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Enhanced cytocompatibility and reduced genotoxicity of polydimethylsiloxane modified by plasma immersion ion implantation

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ABSTRACT

Polydimethylsiloxane(PDMS) is a common industrial polymer with advantages such as ease of fabrication, tunable hardness, and other desirable properties, but the basic $(\text{-OSi}(CH_3)_2\text{-})_n$ structure in PDMS is inherently hydrophobic thereby hampering application to biomedical engineering. In this study, plasma immersion ion implantation (PIII) is conducted on PDMS to improve the biological properties. PIII forms wrinkled "herringbone" patterns and abundant O-containing functional groups on PDMS to alter the surface hydrophilicity. The biocompatibility of the modified PDMS is assessed with Chinese hamster ovarian cells and compared to that of the untreated PDMS. Our results reveal that the PDMS samples after undergoing PIII have better cytocompatibility and lower genotoxicity. PIII which is a non-line-of-sight technique extends the application of PDMS to the biomedical field.

1. Introduction

Polydimethylsiloxane (PDMS), one of the high-performance polymers in many types of applications, has attracted increasing interest from materials scientists and engineers due to its unique physical and chemical properties [1]. PDMS which is flexible, thermo-tolerant, resistant to oxidation, and tunable in hardness is very promising in microelectronics encapsulation [2,3]. Owing to the ease of fabrication and texturing, PDMS is even regarded as the cornerstone of soft lithography thus playing an important role in the development of microfluidics techniques based on soft lithographic patterning on the microscale and nanoscale [4,5]. In the biomedical field, PDMS is particularly suitable as contact lenses and implants [6,7] on account of its transparency, high gaspermeability, and long-term durability in aqueous solutions. Nevertheless, PDMS with the basic structure of $(\text{-OSi}(CH_3)_2\text{-})_n$ has many methyl groups ($\text{-}CH_3$) and the surface free energy of PDMS is quite small (22–25 mJ/m^2) [8].The inherent hydrophobicity and concomitant biocompatibility inadequacy have hitherto restricted the application of PDMS in biomedical engineering.

The hydrophilicity and biocompatibility of PDMS can be enhanced by surface techniques [9–12] such as plasma treatment, silanization, and plasma-enhanced chemical vapor deposition, but the activation tends to be temporary because the modified layer on the surface is typically quite thin and the surface polymer chains of PDMS can migrate into the bulk in a relatively short time. In this respect, energetic ion beam treatment may be more useful, and plasma immersion ion implantation (PIII),a non-line-of-sight plasma- and ion-beam-based surface treatment technique, is particularly suitable for biomedical devices with an irregular shape [13–18].By using different plasma gases, PIII can create different chemical groups on the surface of different types ofsamples including PDMS to enhance the biological performances.

In this work, O_2 PIII is conducted on PDMS in the presence of a grounded conducting grid to reduce surface charging (Fig. S1, Supporting information) [19–21] and improve the effectiveness of the surface treatment. The surface properties and cellular response are studied systematically and our results reveal that PIII is a viable technique to improve the cytocompatibility and reduce the genotoxicity of PDMS.

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2. Materials and methods

2.1. Sample preparation

The PDMS samples were prepared by a commercial polydimethylsiloxane kit (Sylgard-184, Dow Corning) according to the manufacturer's instructions. In brief, the PDMS base was mixed with the cross-linking agent at a ratio of 10:1 and out-gassed in a vacuum chamber. Afterwards, the solution was spin-coated on a clean petri dish (Model WS-400 BZ-6NPP/LITE) at 300 rpm for 2 min and baked at 50 °C for 10 hThe solidified PDMS disk had a diameter of 100 mm and thickness of 1.5 mm.

O_2 plasma exposure and O_2 PIII were conducted on the PDMS samples. In the O_2 plasma exposure experiments,the PDMS disk was cut in half, cleaned by alcohol and distilled water, and exposed to anO_2 plasma for 1 h. The O_2 PIII apparatus consisted of a stainless steel plasma discharge chamber (ϕ600 mm × 300 mm) and stainless steel plasma diffusion chamber (ϕ760 mm × 1030 mm) [Fig. S1, supporting information]. Negative high voltage pulses were applied to the metal sample stage underneath the plasma diffusion chamber and radio frequency (RF) power from 0 to 2 kW was coupled to the plasma discharge chamber. To reduce charging during O_2 PIII, the high voltage sample stage was shielded from the plasma by a metal cage consisting of a 2 mm thick cylindrical tube and a top cover with a 200 mm diameter hole. The hole was covered by a stainless steel mesh (15 meshes per inch and 0.5 mm wire diameter). The PDMS sample was placed on the metal sample stage50 mm away from the top mask. The typical instrumental conditions in O_2 PIII were: sample bias voltage = −10 kV, voltage pulse width = 200 μs, pulsing frequency = 500 Hz, gas flow = 20 sccm, radio frequency power = 1 kW, and treatment time = 1 h. The samples were designated as PDMS (untreated), P-PDMS (plasma exposure), and PIII-PDMS (PIII treatment).

2.2. Surface characterization

The surface morphology was examined by optical microscopy, profilometry (WYKO NT9100, Veeco) and scanning electron microscopy (SEM, JEOL JS-820). The samples were dried and sputter-coated with gold prior to SEM examination. The static contact angles were measured on a Ramé-Hart (USA) instrument by the sessile drop method using distilled water as the medium (10 μl per drop). To evaluate the change in the surface hydrophobicity with time, the water contact angles were measured every 12 h and each data point represents the average of seven measurements conducted on different parts of each specimen to improve the statistics. The surface chemical states were determined by X-ray photoelectron spectroscopy (XPS) on a Physical Electronics PHI 5802 equipped with amonochromatic AlKα source using a constant pass energy of 11.75 eV, take-off angle of 45°, and step size of 0.1 eV.

2.3. Cell culture

The Chinese hamster ovarian (CHO) cells were obtained from ATCC and cultured in a basic medium (Dulbecco's Modified Eagle Medium:F–12 = 1:1, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone) in a humidified atmosphere of 5% CO_2 at 37 °C. Before cell seeding, the PDMS samples were exposed to ambient air for about 5 days, cut into 10 mm × 10 mm pieces, sterilized with 75% alcohol, rinsed twice with sterile phosphate-buffered saline (PBS), and soaked in a complete cell culture medium overnight.

2.3.1. Cell adhesion and morphology

The CHO cells were seeded at a density of 2×10^4 cells per sample on 24-well tissue culture plates. After incubation for 6 h, the attached cells were rinsed twice with PBS and trypsinized before the attached cells were counted by a hemocytometer. After incubation for 2 days, the samples were rinsed twice with PBS and fixed by 2% paraformaldehyde (Sinopharm Chemical Regent) to determine the cell morphology. The cells were stained for the cytoskeleton protein filamentous actin (F-actin) by phalloidin (fluoresceinisothiocyanate labeled, Sigma-Aldrich) and counterstained for nuclei by Hoechst33342 (Sigma-Aldrich). The samples were mounted on slides and images were acquired on a fluorescence microscope (Axio Observer Z1, Carl Zeiss).

2.3.2. Assays of cell viability and genotoxicity

The CHO cells were cultured at a density of 2×10^4 cells per sample to assess the cytotoxicity and genotoxicity on the various specimens. After incubation for 2 days, the cell viability was separately determined by the MTT (Beyotime) assay and Live/Dead assay (Calcein-AM/PI Double Stain Kit, Yeasen) according to the manufacturer's instruction. In the MTT assay, the samples were rinsed twice with PBS and incubated with the MTT solution for 4 h at 37 °C to form formazan which was then dissolved by dimethylsulfoxide. The optical density was determined spectrophoto metrically at 570 nm. In the Live/Dead assay, the CHO cells were trypsinized, rinsed, resuspended in the buffer solution with the same concentration, and co-stained with Calcein-AM and PI for 15 min at 37 °C. Afterwards, the cells in the suspension were quantitatively determined on a microplate reader and dripped onto slides to acquire the fluorescence images.

The corresponding genotoxicity was evaluated by the cytokines block micronucleus (MN) technique [22]. Briefly, the CHO cells were trypsinized and inoculated on the tissue culture dishes using the complete culture medium with 2.5 μg/ml cytochalasin B (Sigma-Aldrich). After incubation for another 12 h, the cells on the dishes were rinsed by PBS, fixed in methanol/acetic acid (v/v = 9:1), and air-dried for further staining with 10 mg/ml acridine orange (Lifetechnology). The MN in the binucleated cells were scored and classified by fluorescence microscopy according to standard criteria [23]. In the genotoxicity assay, at least 1000 binucleated cells were scored on each sample to determine the MN ratio in the binucleated cells.

2.3.3. Assays of reactive oxygen species (ROS)

2×10^4 CHO cells were cultured for 2 days using the completed culture medium added with 0.1% dimethylsulfoxide (DMSO, a scavenger of ROS). The samples were evaluated by the MTT assay and MN test as described above. To determine the level of cellular ROS, 1×10^4 CHO cells suspended in 1 ml complete culture medium were cultured on each specimen for 2 days. The medium in each well was added with 20 μM H_2DCFDA (oxidative stress indicator, Invitrogen) and incubated for 30 min. The samples after H_2DCFDA incubation were washed twice with cold Hank's solution containing 1% serum, added with 0.02% digitonin (Sigma, pH = 4.2), and cultured for 20 min at 37 °C. Afterwards, the mixture was centrifuged at 3000 rpm for 5 min and the supernatant was collected in an ice-bath and analyzed on a microplate fluorescent reader (excitation 486 nm/emission 520 nm). The CHO cells treated with 8.8 mM H_2O_2 served as the positive control, whereas the negative control referred to the cells cultured on the petri dishes. The relative ROS level of each group was normalized to the cell number with the data acquired from the PDMS being set as 1 for easy comparison.

2.3.4. Determination of superoxide anion ($O_2^{\cdot -}$)

1 ml of the culture medium containing 2.0×10^4 cells were added to each specimen and incubated for 1 day. Afterwards, the