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The interplay of plasma treatment and gold coating and ultra-high molecular weight polyethylene: On the cytocompatibility

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ABSTRACT

We have investigated the application of Ar plasma for creation of nanostructured ultra high molecular weight polyethylene (PE) surface in order to enhance adhesion of mouse embryonic fibroblasts (L929). The aim of this study was to investigate the effect of the interface between plasma-treated and gold-coated PE on adhesion and spreading of cells. The surface properties of pristine samples and its modified counterparts were studied by different experimental techniques (gravimetry, goniometry and X-ray photoelectron spectroscopy (XPS), electrokinetic analysis), which were used for characterization of treated and sputtered layers, polarity and surface chemical structure, respectively. Further, atomic force microscopy (AFM) was employed to study the surface morphology and roughness. Biological responses of cells seeded on PE samples were evaluated in terms of cell adhesion, spreading, morphology and proliferation. Detailed cell morphology and intercellular connections were followed by scanning electron microscopy (SEM). As it was expected the thickness of a deposited gold film was an increasing function of the sputtering time. Despite the fact that plasma treatment proceeded in inert plasma, oxidized degradation products were formed on the PE surface which would contribute to increased hydrophilicity (wettability) of the plasma treated polymer. The XPS method showed a decrease in carbon concentration with increasing plasma treatment. Cell adhesion measured on the interface between plasma treated and gold coated PE was inversely proportional to the thickness of a gold layer on a sample.

1. Introduction

Currently, one of the major concerns of human body aging is a joint replacement. Over the last centuries, the average of the human life span and physical activity have significantly increased, as a consequence of which more surgical "adjustments" are required. The total joint replacements have become a routine orthopedic surgery. In developed countries, more than 1000 joint replacements are carried out per 1 million of inhabitants each year [1]. Most often replaced joints are those of hips and knees, which are followed by shoulder and elbow replacements. Metals, ceramics and polymers are commonly used orthopedic materials. Metal and ceramic implants have different stiffness characteristics when compared to that of a bone. Therefore, there is often an increased strain (stress-shielding) on the prosthesis-bone interface which results in the prosthesis release, its shorter lifetime and poorer long-term effectivity [2]. Therefore, materials with low Young modulus, such as polymers, are a better option [3,4].

Ultra-high molecular weight polyethylene (UHMWPE, further "only" PE) is a synthetic polymer widely used in biomedical and technical applications, especially in orthopedics and traumatology. PE is primarily used for its desirable characteristics, such as very low friction coefficient, high wear resistance [5] and impact strength [6]. These qualities and the chemical inertness have made PE an excellent bearing material for artificial hip and knee joints [7]. Nevertheless, PE is a viscoelastic material, performance of which is affected by inherent drawbacks, such as high creeping compared to bone [7–9]. Surface modifications of this material affect its hydrophilicity, morphology, energy, microstructure and roughness, which have a significant impact on its cytocompatibility [10].

The surface properties of a material can be altered by various techniques [11]. One of them is plasma treatment of a surface [12,13]. In biomedicine, plasma treatment has been often used to modify biomaterials for various applications including devices and implants for therapy, such as artificial heart valves, catheters, dialysis membranes, prosthetic

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devices, and materials for bone joint repair and replacement. The capability of plasma to alter only surface physico-chemical properties of a material without altering its bulk properties, especially the mechanical ones, is advantageous in design, development, and manufacturing of biocompatible polymers [14–16]. By either surface modification or thin film deposition, specific surface chemistry can be created to optimize protein-surface interactions and therefore cytocompatibility of a biomaterial. One physical method for enhancing surface properties of a polymer is sputtering. Deposition or grafting of metals, *e.g.* gold, contributes to enhanced cell adhesion and proliferation [17]. Best results were obtained with metal nanolayers with the thickness less than 100 nm [18].

The presented research is focused on surface modification of ultra-high molecular weight polyethylene (UHMWPE) for improvement of its biomedical properties. We have chosen UHMWPE based on the previous measurements [19], in which modification of this material showed good properties for enhanced cell adhesion and proliferation. The aim of our work was to determine the effect of a gold coating in combination with preceding plasma treatment of PE samples on cells growth and adhesion.

2. Experimental

2.1. Material and methods

The ultra-high molecular weight polyethylene, UHMWPE foil (further only PE, the thickness 75 μm, density 0.94 $g \cdot cm^{-3}$, $M_w = 4 \times 10^6$, supplied by Goodfellow Ltd., UK) was used for all experiments. PE samples were plasma-treated and a half of each sample was gold-coated. The PE samples were modified in direct (glow, diode) Ar^+ plasma using Balzers SCD 050 device (BalTec AG, Pfäffikon, CH) under the following conditions: the gas purity 99.997%, flow rate 0.3 $L \cdot s^{-1}$, pressure of 10 Pa, electrode distance of 50 mm with the area of 48 cm^2, chamber volume of approx. 1000 cm^3, and the plasma volume of 240 cm^3. The duration of the plasma treatment was 60 and 240 s and the discharge power was 8.3 W. The gold coating of PE was accomplished by Balzers SCD 050 device from a gold target (the purity 99.95%, supplied by Safina Ltd., CZ). The deposition conditions were: DC Ar plasma, the gas purity 99.995%, sputtering times of 30, 150 and 300 s, the current 40 mA, the discharge power of 15 W, the total Ar^+ pressure about 5 Pa, and the electrode distance of 50 mm. The power density of Ar^+ plasma in our case was 0.13 $W \cdot cm^{-2}$, and the average deposition rate was 0.15 $nm \cdot s^{-1}$. The prepared samples were stored at laboratory conditions (24 °C, 40–60% humidity) [19]. More detailed description of the plasma treatment and gold deposition techniques can be found in [21,22].

2.2. Measurement techniques

The mean thickness of gold deposited layers was measured by gravimetry using Mettler Toledo UMX2 microbalance. The mean thickness was calculated from the difference of sample weights before and after the sputtering using the bulk density of gold, which does not differ much from the density of gold in the nanolayer. The weight gains of deposited layers were converted to an average thickness of the deposited layer h (nm) in accordance with the following equation:

$$h = \frac{V}{S} = \frac{m}{\rho \cdot a \cdot b}$$

where V is volume of the deposited layer (m^3), S is area of sample, m is mass deposited layer based on a sample area (kg), ρ is density of gold ($kg \cdot m^{-3}$), a and b are the length of the sample (m). Obtained results were compared to data obtained by scratch method [23] and they differed by less than 15%. Error of gravimetry measurement was also below 15%.

Wettability of the samples was determined by measuring surface water contact angles (WCA). Further, characterization of structural and compositional changes caused by the prepared samples was determined by Drop Shape Analysis System DSA 100 (KRÜSS GmbH, DE) at room temperature (24 °C, 40–60% humidity) [20]. 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. Water contact angles were then evaluated using the ADVANCE System. At least 7 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" [24,25] for 14 days.

The chemical composition of the prepared samples was determined from X-ray photoelectron spectra (XPS) measured (3 measurements) by Omicron Nanotechnology ESCAProbeP spectrometer (supplied by the Omicron Nanotechnology GmbH, DE) with a relative error of 10%. The dimensions of exposed and analyzed areas were of 2 × 3 mm^2. The measuring conditions were as follows: monochromated X-ray source at 1486.7 eV with the measuring step of 0.05 eV and the take-off angle of 0° with respect to the surface normal (the penetration depth *ca* 1 nm). Characteristic carbon (1s), oxygen (1s) and gold (4f) peaks were searched. Measuring was performed in ultra-light vacuum. The evaluation of acquired spectra was carried out by CasaXPS code [26]. The samples used for measurement were "aged" for 14 days. Before the measurement, the samples were stored under standard laboratory conditions.

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 $mol \cdot L^{-1}$ KCl) and also in buffer solution (PBS). For each measurement, pair of polymer films with the same top layer were fixed on two sample holders (with a cross section of 20 × 10 mm^2 and a gap between them of 100 μm). All samples were measured three times at a constant pH = 6.5 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 [27–29]. All samples used for measurement of zeta potential 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 $N \cdot m^{-1}$ (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.

Inductively coupled plasma with mass spectroscopy detector (ICP-MS) was used to determine the amount of Au ions released into phosphate buffered saline (PBS, pH = 7.4). The trace element analysis of Au leachates was conducted by using Agilent 8800 triple quadrupole spectrometer (Agilent Technologies, Japan) connected to auto-sampler. Sample nebulization was performed using a MicroMist device equipped with a peristaltic pump. The uncertainty of the measurement was less than 3%. The leaches for ICP-MS were prepared by incubation of the samples in PBS in a humidified atmosphere with 5% CO_2 at 37C for 6, 24, and 72 h. The samples were measured under both static and dynamic (shaking at 130 rpm) conditions. The leaches were diluted with distilled water in the ratio of 1:8 and analyzed.

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 gold-coated PE. The samples were sterilized in 70% ethanol in a scintillation counter vials for 1 h, inserted in 12-well plates (VWR, USA, Ø 2.14 cm), washed with PBS and