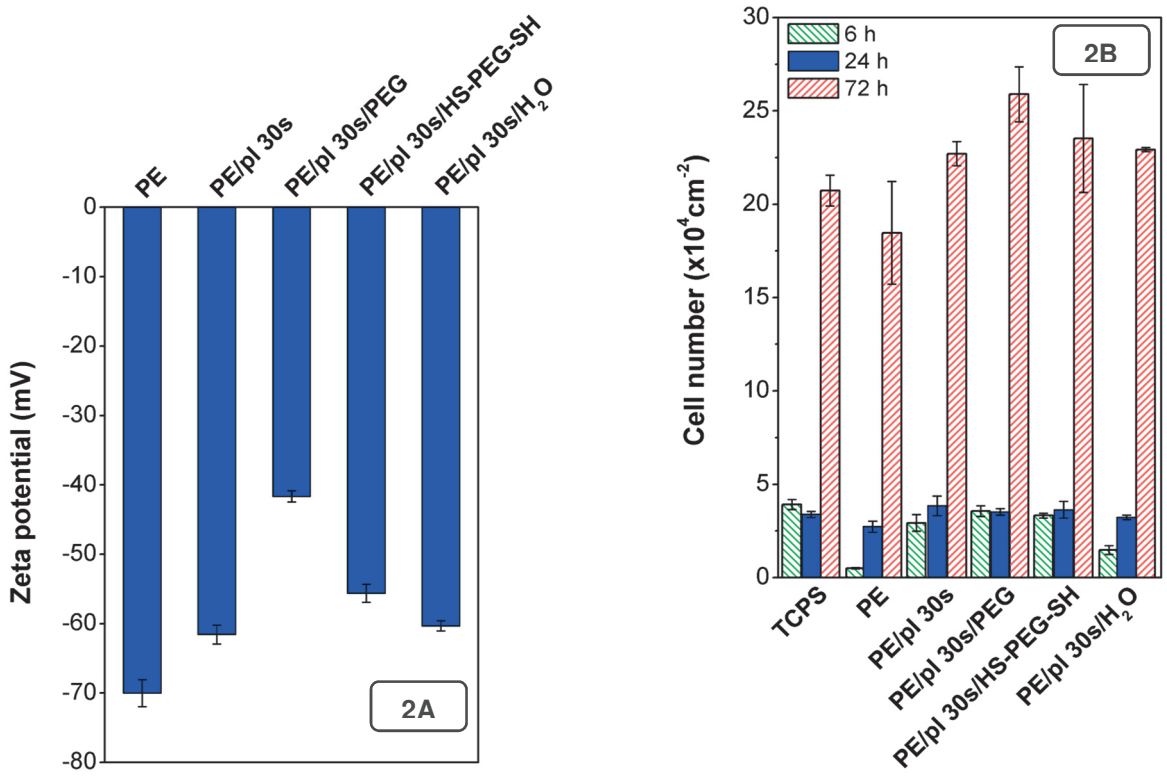

Figure 2a Zeta potential of pristine PE, plasma treated (30 s), grafted by PEG, HS-PEG-SH and distills water samples in 1mM KCl solution. **2b** Number of L929 cells after different cultivation periods (6, 24 and 72 h) on: TCPS, pristine, PE treated by plasma for 30 s and grafted by PEG and HS-PEG-SH

4. CONCLUSION

In this paper, plasma treatment, PEG and HS-PEG-SH grafting were used for surface activation of the PE. Effect of this activation on chemistry, morphology and *in vitro* cytocompatibility of mouse fibroblast (L929) was examined. PEG and HS-PEG-SH increased the surface wettability of PE. XPS showed increase in surface oxygen concentration after plasma treatment due to formation oxidation of the PE; furthermore increased ablation time also enhanced grafting of PEG. It was found that the surface roughness increased grafting with HS-PEG-SH, which creates ripples on the surface, it is apparent from AFM images. This research also shows that PEG with an adequate length of terminal functional groups is suitable material for cell adhesion and growth. Based on these results, we summarize that PEG grafted on plasma activated surface improves the cytocompatibility of PE compared to that of pristine polymer.

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