Enzyme biocatalyst route to superhydrophobic surfaces on microstructured poly (ethylene terephthalate) film

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ABSTRACT

A tunable and green enzyme biocatalyst route to develop superhydrophobic surfaces on microstructured poly (ethylene terephthalate) (PET) films by the tailoring of the micro- and nano scale hierarchical structures is described. Upon the aminolysis of PET films with hexamethylenediamine, the primary amine groups are covalently attached onto the PET surfaces and microstructured pattern is formed. The binding of citrate-stabilized Au nanoparticles onto the PET surfaces via the covalent bond between the gold nanoparticles and the primary amine groups introduced on the PET surfaces was followed spectroscopically. The biocatalytic enlargement of the Au nanoparticles using the enzyme-generated H2O2 as reducing agent for the reduction of AuCl4− at the attached Au nanoparticle seeds on the PET surfaces was followed by spectroscopic means and atom force microscopy (AFM). The AFM experiments indicated that micro- and nano scale hierarchical structures were tailored by the enzyme biocatalyst route. Superhydrophobic surfaces with water contact angles as high as 158.6 ± 2.0° was achieved upon the chemisorption of 1-octadecanethiol as low surface energy material. This route can be potentially applicable to superhydrophobic PET-based microfluidic devices with reduced friction surfaces.

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1. Introduction

Superhydrophobic surfaces, characterized by water contact angles larger than 150° and tilt angles less than 5°, have recently attracted extensive attention for their potential industrial applications including self-cleaning surfaces, anti-adhesive coatings, microfluidic devices with reduced friction surfaces, and many others [1–5]. Inspired by natural systems including the lotus leaf, which accomplish self-cleaning superhydrophobic surfaces through the generation of combined micro- and nano hierarchical structure of the surface and the low surface energy material covered on the structure, researchers have made impressive efforts to prepare similar artificial superhydrophobic surfaces. Various methods, such as plasma etching and polymerization, chemical vapor deposition, electrodeposition, sol–gel, solidification, phase separation, and deposition of nanoparticles, have been developed for the preparation of superhydrophobic surfaces on various substrates, including polymers, metal and semiconductors [6–12].

Poly (ethylene terephthalate) (PET), representing a cheap, chemically stable, and nontoxic materials for diverse technological applications, for example the microfluidic devices, has been intensively studied especially on the surface functionalization [13,14]. Interestingly, regular micro scale patterns on the surfaces of PET films were reported to form upon aminolysis treatments, during which amines attacked the electron deficient carbonyl carbon where chain scission and amide formation occur [15,16]. Although PET represents an attractive material for the applications in microfluidic devices, for which the superhydrophobic surfaces are very important, the report on the construction of superhydrophobic surface on PET film is scarce, particularly under mild conditions. Superhydrophobic coating films prepared on PET substrate using AuCl4− the sol–gel chemistry was reported [17]. Furthermore, superhydrophobic surface from a PET substrate via selective oxygen plasma etching followed by plasma-enhanced chemical vapor deposition using tetramethylsilane as the precursor has been obtained [18]. Although these methods work well for the preparation of superhydrophobic surfaces with high contact angles and low sliding angles, tunable and green methods are still highly desired.

The synthesis and enlargement of particles using enzyme biocatalysts and biological structures of high complexity is an emerging area in nanobiotechnology. Micrometer-long Au metallic wires exhibiting heights and widths in the region of 150–250 nm were fabricated from dip-pen nanolithography patterned “biocatalytic lines” using Au nanoparticle (NP) functionalized glucose oxidize as “ink”. The enzyme-generated H2O2 acted as reducing agent that stimulated the reduction of AuCl4− at the Au nanoparticle seeds associated with the enzyme. The biocatalytic growth process of nanoparticles was tunable with the biocatalytic “development” time-interval [19,20]. Similarly, Ag nanowires were prepared using alkaline phosphatase produced p-amino-phenol as reducing agent for Ag+ [19,20]. Furthermore, the exposure of the fungus Verticillium sp. to an aqueous solution of AuCl4− resulted in the reduction of the salt to gold nanoparticles [21]. Also, cell extracts from the

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lemongrass plant yield, in the presence of AuCl₄⁻, single crystalline gold nanotriangles and nanoprisms [22]. We conceived that the enzyme biocatalyst route for the enlargement of particles is a tunable and green way to fabricate surfaces with tailored hierarchical architecture in micro- and nano scales.

Our goal here is to develop superhydrophobic surfaces based on the tailoring of the micro- and nano scale hierarchical structures on microstructured PET films for potential microfluidic device-based applications. In contrast to previous studies, here we adopted a tunable and green enzyme biocatalyst route for the construction of the superhydrophobic surfaces. We also showed that the enzyme biocatalyst route is a rapid and versatile method to construct superhydrophobic surfaces on various substrates.

2. Materials and methods

2.1. Materials

PET films (Mylar®, DuPont) with thickness of 100 µm were obtained from DuPont. Cetyltrimethylammonium chloride, glucose oxidase and HAuCl₄ was purchased from Aldrich and used without further purification. β-D(+) glucose and sodium citrate with AR purity obtained from Shanghai Chemical Reagent Company of China and used as received. Other reagents were purified by conventional methods. All the water used in this work is distilled and deionized water.

2.2. Instruments and measurements

Transmission electron microscopy (TEM) analysis was performed on a JEM-1200EX TEM operating at 200 kV in bright field mode. UV–vis spectra were carried out with a UV–vis Shimadzu UV-2055 spectrometer using quartz cuvettes. Atomic force microscope (AFM) measurements were performed in the tapping mode under ambient conditions using a commercial scanning probe microscope, Seiko SPI3800 N, equipped with a silicon cantilever, nanosensors, typical spring constant 40 N/m. Scanning electron microscopy (SEM) were performed on Jeol JSM-35C. The contact angle of the samples toward distilled water was measured by the sessile drop technique using contact-angle measurement equipment, model KRUSS DSA 10-MK2.

2.3. Aminolysis reaction of PET substrates [15,16]

PET film was cut to 1 × 2 cm samples. The samples were cleaned with acetone, ethanol and triple-distilled water using ultrasonication for 15 min and dried in a dessicator before aminolysis. The aminolysis reactions were carried out in tubes. PET samples were added to tubes containing 10 mL of 10% hexamethylenediamine solution in i-propanol, which were previously thermostated in an oil bath at 65 °C. Moderate agitation was used during the reaction. The PET samples were removed from the solution after the desired reaction time, washed with methanol, and then dried in vacuum at room temperature for at least 8 h.

2.4. Preparation of Au-NPs seeds [23]

In a 250 mL round-bottom flask equipped with a condenser, 100 mL triply distilled water was added. After boiling, 4.12 mL of 10 mg/mL HAuCl₄ was added with vigorous stirring. Rapid addition of 11.57 mL of 38.8 mM sodium citrate to the boiling solution resulted in a color change from pale yellow to burgundy. Boiling was continued for 10 min; the heating mantle was then removed, and stirring was continued for additional 15 min. The UV–vis spectrum exhibits a characteristic plasmon band at 520 nm. The diameter of the particles, measured from TEM images, was 16 nm ± 2 nm.

2.5. Attachment of Au-NPs onto the aminolysed PET substrates [24]

The aminolysed PET substrates were immersed into the citrate-stabilized Au-NPs for desired reaction time. The PET substrates that included the Au-NPs films were rinsed with water.

2.6. Biocatalytic enlargement of Au-NPs on the PET substrates [25]

In a 250 mL growth solutions consist of 2 × 10⁻⁴ M HAuCl₄ in 0.01 M phosphate buffer, pH = 7.2, 2 × 10⁻³ M cetyltrimethylammonium chloride (CTAC) and 3 × 10⁻¹ M β-D(+)-glucose with 50 µg mL⁻¹ glucose oxidase (GOx). For the catalytic growth of the Au-NPs, Au-NPs attached PET substrates were then soaked in the above-described growth solution. The experiments were performed at ambient temperature (30 ± 2 °C). The absorbance features of the resulting modified PET substrates were recorded in water.

2.7. Chemisorption of 1-octadecanethiol on the gold-coated PET substrates

Control PET samples were prepared by sputtering deposition Au on pristine PET. The sputter-coated and the Au-NPs attached PET substrates were then soaked in 1 mM 1-octadecanethiol for 0.5 h and washed with ethanol carefully.

3. Results and discussion

Aminolysis was used to introduce primary amine groups onto the PET films. During aminolysis amines attack the electron deficient carbonyl carbon where chain scission and amide formation occur, which results in a reduction of the molecular weight of the sample. It was assumed that the ordered or crystalline regions are insoluble while the amines predominantly react with the noncrystalline regions. This surface etching techniques has been used in several studies and is used in this study to prepare regular pattern on PET films [26–28]. Then, Au nanoparticles were covalently bonded onto the PET surfaces via the binding between the gold nanoparticles and the primary amine groups. Micro- and nano scale hierarchical structures were then tailored by using the enzyme-generated H₂O₂ as reducing agent for the reduction of AuCl₄⁻ at the attached Au nanoparticle seeds on the PET.
surfaces. Finally, the chemisorption of 1-octadecanethiol as low as surface energy material onto the enlarged gold NP surfaces was adopted to achieve superhydrophobic surfaces, as schematically illustrated in Scheme 1.

The aminolysis of PET films was carried out by incubating the PET films with 10% hexamethylenediamine in i-propanol at 65 °C. The success of introduction of primary amine groups onto the PET films was indicated by Fourier transform infrared. The carbonyl stretch at 1645 cm⁻¹ and amino bending at 1558 cm⁻¹ were developed after the aminolysis of PET films. The influence of the aminolysis process on the morphology of the substrate was followed by the SEM. While the cleaned pristine PET film did not show any micro scale structure, Fig. 1(A), the incubation of the PET substrate in the hexamethylenediamine solution for 16 h resulted in banded structures exhibiting widths in the region of 50–100 µm, Fig. 1(B). Increase of the incubation time leads to the change of the micro scale structures from the banded structures to lumpy structures, Fig. 1(C) and (D). Control experiment indicated that the immersing of PET films in i-propanol without hexamethylenediamine did not lead to any change of the morphology of the PET films.

Au-NPs with diameter of ~16 nm were prepared by reduction of gold chloride (HAuCl₄) with freshly prepared sodium citrate under reflux conditions and capped with citrate. The Au-NPs were then attached onto the aminolised PET substrate via the covalent bond between the gold nanoparticles and the primary amine groups [24]. The color of the PET substrate changed to red upon the immersion of the aminolised PET substrate into the 16 nm Au-NPs solution (1.7 × 10⁻⁹ M) and this permits to follow the attaching process by UV spectroscopy, Fig. 2. The absorbance of the Au-NPs-functionalized PET substrates increased with time and reached a steady-state value after ~90 min, Fig. 2, inset.

The attached 16 nm Au-NPs were then used as seeds for the enzyme biocatalytic growth of Au-NPs. The glucose oxidase (GOx)-biocatalyzed oxidation of glucose leads to the formation of H₂O₂ that acts as reducing agent for the catalytic deposition of Au on the Au-NPs associated with the PET substrate. The enlargement of the particles was then followed spectroscopically, Fig. 3. Since the amount of the formed H₂O₂ is tunably

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**Fig. 1.** SEM images for the pristine PET (A), and the PET aminolised for 16 h (B), 24 h (C) and 40 h (D).

**Fig. 2.** Absorbance spectra of the PET substrates upon reaction with 16 nm Au-NPs (1.7 × 10⁻⁹ M) for 30 min (A), 60 min (B), 90 min (C), and 120 min (D). Inset: absorbance at 540 nm of Au-NPs on PET substrates as a function of reaction time.

**Fig. 3.** Absorbance spectra of the Au NP-functionalized PET substrates upon reaction with 2 × 10⁻⁴ M HAuCl₄ and 50 µg ml⁻¹ GOx in 0.01 M phosphate buffer that includes CTAC (2 × 10⁻³ M) and β-D(+) glucose (3 × 10⁻³ M) at 30 °C for different reaction time-intervals: (A) 0 min, (B) 5 min, (C) 25 min, (D) 50 min, (E) 120 min, (F) 180 min, (G) 210 min. The samples were inserted in the cuvette in the way that the light can vertically pass the sample. Inset: calibration curve corresponding to the peak absorbance of the Au NP-functionalized PET substrates upon different reaction time-interval.
controlled by the reaction time-interval, the absorbance intensities of the resulting NPs are faciley regulated by the reaction time-interval, and the calibration curve, Fig. 3, inset, was extracted. The absorbance of the Au-NPs-functionalized PET substrates increased with time and reached a steady-state value after ~180 min. Control experiments indicated that the immersing of Au-NPs-attached PET films in biocatalytic growth solutions without glucose oxidase or glucose did not lead to the change of absorbance intensities in UV spectroscopy. Also, the immersing of PET without the attached Au-NPs failed to produce Au-NPs on the PET substrates.

Fig. 4 showed the AFM images of the PET substrates after aminolysis, attachment of Au-NPs and the enlargement of the Au-NPs on the flat area.

![AFM images of PET substrates](image)

**Fig. 4.** (A) SEM image of the aminolised PET substrate with micro scale pattern, the sample was sputter-coating with gold and observed with SEM operating at 200 kV in bright field mode. The flat and smooth area of the patterned PET samples was observed with AFM after the aminolysis treatment for 16 h (B), the attachment of Au-NPs (C) and the enlargement of the Au-NPs (D). The samples for the AFM were dried with nitrogen blowing and the measurements were performed in the tapping mode under ambient conditions.

![Water droplets on various substrates](image)

**Fig. 5.** Water droplets (3 µL) on various substrates: (A) Au-NPs enlarged PET after chemisorption of 1-octadecanethiol; (B) PET aminolised for 16 h; (C) Au-NPs attached PET after chemisorption of 1-octadecanethiol; (D) Pristine PET functionalized by sputtering deposition Au and chemisorption of 1-octadecanethiol.
of the aminolysed PET substrates. Although the micro scale lumpy patterns were introduced onto the PET substrates after aminolysis for more than 16 h, Fig. 4(A), the root mean square (RMS) surface roughness was calculated to be 2.17 nm. After immersing the substrates into the Au-NPs solution, the attached Au-NPs on the PET substrates were clearly indicated, Fig. 4(C). The presence of Au-NPs on the PET films increased the RMS surface roughness to 9.45 nm. Intriguingly, the enzyme biocatalytic enlargement of the Au-NPs, using the glucose oxidase produced H2O2 as reducing agent and the attached Au-NPs as the enzyme biocatalytic enlargement of the Au-NPs, increased the RMS surface roughness to 9.45 nm. Fig. 4(D). This enzyme biocatalyst route provided a tunable and green method for the tailoring of the micro- and nano scale hierarchical structures on the PET substrates.

Up to now, a hierarchically structured surface with tailored micro- and nano scale structures has been fabricated. Chemisorption of 1-octadecanethiol on the hierarchically structured surfaces via the covalent bond between the gold nanoparticles and the thiol groups was fabricated. Chemisorption of 1-octadecanethiol on the hierarchically structured surfaces via the covalent bonds between the gold nanoparticles and the thiol groups was investigated. A Poly (ε-caprolactone) substrate was chosen to be appropriately functionalized sequentially by aminolysis, attachment of the Au-NPs, enzyme biocatalytic enlargement of the Au-NPs and chemisorption of 1-octadecanethiol. A surface with water contact angle of 111.3±2.3°, Fig. 5(C). Pristine PET functionalized by sputtering deposition Au and chemisorption of 1-octadecanethiol, which characterized by a rather flat surface with RMS surface roughness less than 5 nm, produced a surface with water contact angle of 100.2±1.8°, Fig. 5(D). Accordingly, it is reasonable to deduce that the hierarchical structures tailored by the enzyme biocatalytic enlargement of the Au-NPs contribute to the formation of the superhydrophobic surfaces. This route to fabricate the superhydrophobic surfaces is, interestingly, feasible for other polyester based materials. A Poly (ε-caprolactone) substrate was chosen to be appropriately functionalized sequentially by aminolysis, attachment of the Au-NPs, enzyme biocatalytic enlargement of the Au-NPs and chemisorption of 1-octadecanethiol. A surface with water contact angle of 170.1±3.2° was obtained. Also, superhydrophobic surfaces with water contact angle of 158.6±2.0° was fabricated on the PET substrates via the sequential functionalization by aminolysis, attachment of the Au-NPs, enzyme biocatalytic enlargement of the Au-NPs and chemisorption of 1-octadecanethiol. The superhydrophobic surfaces from enzyme biocatalyst route are of practical significance for various applications including self-cleaning surfaces, antifouling coatings, coatings for microfluidic channels and biosensors, and others.

4. Summary

In conclusion, the present study has demonstrated an enzyme biocatalyst route to develop superhydrophobic surfaces on microstructured PET films by the tailoring of the micro- and nano scale hierarchical structures. Superhydrophobic surfaces with water contact angle that reached as high as 158.6±2.0° was fabricated on the PET substrates via the sequential functionalization by aminolysis, attachment of the Au-NPs, enzyme biocatalytic enlargement of the Au-NPs and chemisorption of 1-octadecanethiol. The superhydrophobic surfaces from enzyme biocatalyst route are of practical significance for various applications including self-cleaning surfaces, antifouling coatings, coatings for microfluidic channels and biosensors, and others.

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