Chinese Journal of Polymer Science Vol. 35, No. 7, (2017), 887–896

Chinese Journal of Polymer Science
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Institute of Chemistry, CAS
Springer-Verlag Berlin Heidelberg 2017

Fouling-resistant Behavior of Liquid-infused Porous Slippery Surfaces*

Dan Zhao[a, b], Xiao-dong Xu[a**], Shuai-shuai Yuan[c**], Shun-jie Yan[b], Xiang-hong Wang[b], Shi-fang Luan[b**] and Jing-hua Yin[b]

[a] *Key Laboratory of Superlight Materials & Surface Technology, Ministry of Education, College of Materials Science and Chemical Engineering, Harbin Engineering University, Harbin 150001, China*

[b] *State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China*

[c] *School of Polymer Science and Engineering, Qingdao University of Science and Technology, Qingdao 266042, China*

Abstract Marine economy is seriously affected by marine biofouling, which has plagued people for thousands of years. Although various strategies have been developed to protect artificial surfaces against marine biofouling, cost-effective biofouling-resistant coating is still a goal in pursue. Herein, a cost-effective liquid-infused porous slippery surface (LIPSS) was facilely prepared by using poly(styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene) (SEBS) elastomer to form microsphere surfaces, followed by infusing fluorocarbon lubricants into the porous structure. The as-prepared slippery surfaces were characterized by static water contact angle, sliding velocity and sliding angle analysis. We also investigated the adhesion behavior of Escherichia coli (*E. coli*) and limnetic algae on different surfaces. It is confirmed that the slippery surfaces have better anti-biofouling properties than the porous SEBS reference. This cost-effective approach is feasible and easily produced, and may potentially be used as fouling-resistant surfaces.

Keywords Porous surfaces; Slippery surfaces; Antifouling; Bacteria; Algae

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s10118-017-1930-9.

INTRODUCTION

The adhesion and proliferation of marine organisms on artificial surfaces is a global problem for maritime industries[1, 2]. The adherence and subsequent colonization of marine organisms results in many problems, *e.g.*, accelerated rate of corrosion or degradation as well as increased drag resistance owing to surface roughening[3]. The primary strategy for combating marine fouling is to use biocidal coatings containing copper, organotin, or other organic biocides[4–7], but increasingly restrictive regulations are driving science and technology towards solely non-biocidal methods[8–11]. At present, environmental friendly anti-biofouling coatings based on amphiphilic polymers[12], PEG-containing polymers[13, 14], zwitterionic polymers[15], fluoropolymers[16] have been

* This work was financially supported by the National Natural Science Foundation of China (No. 51473167), the Natural Science Foundation of Heilongjiang Province of China (No. E201419), the Open Research Fund of State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, and China Postdoctoral Science Foundation (No. 2016M602106).

** Corresponding authors: Xiao-dong Xu (徐晓冬), E-mail: xuxiaodong@hrbeu.edu.cn
Shuai-shuai Yuan (袁帅帅), E-mail: ssyuan@qust.edu.cn
Shi-fang Luan (栾世方), E-mail: sfluan@ciac.ac.cn

Received December 5, 2016; Revised December 23, 2016; Accepted December 28, 2016
doi: 10.1007/s10118-017-1930-9

explored. Coatings that persistently resist biofouling can be achieved by means of surface chemistries[15, 17, 18], or topography[19–21]. Although great efforts have been made for several decades, cost-effective marine biofouling-control coating is still a goal in pursue.

Inspired by Nepenthes pitcher plant, Aizenberg's group firstly proposed a slippery liquid-infused porous surface (SLIPS)[22, 23]. They confirmed that the SLIPS had good repellency against blood cell, protein, and bacterial over a far long time[24–28]. The surfaces that possess nanoscale dynamic features may prevent interactions between the biofouling and the solid surfaces. Moreover, the SLIPS anti-biofilm surfaces are stable when submerged, under extreme pH, salinity and other conditions, and can be formed on arbitrarily shaped surface contours[27]. So the SLIPS can be used for the prolonged marine anti-biofouling. Recently, Levkin and co-workers reported that SLIPS provided another possible strategy to obtain promising fouling-resistant marine coating[29]. Microporous butyl methacrylate-ethylene dimethacrylate surfaces were prepared *via* photo-initiated free-radical polymerization. Subsequent infusion of fluorocarbon lubricants into the porous microtexture resulted in liquid-repellent slippery surfaces. The as-prepared surface maintained the water-repellent slippery property after 1 month of incubation under shaking, and revealed a significant inertness in inhibiting attachment of zoospores and cyprids. However, the preparation of microporous butyl methacrylate-ethylene dimethacrylate surfaces involved complex synthetic procedure and relatively stringent experimental conditions.

In this paper, we propose a facile and cost-effective strategy to fabricate a slippery surface. Firstly, highly adherent poly(styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene) (SEBS) elastomer solution in a mixed solvent of decanol and xylene was casted onto the glass to form a porous structure surface[30]. SEBS is widely used in marine antifouling coatings because it possesses important properties for marine fouling release performance, such as good thermal stability, outstanding mechanical performance, excellent adhesive property on different substrates, and easy control of the elastic properties of the coating[14, 31, 32]. Secondly, fluorocarbon lubricants were infused into the porous microtexture to obtain liquid-repellent slippery surfaces. The as-prepared slippery surfaces were challenged with Chlorella ellipsoidea and bacteria to test their resistance for biofouling.

EXPERIMENTAL

Materials

Hydrogenated poly(styrene-*b*-(ethylene-*co*-butylene)-*b*-styrene) (SEBS G1652, powder) with 29 wt% styrene and 44 wt% ethylene was obtained from Kaneka Corporation. Decanol and xylene were purchased from Aladdin Industrial Corporation and used without further purification. Perfluorodecalin (J.K. Scientific Ltd., 2.63 cSt) and perfluoropolyethers (Dupont Krytox GPL 105, 160 cSt) were used as lubricants. Rhodamine B, crystal violet, phosphate buffered solution (PBS, pH 7.4), Gram-negative Escherichia coli (*E. coli*, ATCC 25922), Luria-Bertani (LB) broth and BG11 medium were obtained from Dingguo Biotechnology Co., Ltd. Chlorella ellipsoidea (GY-D20) were purchased from Guangyu Biotechnology Co., Ltd.

Preparation of Liquid-infused Porous SEBS Coating

10 wt% of SEBS solution in mixed solvent of xylene and decanol at various ratios was coated on glass substrate. The samples were placed in air to allow partial solvent evaporation, and then the residual solvent was washed out by ethanol. The samples were dried overnight under vacuum at 30 °C to prepare the porous SEBS surfaces, denoted as PSEBS. PSEBS were infused with perfluorodecalin and perfluoropolyethers, denoted as SEBS-PD and SEBS-PP, respectively. The samples were tilted to 90° for 5 min to remove the excess lubricant by gravity-driven processes and then flat-wised at room temperature before experiment.

Characterization of Surface Morphology and Property

The structure of samples was examined by field emission scanning electron microscopy (FESEM, XL 30 ESEM, FEI Company). The static water contact angle (WCA) was measured on Drop Shape Analysis System (DSA, Kruss Gmbh, Germany) by a sessile water drop method with 2 μL water drops. The advancing (θ_A) and receding (θ_B) water contact angles were measured by a water drop method with 4 μL water drops at a slip tilting table. The difference between θ_A and θ_B is termed as contact angle hysteresis (CAH). The aqueous droplet of

rhodamine B dye (50 μL) was used to characterize the slippery property of prepared samples at the slip tilting angle of 20°[26]. All the samples were dip-coated in lubricating liquid before measurement and tilted to 90° for 5 min to remove the excess lubricant by the gravity-driven processes at room temperature.

Antibacterial Assay

E. coli was cultivated in growth broth (LB broth) at 37 °C for 24 h. The bacteria were resuspended to a concentration of about 1×10^8 cells/mL after centrifugation at 3000 r/min for 10 min. Bacterial cell concentration was calculated by testing the optical density with a microplate reader (Tecan Sunrise, Swiss) at 540 nm relative to a standard calibration curve[25]. It is confirmed that an optical density of 1.0 at 540 nm is equivalent to a bacteria concentration of ~10^9 cells/mL.

The samples (1 cm × 1 cm) for spread plate experiment were transferred to a 24-well plate and incubated with bacterial suspension (50 μL, 10^7 cells/mL) for 2 h or 24 h at 37 °C. After incubation, the samples were rinsed with 2 mL of PBS solution three times to remove non-adherent bacteria. Then the samples were put into 1 mL of PBS solution and subjected to ultrasonic treatment for 20 min to release the adherent bacteria cells. The resulting bacteria containing PBS suspension (50 μL) were spread on gelatinous LB agar plates and cultured at 37 °C for 24 h. Then the bacteria adherence and growth situation was observed.

The samples for bacterial adherence were incubated in 2 mL of bacterial suspension (10^8 cells/mL) for 2 h or 24 h at 37 °C. After incubation, the samples were rinsed with 2 mL of PBS solution to remove non-adherent bacteria. Then the samples were fixed with 3 vol% paraformaldehyde and dehydrated with a series of ethanol aqueous solution (10 vol%, 30 vol%, 50 vol%, 70 vol%, 90 vol% and 100 vol%). The adhered bacterial cells on the samples were observed by FESEM.

The amount of adherent bacteria was quantitatively determined by a crystal violet staining method. The samples after 24 h incubation were stained by crystal violet (0.1 wt%) for 1 h. Then, the stained samples were rinsed with distilled water six times. Acetic acid (10 vol%) was used to dissolve the bound crystal violet for 10 min. 300 μL of crystal violet-acetic acid solution was pipette into a 96-well plate and measured by a microplate reader (Tecan Sunrise, Swiss) at 590 nm.

Toxicity Test

Bacterial suspension (*E. coli*, 10^7 cells/mL) was used to test the toxicity of the lubricants. Shaken cultures of *E. coli* were grown in the presence of perfluorodecalin and perfluoropolyethers (1 vol%). Control sample containing only *E. coli* in LB and negative control sample ($AgNO_3$, 0.1 wt%) were also investigated. The samples were then cultured in a thermostatic shaker (37 °C, 110 r/min). The optical density of the samples at 0, 1, 5, 10, 24 and 30 h was measured by a microplate reader at 540 nm[27].

Anti-algae Assays

Limnetic algae (Chlorella ellipsoidea) were cultured in conical flasks using autoclaved sterilized distilled water in BG11 growth medium at 25 °C for 10 days. Samples were put into the beakers with algae solution. The adhesion of algae on the surface of the samples was observed at regular intervals. The samples were gently dip-rinsed with sterile distilled water to remove the non-adherent algae, and dried with a nitrogen flow. Finally, the samples were observed by a confocal laser scanning microscopy (CLSM, LSM 700, Carl Zeiss) and photographed with a digital camera. The percentage of the occupied area of adhered algae from the images were counted using Image J software[33].

Statistical Analysis

All data are presented as mean ± standard deviation (SD). The statistical significance was assessed by analysis of variance (ANOVA), *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$). Each result is an average of at least three parallel experiments.

RESULTS AND DISCUSSION

Random networks of SEBS microsphere surfaces were used to fabricate LIPSS (Fig. 1). To generate the

microsphere surface, SEBS was firstly dissolved in a mixed solvent of xylene and decanol, and then the solution was casted onto glass substrates at room temperature. After evaporation of the solvent, stable hydrophobic micro-structure surfaces were fabricated. In order to prepare the lubricating film, we chose two different viscosity fluorocarbon lubricants of perfluorodecalin and perfluoropolyethers. The LIPSS was generated through liquid imbibition into the microsphere surfaces. The sliding characteristics and anti-biofouling behavior of slippery coatings were subsequently studied.

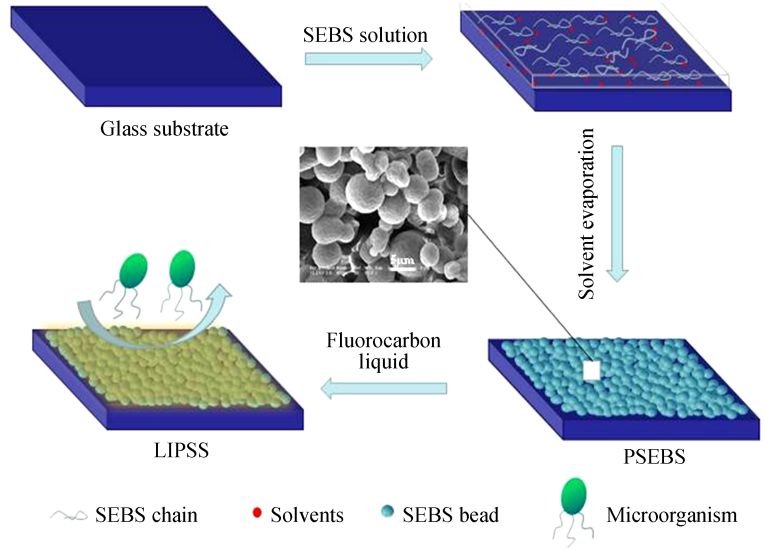


Fig. 1 The preparation procedure of liquid-infused porous slippery surface (LIPSS)

Porous SEBS Surface Characterization

The morphology of the samples is shown in Fig. 2. The content of decanol is critical on the surface morphology and hydrophobicity. As shown in Figs. 2(a) and 2(d), the flat SEBS film prepared by using xylene alone is

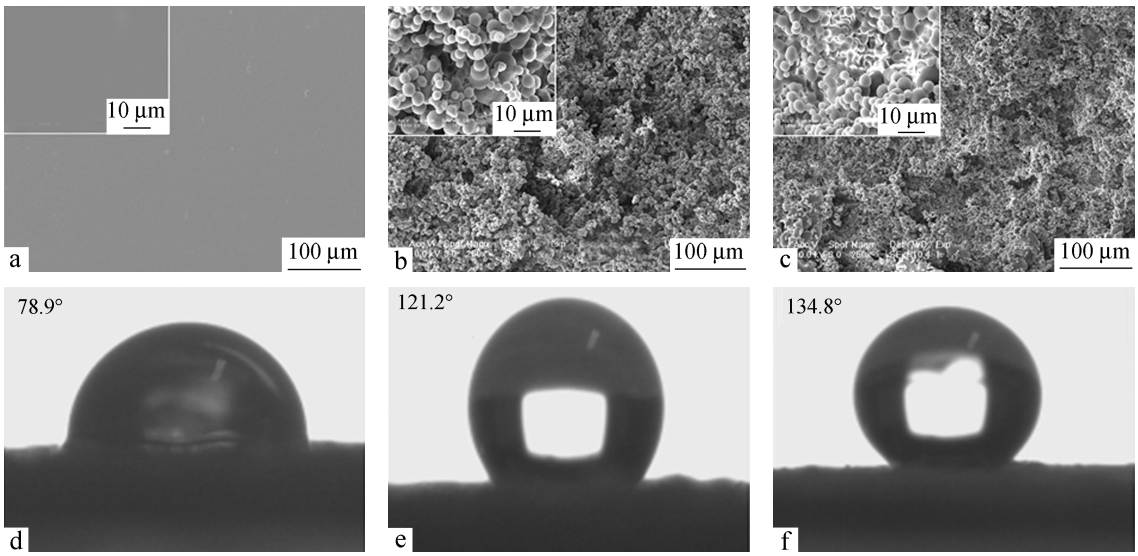


Fig. 2 The SEM images of surface morphology of SEBS samples with different volume ratios of xylene and decanol and corresponding WCA values: (a) and (d) xylene:decanol = 1:0; (b) and (e) xylene:decanol = 1:1; (c) and (f) xylene:decanol = 4:3

hydrophobic with a WCA of about 80°. Microsphere morphology was observed for the samples prepared by using a mixed solvent of xylene and decanol with a 1:1 volume ratio (Fig. 2b). The WCA increased to about 121.1° because of the increase of roughness (Fig. 2e). The homogeneity of the pores on the surface increased as a result of changing the volume ratio of solvents (xylene:decanol = 4:3) (Fig. 2c), resulting in an increase of WCA value to about 134.9° (Fig. 2f).

Porous SEBS surfaces (xylene:decanol = 1:1, denoted as PSEBS) were used to wick lubricating liquids. Due to the molecular smoothness interface between the water droplet and the thin oil layer, the WCA values became smaller after infused with fluorocarbon lubricants[26]. PSEBS infused with perfluorodecalin and perfluoropolyethers was denoted as SEBS-PD and SEBS-PP, respectively. Compared to PSEBS, the WCA of SEBS-PD and SEBS-PP decreased about 44° and 72° after infusing of fluorocarbon lubricants (Fig. 3).

The change of WCA with time was also examined to evaluate the stability of SEBS-PD and SEBS-PP (Fig. 4). The WCA of SEBS-PD obviously changed in 30 min, and the variation tendency became slow with time elapsing until perfluorodecalin ran off completely. In contrast, the SEBS-PP had a smaller change and a less wastage rate than SEBS-PD after 24 h. These results suggest that the samples infused with larger viscosity fluorocarbon lubricants possess a better stability.

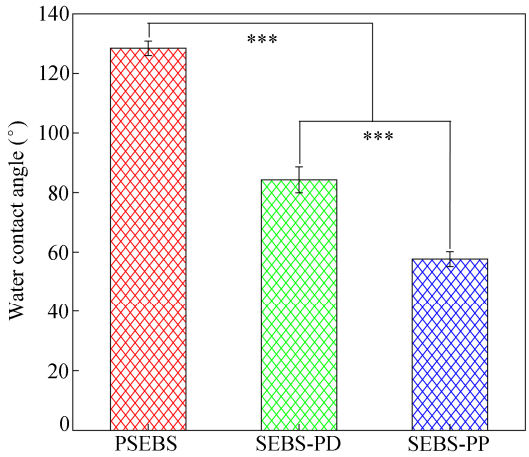


Fig. 3 The static water contact angle of the samples (Data shown are the average ± standard deviations.) Significant difference *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)

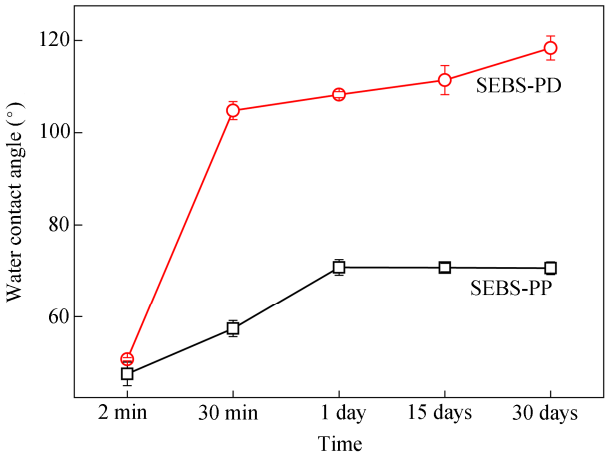


Fig. 4 The change of WCA of SEBS-PD, SEBS-PP with elapsing of time at room temperature (Data shown are the average ± standard deviations.)

Characterization of Slippery Property

To reflect the slippery property of the lubricant infused samples, the sliding velocity of 50 μL of an aqueous rhodamine B on the surfaces was measured. The sliding velocity of the samples with infused lubricant significantly increased due to the dependence of sliding velocity on the smooth degree of sample surface. As shown in Fig. 5, the velocity is 0.0, 4.8 and 0.2 cm/s for PSEBS (Video S1 in supporting information, SI), SEBS-PD (Video S2 in SI) and SEBS-PP (Video S3 in SI), respectively. Water droplet on PSEBS surfaces remained pinned as it was tilted to 90°. The experimental results demonstrate that water droplets exhibit Wenzel state in which water droplets are in full contact with the rugged surfaces. To further explore the slippery of SEBS-PD and SEBS-PP, the dynamic water contact angle was also tested. A liquid droplet can form different static contact angle with one surface, but unique maximum and minimum angles (dynamic advancing (θ_A) and receding angles (θ_B)) can be measured[26]. The difference between θ_A and θ_B is termed contact angle hysteresis. Our results are summarized in Table 1, and show that SEBS-PD (~4.7°) and SEBS-PP (~6.4°) exhibit low contact angle hysteresis. In combination with the data in Fig. 5, it proved that the sliding velocities decreased substantially with increase in hysteresis.

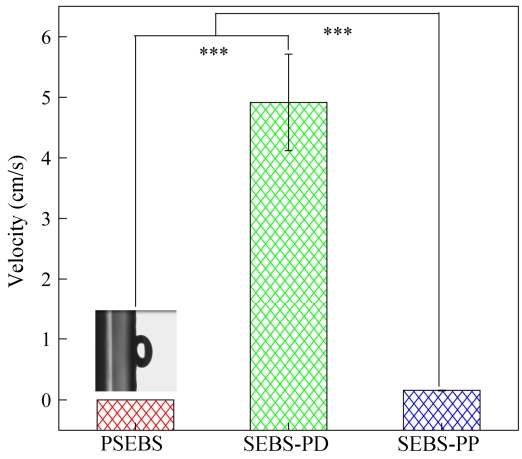


Fig. 5 The velocity for 50 µL of an aqueous rhodamine B droplet to slide 6 cm on PSEBS, SEBS-PD and SEBS-PP (tilt angle = 20°) (Data shown are the average ± standard deviations.)
Significant difference *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)

Table 1 Dynamic water contact angle of the samples

Samples	θ_A (°)	θ_B (°)	Hysteresis (°)	Sliding angle (°)
SEBS-PD	96.0 ± 0.6	91.3 ± 0.5	4.7 ± 0.8	15.0 ± 0.5
SEBS-PP	88.7 ± 0.5	82.3 ± 0.7	6.4 ± 0.9	10.0 ± 0.2

Biofouling Assays

The antifouling properties of LIPSS were assessed by using two typical microorganisms, *E. coli* and Chlorella ellipsoidea. Chlorella ellipsoidea was used to gain a better understanding of the antifouling properties of LIPSS against marine biofouling organisms.

Antifouling behavior against E. coli

Figure 6 shows the digital photographs of agar plates incubated with the diluted *E. coli* suspension, which has been in contact with PSEBS, SEBS-PD and SEBS-PP for 2 and 24 h. Compared to PSEBS (Fig. 6a), SEBS-PD

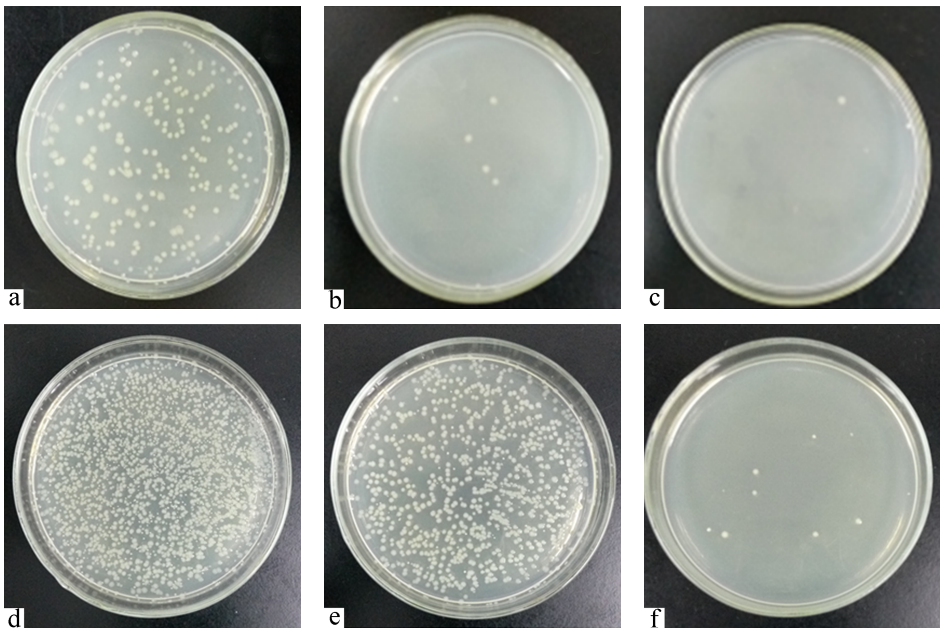


Fig. 6 Photographs of agar plates for 10^7 cells/mL *E. coli* suspension recovered from different samples (a) PSEBS, (b) SEBS-PD and (c) SEBS-PP for 2 h; (d) PSEBS, (e) SEBS-PD and (f) SEBS-PP for 24 h

(Fig. 6b) and SEBS-PP (Fig. 6c) can effectively inhibit bacterial attachment in 2 h incubation. The number of bacteria adhered on the surface of SEBS-PD (Fig. 6e) increased to a level equal to that of the PSEBS control (Fig. 6d) because of perfluorodecalin running off with the elapsing time. The FESEM images show the similar results (Fig. 7).

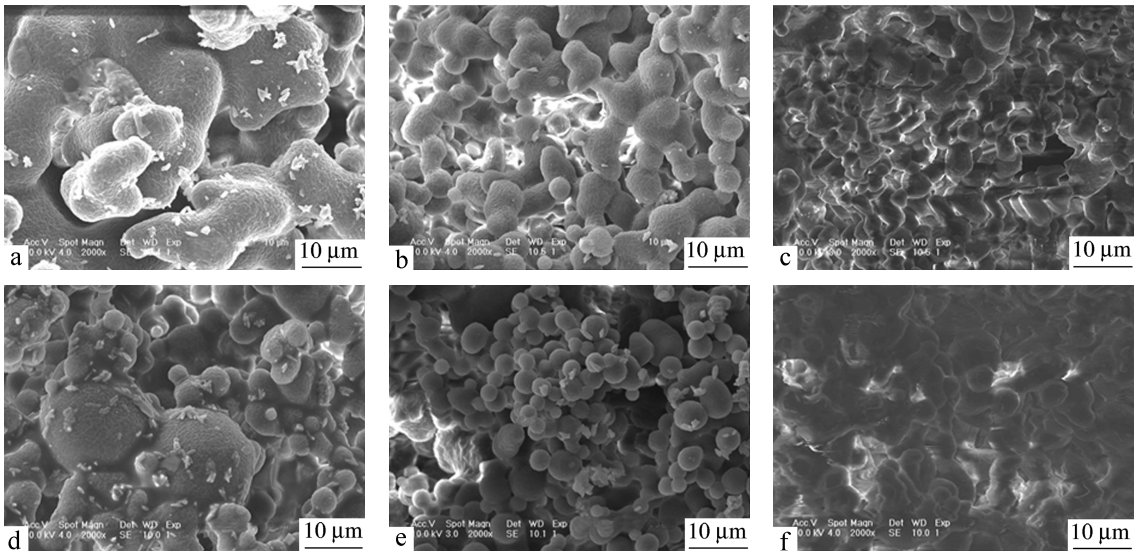


Fig. 7 The FESEM images of 10^8 cells/mL *E. coli* adherent on (a) PSEBS, (b) SEBS-PD and (c) SEBS-PP for 2 h; (d) PSEBS, (e) SEBS-DP and (f) SEBS-PP for 24 h

Besides, crystal violet staining method was used for quantitatively investigating the amount of *E. coli* attached on the surfaces (Fig. 8). Compared to the PSEBS, the amount of attached *E. coli* on the different viscosity fluorocarbon lubricants infused surfaces was reduced by ~67% (SEBS-PD) and ~71% (SEBS-PP), respectively. These results demonstrate that LIPSS is more effective as antifouling surfaces against *E. coli* as compared to PSEBS.

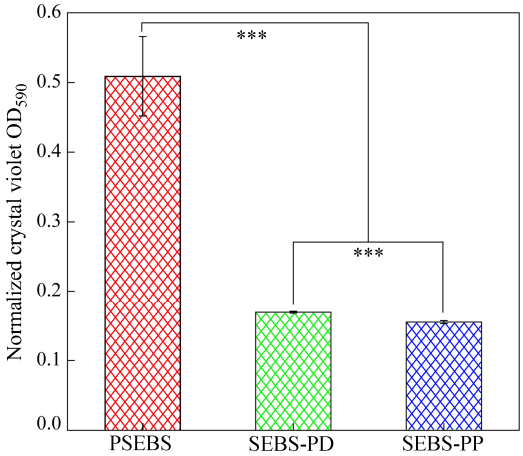


Fig. 8 Quantitative count of 10^8 cells/mL *E. coli* on the samples' surface following 24 h of growth (Data shown are the average ± standard deviations.)
Significant difference *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)

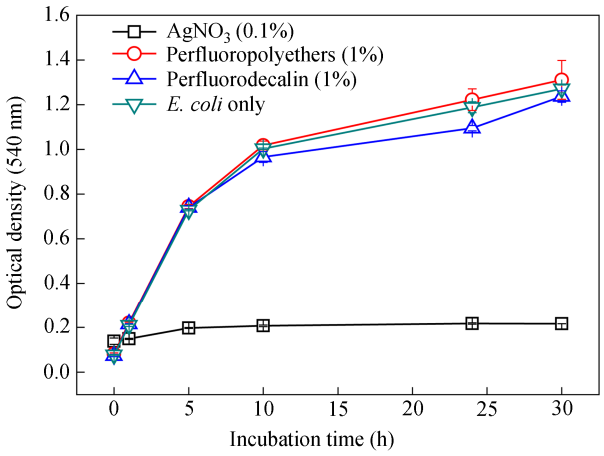


Fig. 9 Growth curves of *E. coli* incubated in LB medium with perfluorodecalin (1 vol%), perfluoropolyethers (1 vol%), $AgNO_3$ (0.1 vol%), and by contrast with only *E. coli* incubated in LB medium at 0, 1, 5, 10, 24, and 30 h (Data shown are the average ± standard deviations.)

Lubricant toxicity tests

To confirm that the prevention of bacterial attachment on LIPSS is not due to cytotoxicity of the lubricating liquids, the effects of lubricants on the growth of *E. coli* were investigated. As shown in Fig. 9, statistically indistinguishable bacterial growth occurred at 0, 5, 10, 24, and 30 h for all LIPSS liquids as compared to the control culture with no lubricant. Negative control of silver nitrate was also investigated. As expected, the lubricants have no significant effect on the growth of *E. coli*.

Antifouling behavior against Chlorella ellipsoidea

Experiments were performed using Chlorella ellipsoidea to evaluate the antifouling effectiveness of the samples exposed to Chlorophyta. The samples were placed in Perish dishes containing Chlorella ellipsoidea culture. After 1 day or 10 days, the samples were lifted out of the medium and photographed with a camera (Fig. 10). By comparing Figs. 10(b), 10(c) and 10(d), few Chlorella algae were observed on SEBS-PP surfaces, and SEBS-PP exhibited good stability during 10 days incubation.

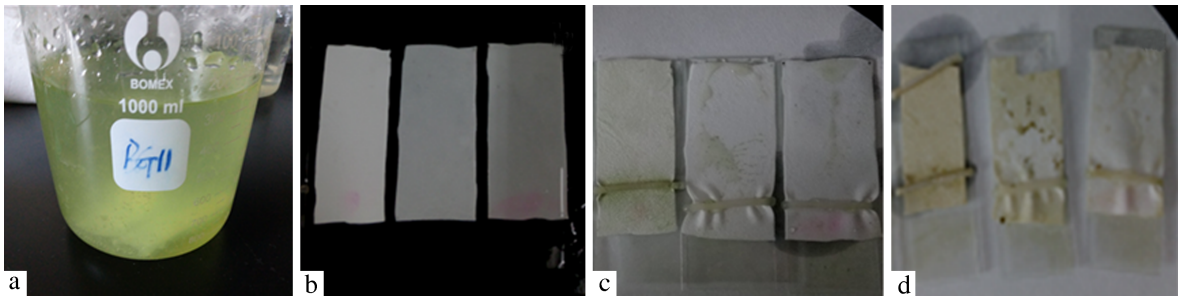


Fig. 10 The images of (a) Chlorella algae of 10 days old, and samples immersed in Chlorella algae for (b) 0 day, (c) 1 day and (d) 10 days (The order of samples from left to right: PSEBS, SEBS-PD, SEBS-PP)

Furthermore, CLSM images of the samples at different incubation time points are shown in Fig. 11. The images were all shot in the same luminosity. Besides, the coverage of Chlorella algae settled on samples after immersion for 1 day and 10 days is shown Fig. 12. It can be seen that the Chlorella algae coverage of SEBS-PD (4%) and SEBS-PP (2%) is less than that of PSEBS (22%).

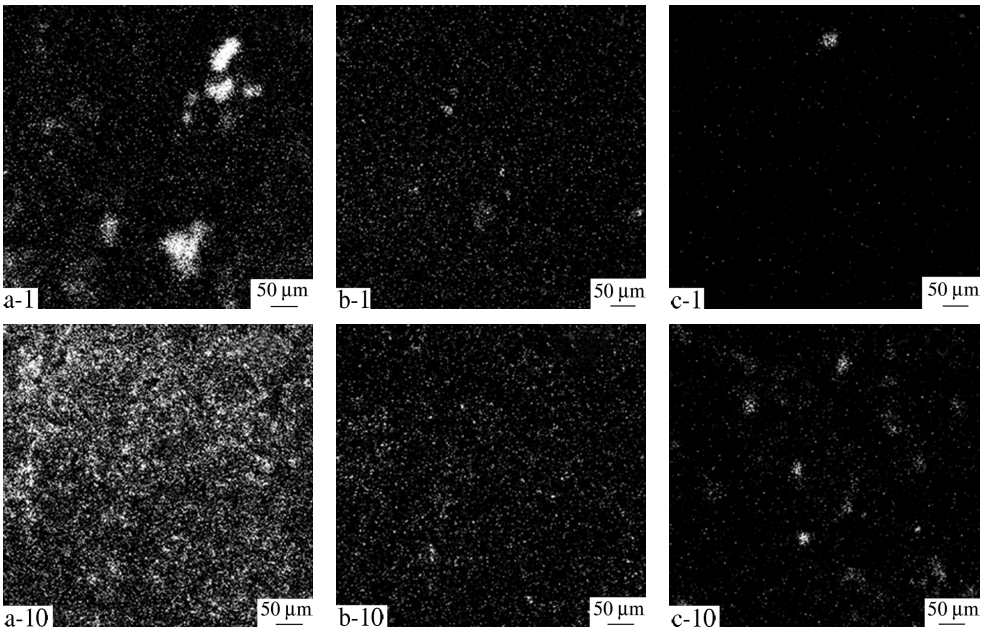


Fig. 11 CLSM images of the samples placed in chlorella algae for 1 day (the top) and 10 days (the bottom): (a-1, a-10) PSEBS, (b-1, b-10) SEBS-PD and (c-1, c-10) SEBS-PP

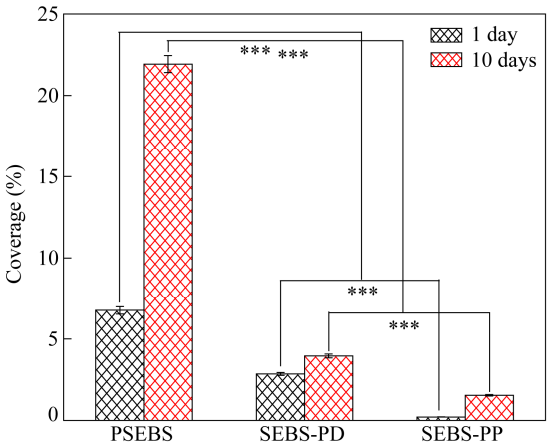


Fig. 12 Coverage of Chlorella algae settled on the samples after immersion for 1 day and 10 days (Data shown are the average ± standard deviations.)
Significant difference *($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)

CONCLUSIONS

In brief, we have fabricated slippery surfaces through a facile and cost-effective method. Because of the porous structure of SEBS substrate and the composition of fluorocarbon lubricants, the as-obtained LIPSS exhibits excellent biofouling-resistant properties. The stability of LIPSS is significantly affected by the viscosity of the lubricants. We have also found that the slippery surfaces prevent chlorella ellipsoidea attachment up to 10 days. We anticipate that this method will be potentially used for fouling-resistant surfaces.

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