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Nanostructured surfaces from high-density grafted poly (acrylic acid) with liquid-like property

Tianchi Liu, Jun F. Liang*

Department of Biomedical Engineering, Chemistry, and Biological Sciences, Schaefer School of Engineering and Science, Stevens Institute of Technology, 507 River Street, McLean Hall, Room 509, Hoboken, NJ 07030, USA

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ABSTRACT

Nanostructured surfaces have unique properties that can be used in many applications. Among different methods on creating nanostructured surfaces, we successfully assembled various nanostructures on one and the same high-density poly (acrylic acid) (PAA) grafted surfaces by conducting vacuum-mediated rapid phase separation in appropriately selected solvents. This not only increased the efficiency of creating surfaces with multiple structure patterns, also such nanostructured surfaces had improved biocompatibility and demonstrated liquid-like properties. Conducted experiments also proved that lanthanide ion Ce immobilized on nanostructured PAA grafted surfaces behaved the same as in the aqueous solution, attaining high illumination. In addition, good cell stretching and attaching experiment also present its potential in tissue engineering applications.

1. Introduction

Nanostructured surfaces have received great interest because novel and unique properties–catalytic, magnetic, ferroelectric, mechanical, optical and electronic–occur only when the scale is decreased to nano size. Applications have been found for nanostructured surfaces in energy conversion and storage [1], wave energy harvesting [2], surface-enhanced Raman spectroscopy (SERS) and sensing [3], tissue engineering and drug delivery [4–6]. With the above promising applications, scientists are putting more and more effort into this field.

Commonly, nanostructured surfaces are created on materials through chemical etching [7], lithography [8] and templated polymerization [9]. These methods can produce nearly perfect structure patterns. However, they usually require specific equipment with extra high accuracy and are limited to certain hard solid substrates, such as silicon and metals. Approaches like self-assembling [10] and electrospinning [11,12] are suitable for more materials, such as polymer substrates, but the resulting surfaces structures made from nanofibers can hardly offer us regular structures compared with previous etching, etc., methods. What's more, these structures are assembled through weak physical interactions. In general, although nanostructured surfaces created by above methods have their specific fields of application, however, these methods all have their own challenges to overcome to improve their materials.

Based on our previous research and the technique of grafting polymer brushes on surfaces utilizing discharged argon plasma [13], we report a simple and unique surface nano-structuring method through controlled phase separation on thin PET films. During this process, the grafted polymer brushes self-assembled into different structures driven by the interactions with applied solvents, and vacuum involved phase separation helped the structures remain their morphologies on the surfaces. As a result, simply by controlling applied solvents, we successfully constructed different nanostructured surfaces from the exact same polymer grafted surface. This method indicates that single type of grafted surface could be more versatile to meet wider requirements. Moreover, our nanostructured surfaces from grafted poly (acrylic acid) (PAA) had similar polar microenvironments as in the aqueous solution. Among the variety of applications, we examined its lanthanide illumination and cell attaching properties.

2. Experimental

2.1. Chemicals and reagent

Acrylic acid (anhydrous, contains 200 ppm MEHQ as inhibitor, 99%), Pyrene, Cerium (III) chloride were all from Sigma Aldrich; PET (polyethylene terephthalate) films with thickness 0.023 mm was from Good fellow Company; All solvent ethanol, methanol, acetone, ethyl acetate and DI water were all supplied form Stevens Institute of Technology; GFP transfected MB 231 cells cell lines and culture

* Corresponding author.
E-mail address: jliang2@stevens.edu (J.F. Liang).

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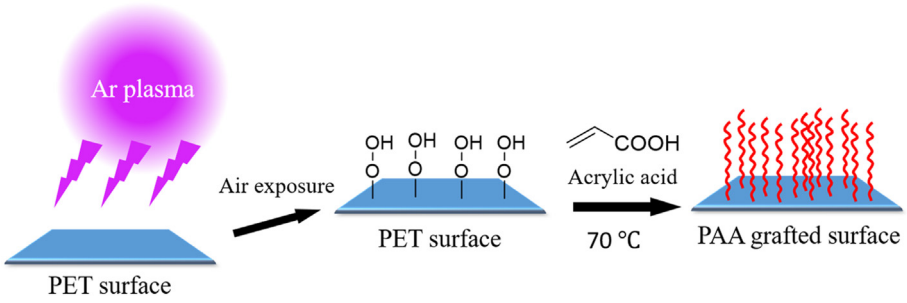

Scheme 1. Surface grafting process.

medium were from American Type Culture Collection (ATCC); A LIVE/DEAD staining kit was purchased from Invitrogen Life Technologies (Carlsbad, CA) for cell staining.

2.2. PAA grafting

A plasma-initiated surface polymerization method established in our previous studies was used (Scheme 1). Briefly, clean polyethylene terephthalate (PET) films, with thickness 0.023 mm, were placed in the Pyrex inner chamber (5 cm away from the gas outlet) of Plasma Prep III device. Pure argon gas was introduced at a flow rate of 1.0 SCFH, and the whole system was evacuated to 28–31 Pa (210–230 m Torr). The plasma treatment was conducted at 80 W for 5 min. After exposure to air for 1 h, samples were quickly transferred into degassed 30% (v/v) acrylic acid aqueous solution. The graft polymerization was carried out at 70 °C under vacuum atmosphere −88 kPa (−26 in Hg) in Isotemp vacuum oven A281 (Fisher Scientific) for 18 h. Grafted samples were washed thoroughly with DI water. PAA grafting quantity and homogeneity were tested using Toluidine Blue O as described before [13]. And ATR-FTIR was also conducted for film characterization.

2.3. Surface nano-structuring

The PAA grafted PET films were submerged in a solvent, such as water, ethanol, methanol, etc., and removed from the solvent after 2 h. Next, while the surfaces were still wet, we placed them into a petri dish and then into the Pyrex inner chamber of the Plasma Prep III device. The vacuum was turned on until the system evacuated pressure to 24 Pa (179 m Torr). After the films were completely dried, we removed the samples. Through this simple step, nanostructured surfaces were formed. Scanning Electron Microscope (SEM) was used to study the surface pattern in nanoscale. Due to the insolation property of PET films, spin gold coating was necessary before loading samples in SEM. Finally, pyrene was used to characterize the environment of the created nanostructured surfaces.

2.4. Immobilization of pyrene and Ce ions on PAA grafted surfaces

The PAA grafted surfaces were immersed in pyrene (1 mM) or $CeCl_3$ (10 mM) for 2 h. Then films dried either naturally in the air (control) or through the phase separation (nanostructured) after taken out of the solution while wet.

2.5. Cell adhesion assay

Nanostructured PET films ($0.5 \times 0.5\,cm^2$) were prepared in the presence of water and confirmed under SEM. Samples were spin coated with gold for 1 min before cell culture. PET films were placed in 24-well cell culture plates, and 100 µl of GFP transfected MB 231 cells (5×10^4 cell/ml) were loaded on the sample surface. After being cultured in a 37 degree Celsius, 5% of CO_2 environment for 6 h, PET films were removed and washed carefully. Adhered cells on PET surfaces were observed and counted under fluorescence microscopy by setting excitation wavelength at 488 nm.

3. Results and discussion

3.1. Construction of high-density PAA grafted surfaces

By utilizing glow discharge plasma, we have developed a method to create polymer grafted surfaces with high density with distinguished chemical properties. We applied this technology and successfully grafted high-density poly (acrylic acid) brushes on silicon wafers and PET films as analyzed by using toluidine blue O to quantify total surface carboxyl groups (Fig. 1a, b). PET films were selected because of its good flexibility, optical properties, biocompatibility, and wide biomedical applications [14–16]. Silicon wafers were used occasionally for certain assays requiring extra-smooth surfaces and confirmed results from PET films.

Successful surface grafting was further confirmed by ATR-FTIR analysis: a strong peak at 1710 cm^{-1} was assigned to the C═O stretch of dimer in the solid state; peaks at 505 cm^{-1} and 723 cm^{-1} were due to C—C═O bending in the carboxyl group: the peak at 3456 $cm^{-1}$and the very broad peak between 3300 and 2400 cm^{-1} belonged to OH stretch; the peaks of 3068 $cm^{-1}$and 2968 cm^{-1}were from CH stretches. In addition to that, the presence of the strong peak at 723 cm^{-1} indicated the length of methylene groups was over four, which also confirmed the polymer chains bone structure.

3.2. Phase separation induced nanostructures on PAA grafted surfaces

Conversion of a single-phase system into a multi-phase system can lead to the formation of micro-scale or even nano-scale structures on polymeric materials. We performed phase separation on grafted PAA under vacuum. The solvent, frozen by vacuum, helped PAA maintain the chain-chain interactions in the liquid phase. As phase separation removed the solvent through sublimation, surface grafted PAA brushes were fixed to form nano- and micro-sized porous structures. Compared with sample underwent naturally drying procedure (Fig. 2a), when water was used as a solvent, the structural pattern presented the porous structure size ranged from 600 nm to 1100 nm, and the nanofiber diameter was about 80–100 nm (Fig. 2b). When ethanol and methanol were used, aggregation of polymer chains occurred and formed polymer ridges, knots and semi-porous structures (Fig. 2c–d). But when acetone was applied, porous structure with pore size range from 50 to 200 nm was obtained (Fig. 2e).

Solvent properties play important roles in the swelling process. Because polymers displayed maximum swelling in solvents with a similar polarity to their polymeric backbone and further affected the crosslinking degree and hydrogen bond formation [17], the carboxylic acid had very polar functional groups. The polymer brushes interacted with solvent molecules differently in the solvation process with the increasing polarity (dipole) of the solvents in the order of acetone, ethanol, methanol, and water. In the other aspect, the solubility