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Emphasizing the role of surface chemistry on hydrophobicity and cell adhesion behavior of polydimethylsiloxane/TiO_2 nanocomposite films

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ABSTRACT

Improving the bioinertness of materials is of great importance for developing biomedical devices that contact human tissues. The main goal of this study was to establish correlations among surface morphology, roughness and chemistry with hydrophobicity and cell adhesion in polydimethylsiloxane (PDMS) nanocomposites loaded with titanium dioxide (TiO_2) nanoparticles. Firstly, wettability results showed that the nanocomposite loaded with 30 wt.% of TiO_2 exhibited a superhydrophobic behavior; however, the morphology and roughness analysis proved that there was no discernible difference between the surface structures of samples loaded with 20 and 30 wt.% of nanoparticles. Both cell culture and MTT assay experiments showed that, despite the similarity between the surface structures, the sample loaded with 30 wt.% nanoparticles exhibits the greatest reduction in the cell viability (80%) as compared with the pure PDMS film. According to the X-ray photoelectron spectroscopy results, the remarkable reduction in cell viability of the superhydrophobic sample could be majorly attributed to the role of surface chemistry. The obtained results emphasize the importance of adjusting the surface properties especially surface chemistry to gain the optimum cell adhesion behavior.

1. Introduction

Controlling the cell attachment and proliferation is being considered as a fundamental issue in various areas of science and technology including biomedicine, tissue engineering, microfluidics or high-throughput screening devices [1]. The cell adhesion could be governed by adapting the surface properties including morphology, topography and chemical composition to the desired cell behavior. In a cell adhesion process, the proteins are firstly adsorbed onto the surface of the material, and then, cellular membrane receptors are bound to the proteins' chemical groups or directly to the chemical groups of the substrate [2]. It is well established that topography, wettability, surface charge, functional groups and even the stiffness of a material are influential on the protein adsorption and thus cell attachment behavior [3–7]. Generally, the hydrophilic surfaces possess a higher affinity towards cells but lower affinity for proteins as compared with the hydrophobic surfaces [8]. Regarding the influence of roughness on the cell adhesion behavior, some contradictory results have been published in the literature [9–12]. Mainly, the effect of roughness is dependent on whether the surface energy is high or low leading to the extreme wettabilities, namely, superhydrophilic and superhydrophobic states. For instance, Oliveira et al. [13] have patterned superhydrophilic spots on superhydrophobic surfaces such that the proliferation of SaOs-2 cells was possible on the superhydrophilic spots. They also found that the SaOs-2 and ATDC5 cell lines were not able to proliferate on superhydrophobic surfaces. In another study, Lourenco et al. [14] have synthesized superhydrophobic polystyrene and polylactic acid surfaces with different micro/nano topographies and found that the protein adsorption onto the roughened surfaces was lower as compared with the smooth surfaces of the same material. Moreover, they reported that the superhydrophobic surfaces allowed the cells to adhere but inhibited their proliferation. In another study, both adhesion and proliferation of bone marrow-derived cells were prevented on superhydrophobic surfaces based on polylactic acid [15]. Superhydrophobic materials with a water contact angle (WCA) above 150° as well as a very low value of WCA hysteresis (<10°) have gained much attention and have a promising potential in both industrial and biological applications [16–20]. Similar to many other scientific phenomena, superhydrophobicity has also been inspired by nature. In fact, many plants and insects, for example, lotus leaves and butterfly wings possess self-cleaning surfaces. Up till now, numerous methods have been proposed for the fabrication of superhydrophobic surfaces including phase separation [21], surface embedding [22], the sol-gel process [23] and others. Conventionally, bioinspired super-

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hydrophobic surfaces are produced in two ways. One is to create a rough structure on a hydrophobic surface, and the other is to modify a rough surface using materials with low surface free energy [24]. In the current research, the former method is used based on which the intrinsically hydrophobic PDMS surface is converted into a super-hydrophobic surface via induction of a rough structure based on the surface aggregation of nanoparticles. Therefore, morphology, roughness and surface chemistry of the PDMS-based surfaces are of critical importance on the final hydrophobicity of the films. Despite establishing correlations among the level of cell adhesion with the surface properties of the PDMS/TiO_2 nanocomposite films, it was also attempted to emphasize the role of surface chemistry on super-hydrophobicity and thus cell adhesion especially once the surface roughness profiles are quite similar. All in all, the main aim was to fabricate a cell-repellent material which could have potential applications in many biomedical devices such as those in drug delivery and blood contacting applications as well as biosensors.

2. Materials and methods

2.1. Materials

Vinyl-terminated PDMS with average molecular weight of 25000 g mol^{-1} and density of 0.965 g/mL was purchased from Sigma-Aldrich (St Louis, MO). Trimethoxymethylsilane as crosslinking agent for PDMS and hexane as solvent were purchased from Merck (Darmstadt, Germany) and used as received. The hydrophobic fumed titanium dioxide (titania) used in this study is a commercial product (AEROXIDE® TiO_2 T805) which was purchased from Evonik Industries (Essen, Germany) and used as received. AEROXIDE® T805 has a specific surface area of $45 \pm 10\ m^2/g$ and primary particle size of 12 nm. It was produced by treating TiO_2 with octylsilane ($C_8H_{17}SiH_3$).

2.2. Preparation of samples

First of all, the glass slides ($2 \times 3\ cm \times cm$) were sonicated in deionized water for 30 min followed by heating in the oven at 100 °C for 30 min. Then, they were sonicated in acetone for 30 min followed by drying in the oven at 100 °C for another 30 min. To prepare the nanocomposite samples, a certain amount of PDMS (0.327 g) was dissolved in 50 ml of hexane to make a polymer solution with concentration of 1 wt.%. To achieve a homogeneous solution, the magnet stirring was conducted for 2 h. After that, 10, 20 and 30 wt.% of TiO_2 nanoparticles, with respect to the PDMS content, was added to the solution, and the stirring was performed for another 6 h followed by dispersion in an ultrasonic bath to achieve a stable and homogeneous suspension. After adding a certain amount of curing agent, the suspension was stirred for another 10 min, and then, a few drops of the mixture were casted onto the glass slides and left to dry at ambient conditions for 48 h to complete the curing reaction. For simplicity, the samples were named under the code of P_XT_Y in which X and Y denote the concentrations of PDMS and TiO_2 nanoparticles, respectively.

2.3. Characterization

A video-based contact angle measurement system (OCA 15, DataPhysics Instruments GmbH, Filderstadt, Germany) was employed to determine the WCA values of the samples. The WCA measurements of each sample were conducted at least three times across the samples' surfaces using the sessile drop method by dispensing 4 μl drops of de-ionized water on the samples. All WCA values were measured under the ambient laboratory conditions at a temperature of 25 °C. Morphologies of the films' surfaces were evaluated on a digital scanning electron microscope coupled with energy dispersive X-ray spectroscopy (EDX) (VEGA//TESCAN instrument, Czech Republic) operated at 25 kV. To avoid electric charging, all the samples were plated with gold coating. All the roughness parameters were acquired at magnification of 20 × by means of a 3D confocal microscope μsurf explorer, provided by NanoFocus AG, Oberhausen, Germany. X-ray photoelectron spectroscopy (XPS) analysis was conducted via an Axis Ultra photoelectron spectrometer (Kratos Analytical, Manchester, UK). The spectrometer was equipped with a monochromatic Al Kα ($h\nu = 1486.6$ eV) X-ray source of 300 W at 15 kV. During all measurements, electrostatic charging of the samples was avoided by means of a low-energy electron source working in combination with a magnetic immersion lens.

2.4. Cell culture

The SW 480 human colon cancer cell line (National cell bank of Iran, Pasteur institute, Tehran, Iran) was cultured in RPMI 1640 medium (Gibco BRL Inc., Grand Island, NY, USA), supplemented with adding 10% heat-inactivated fetal bovine serum, and 2 mM L-glutamine, and maintained in humidified incubator at 37 °C in a 5% CO_2 atmosphere. The cell suspensions of greater than 90% viability were prepared from subconfluent cultures with 0.25% trypsin (Invitrogen, CA, USA) and 0.02% Ethylenediaminetetraacetic acid (EDTA). To prepare samples for SEM observation, at the end of incubation (24 h), samples were washed with Phosphate-buffered saline (PBS) to remove the unattached cells. The adhered cells were then fixed by glutaraldehyde solution (2.5%) for 10 min and dehydrated through a series of ethanol/water mixtures (10, 30, 50, 70, 80, 90, and 100%) each for 10 min. Dehydration was followed by air drying overnight.

2.5. MTT assay

MTT assay was employed to determine the viable cell numbers in the current study. This assay is based on the mitochondrial conversion of the tetrazolium salt, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltertrazolium bromide (MTT). Briefly, 500 μl serum free medium and 40 μl MTT solution were added to each sample, and incubated at 37 °C for 1 h for MTT formazan formation. The medium and MTT were replaced by isopropanol solution (containing 10% formic acid; Sigma, St. Louis, MO), 400 μl per well, the samples were incubated at 37 °C for an additional 5 min, to solve the MTT formazan, also mildly shaken for 10 min to ensure the dissolution of formazan. MTT dissolved formazan solution (100 μl) was added into 96-well plates per well and the absorbency values were measured using an ELISA reader (Dynatech 5000, Dynatech, Billinghurst) at wavelength 550 nm, blanked with isopropanol solution.

2.6. Statistical analysis

Multiple samples were tested and the results were reported are average values ± standard deviation. The data were analyzed using a one-way analysis of variant (ANOVA) by means of the SPSS software and differences among mean values were processed by the Duncan's multiple range tests. Significance was defined at $P < 0.05$.

3. Results and discussion

3.1. Wettability

The wettability of the prepared samples was investigated via water contact angle (WCA) measurement, and the results are reported in Table 1. The WCA value was notably increased from 99° for the pure PDMS film to 123° for P_1T_{10} which can be ascribed to