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Stable lotus leaf-inspired hierarchical, fluorinated polypropylene surfaces for reduced bacterial adhesion

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ABSTRACT

Polypropylene (PP) is used in a wide variety of medical components, but is susceptible to bacteria surface colonization and biofilm formation, which leads to infections and inflammations. In this study, we report on the micro-/nanostructuring and surface functionalization of PP substrates through various oxygen and fluorine reactive ion etching (RIE) treatments and their effects on wettability and bacteria adhesion. We found that oxygen treatment creates a hydrophilic surface that reduces bacteria adhesion by 68.7% compared to the control, but additional nanostructuring reduces the surface's anti-biofouling properties due to increased microscale roughness and air pockets that reduce the effectiveness of the liquid barrier. We demonstrate that a fluorine etch chemistry may be utilized to create lotus leaf-inspired, low surface energy, hierarchical microstructure/nanofibrils in PP. Due to the low surface energy and hierarchical morphology, the surface exhibits lotus-leaf wetting (high contact angle ~155° and low contact angle hysteresis < 10°) where water droplets easily roll off the surface in contrast to other PP samples. The lotus leaf-inspired hierarchical, fluorinated surfaces exhibit a 99.6% reduction of *E. coli* cell adhesion compared to untreated PP. These surfaces demonstrate water contact angle stability over a week in contrast to hydrophilic samples, where the contact angle degrades after just a few days. These new surfaces may help reduce the spread of infections from various plastic medical components without the need for the loading of antibacterial agents that eventually deplete from the surface.

1. Introduction

Plastics are used in a wide range of medical components such as prosthetics, implants, catheters, and syringes, due to their chemical resistance, versatility in manufacturing, high specific strengths, and low cost [1]. However, contaminating bacteria can attach to plastic surfaces and grow and form biofilms that lead to healthcare associated infections [2]. The consequences on patients and their families are serious, as infections can extend hospital stays, create long-term disability, increase healthcare costs, and even result in unnecessary deaths [3,4]. In the United States alone, there are 90,000 deaths associated with healthcare-associated infections every year [5]. These issues are even worse in developing countries where resources and accountability are poor [6].

Bacteria cause infections by attaching to a surface and forming organized and multicellular biofilms. Two strategies for creating antibacterial surfaces are (1) bactericidal surfaces that kill bacteria cells that come in proximity of or contact the surface and (2) anti-biofouling surfaces that make the bacterial attachment process difficult. In this study, we focus on modifying polypropylene (PP), which is a thermoplastic suitable for use in clinical environments due to its unique rigidness, chemical solvent resistance, and ability to withstand high temperatures compared to other polymers [7]. PP is commonly used for injectors, syringes, medical packaging and cases for contact lenses [8–12] and is expected to be the fastest growing plastic for medical packaging [13]. Many bactericidal PP surfaces have been studied by incorporating silver nanoparticles-zeolite plastics [14], copper nanoparticles [15,16], and silver nanoparticles [17,18]. However, nanoparticles are easily removed by abrasion [19,20] and the metal ions eventually leach out of the surface completely, rendering the surface sterile against bacteria. Metal ions may also be toxic to aquatic organisms and the environment [21,22]. Furthermore, bactericide agents must be used in high concentration because many bacteria can sustain growth in low concentrations [23]. Anti-biofouling PP surface

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modifications have been investigated to combat these issues. PP membranes have been fabricated by the UV-induced grafting of zwitterionic anti-fouling polymers and demonstrated a 77% reduction in *Escherichia coli* (*E.Coli*) adhesion [24]. Anti-biofouling PP surfaces have also been prepared by thermal annealing to create microscale patterns of rice that reduce the adhesion of *E. coli* by 53% compared to controls [25].

In this study, we examined how different reactive ion etching (RIE) processes affect the anti-biofouling properties of PP samples. Our reactive ion etching method provides a single step approach without the need for patterning or the incorporation of additional materials. We studied how different oxygen and fluorine reactive ion etching (RIE) treatments affect the surface chemistry, morphology, and wettability, and bacteria adhesion of PP. We found that a light power oxygen etching treatment creates a hydrophilic surface that reduces bacteria adhesion of *Escherichia coli* (*E. coli*) by 68.7% compared to untreated PP. Etching a PP surface with high power oxygen creates a surface with about the same surface energy, but nanofibril structures with microscale roughness. These structures exhibit increased bacteria adhesion due to a combination of greater microscale roughness and air pockets that reduce the effectiveness of the liquid barrier. In contrast, we demonstrate substantially reduced bacteria adhesion through lotus-leaf-inspired low surface energy, hierarchical microstructure/nanofibrils in PP. These surfaces exhibit lotus-leaf-like wetting with high static water contact angle (~155°) and low hysteresis (< 10°). Water droplets easily roll off these surfaces as opposed to the other PP samples. Furthermore, these lotus-leaf-like surfaces reduce *E. coli* by 99.6% compared to untreated control samples. These surfaces demonstrate water contact angle stability over a week in contrast to hydrophilic samples, where the contact angle degrades after just a few days.

2. Experimental section

2.1. Materials

PP sheets were purchased from an online vendor (Small Parts), which have a standard tolerance and meet ASTM D4101-PP0112 specifications [26]. The thickness of the PP sheet was 1.57 mm. Electronic grade acetone (99.5%), methanol (99.9%) and isopropyl alcohol (99.5%) were bought from VWR. Diiodomethane (99%) was bought from Sigma-Aldrich. Deionized water was obtained from a Millipore Academic A10 system with total organic carbon below 40 ppb.

2.2. Sample preparation

Circular coupons with 12.7 mm diameter were made from the PP sheet using a custom-made hole punch. Afterwards, the samples were cleaned with acetone, methanol, and isopropyl alcohol and dried with nitrogen gas. All samples including the control were cleaned with a low power argon plasma (Diener Electronic GmbH) using diffusion process plasma cleaning. The plasma clean parameters for all samples were set to power = 20 W, pressure = 100 mTorr, flow rate = 30 sccm and duration = 70 s. Then, experimental samples were treated by reactive ion etching (Trion III). Two types of samples were prepared by oxygen treatment. Both oxygen treatments were performed under pressure = 100 mTorr, O_2 flow rate = 98 sccm, and duration = 120 s. However, one treatment had high power (HP) of 200 W and the other had a low power (LP) of 25 W. The fluorinated samples were initially treated with oxygen for 70 s and then treated with CF_4 and SF_6 gasses in order to maximize fluorination at power = 200 W, pressure = 250 mTorr, CF_4 flow rate = 86 sccm, SF_6 flow rate = 52 sccm, and duration = 1800 s (or 30 min).

2.3. Surface characterization

2.3.1. Morphology characterization

The physical morphology of PP surfaces was characterized by scanning electron microscopy (SEM, Zeiss Sigma 500 VP) and atomic force microscopy (AFM, Multimode SPM with a Digital Instruments Nanoscope III controller). For SEM imaging, the samples were sputter coated with 7 nm gold/palladium (80:20) using a sputter coater (Hummer), as the polymer samples were non-conductive. For AFM, the PP surfaces were imaged in tapping mode, using silicon nitride tips to assess surface topography and roughness in 20 μm by 20 μm areas. The AFM tip had a radius of 8 nm, and the total tip height was 12–18 μm. Data analysis was performed with Digital Instruments version v720 and Gwyddion software. Additionally, dimensional stability was tested with the ASTM D1204 standard (except with 3 in. by 3 in. samples) by comparing the dimensions of samples before and after RIE treatment [27]. Measurements of the sample width and length before and after plasma treatments were conducted using a digital vernier scale caliper.

2.3.2. Contact angle measurements and surface energy calculation

Static water contact angles (WCA) for all the surfaces were measured using a video contact angle goniometer (VCA 2000 Optima XE). This goniometer utilizes a precision camera and advanced PC technology to capture static or dynamic images of the droplet and determine tangent lines for the basis of contact angle measurement. Contact angle measurements were taken in ambient air at 22–25 °C and 20–30% relative humidity. Contact angle measurements were taken from 5 μl droplets of deionized water. Similarly, the hysteresis was tabulated for each treatment by measuring the advancing and receding contact angles during syringe controlled water dispersion and withdrawal, respectively. Hysteresis is defined as the difference between the advancing and receding contact angle. The fractional surface areas in analyzing different wetting states were calculated using MATLAB Image Processing Toolbox.

2.3.3. X-ray photoelectron spectroscopy

All samples were analyzed by X-ray photoelectron spectroscopy (XPS, Thermo Fisher ESCALAB 250 Xi multichannel) with monochromatic Al K radiation. XPS was performed at an acceleration voltage of 15 kV with an emission current of 15 mA, in a residual vacuum of approximately 1×10^{-9} Torr. The analyzer was used in fixed analyzer transmission (FAT) mode. The spectra were taken from two areas on each sample, and a minimum of two replicate samples were analyzed for each recipe. Sample surface compositions were determined from the average of these measurements.

2.4. Bacterial adhesion experiments

Adhesion to surfaces was tested using a fluorescent bacterial strain. To generate a fluorescent bacteria, *E. coli* K-12 strain W3110 was transformed by electroporation with GFP expressing plasmid pGFPmut2 [28,29]. Cultures of the fluorescent *E. coli* were grown in 5 ml of LB broth at 30 °C for 18–20 h with aeration and normalized to OD_{600} = 0.1 using saline (NaCl 0.9%) in a spectrophotometer (SpectraMax M3) [30]. Coupons were glued to the bottoms of the wells of 12 wells plates as previously described and the silicon sealant was allowed to dry for 30 min [31]. The wells were then filled with 2.5 ml of the fluorescent bacteria in saline. After 30 min at 37 °C, the saline was removed, and the coupons were rinsed three times with 2.5 ml of saline to remove non-adhered bacteria. The exposed coupon surface was then placed under a coverslip and observed by fluorescent microscopy (Nikon TE2000-E microscope with a Photometrics CoolSNAP HQ-camera and a 20× objective). NIS-Elements 3.2 software was used to obtain digital images that were then analyzed for the number of attached bacteria using ImageJ software (NIH). In some cases a few loosely attached bacteria cells were observed, which were moving on the surface. Those