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ARTICLE

Superhydrophobic Coating of Elastomer on Different Substrates with a Liquid Template to Construct a Biocompatible and Antibacterial Surface

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Construction of biocompatible and antibacterial surfaces becomes urgent needed recently. However, most of existing techniques require multi-step procedures, stringent conditions and specific substrates. Here, we present a facile method to create a biocompatible and antibacterial surface on virtually any substrate under an ambient condition. The strategy is based on casting a highly adherent elastomer, styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS), from solvent mixtures of xylene and decanol, where decanol acts as both a polymer precipitator to induce phase separation and a "liquid template" to stabilize superhydrophobic structure. The stable and durable superhydrophobic surface shows high biocompatibility and antibacterial properties.

1. Introduction

Biomaterials and biomedical devices need biocompatibility and antibacterial properties. Numerous approaches based on surface modification have been made to create such surfaces, however, most of them require chemical reactions, multi-step procedures, stringent conditions and specific substrates.[1-6] Obviously, development of facile methods to construct a biocompatible and antibacterial surface, which can be equally applicable to different substrates under ambient atmosphere, is important to facilitate the applications of biomaterials. Superhydrophobic coating holds great promise in successful creation of biocompatible and antibacterial surface. It has been reported that superhydrophobic surfaces with water contact angles (CAs) larger than 150° possess unique water repellent, self-cleaning properties, anti-fouling and anti-bacterial.[7-11] Additionally, formation of the superhydrophobic surface with casting method is a facile and versatile method.[8,12-14] However, unstable and weakly adherent coating on different substrates is a big obstacle to the progress. Thus, casting with highly adherent polymer will bridge the gap between facile superhydrophobic coatings and stable surfaces with high biocompatibility and antibacterial properties. Elastomers are promising candidates for superhydrophobic coating with casting method due to their strong adhesion with other substrate[15-17] and good abrasion resistance of superhydrophobic surface.[18] However, because elastomers are amorphous and soft at ambient temperature, the microscale structures are more prone to collapse during drying as the size is reduced,[9] failing in creation of superhydrophobic surface. Thus, construction of a superhydrophobic surface of elastomer simply by casting method without the need of any complex assistance, such as lithography[11] and template-based techniques,[14] remains challenging.

Here, we present a facile method to construct a biocompatible and antibacterial surface on virtually any substrate under an ambient condition. An elastomer, styrene-b-(ethylene-co-butylene)-b-styrene elastomer (SEBS) is used due to its good biocompatibility, thermal stability, outstanding mechanical performance and stable coating on different substrates.[19-21] The strategy is based on casting highly adherent SEBS from solvent mixtures of decanol and xylene, where decanol acts as both a polymer precipitator to induce phase separation and a "liquid template" to stabilize superhydrophobic structure. We demonstrate superhydrophobic surface is achieved by the formation of porous structure on the surface, which can be controlled by varying the decanol/xylene ratio. In addition, the stable and durable superhydrophobic surface shows high resistance to bacterial adhesion, protein adsorption, platelet adhesion, elongation of blood clotting and reduced hemolysis. Our work thus establishes a universal principle to develop antifouling, antibacterial and biocompatible surfaces.

2. Materials and methods

2.1 Chemicals and Materials

SEBS copolymer with 29 wt% styrene (Kraton G 1652, M_n=74800) was purchased from Shell Chemicals (USA). Ethanol, decanol, *p*-xylene and cyclohexanol were provided by Aldrich and used without further purification. Phosphate-buffered saline (PBS 0.9 % NaCl, 0.01 M phosphate buffer, PH 7.4) used for hemocompatibility evaluation was freshly prepared.

2.2 Preparation of Superhydrophobic Surface of SEBS

SEBS was dissolved in the solvent mixtures that contain *p*-xylene and decanol with the ratio of 4/2, 4/3, and 4/4, respectively. The SEBS solution was casted onto different substrates under ambient atmosphere (~ 60 % RH room temperature). To accelerate the evaporation of decanol, the solid films were either washed in ethanol with ultrasound or dried in vacuum. After solvent evaporation, the morphology of the film was observed by scanning electron microscopy (SEM). The static water contact angle (WCA) of sample was measured at room temperature and 60 % relative humidity using a contact angle goniometer (DSA, KRUSS GMBH, Germany) by a sessile drop method with a 2 μL water droplet. The dynamic contact angles of superhydrophobic SEBS surface were also recorded by DSA. A 2 μL droplet of water was initially deposited onto the film, and then the advancing and receding contact angles were recorded while the water was added to and withdrawn from the drop at a constant rate (30 μL /min) to advance and recede the contact line, respectively.

2.3 Protein adsorption

Bovine serum fibrinogen (BFg) was dissolved in PBS (pH 7.4) at concentration of 0.1 mg/ml. Then it was deposited onto the surface of original SEBS film and casted films, and incubated for 1.5 h at room temperature. Then, the films were rinsed with PBS and deionized water to remove any physical adsorption protein. All the samples were completely dried in a vacuum oven before measurements. The amount of protein adsorption was determined by X-ray photoelectron spectroscopy (XPS) (VG Scientific ESCA MK II Thermo Avantage V 3.20 analyzer with Al/K anode mono-X-ray source). The take-off angle for photoelectron analyzer was fixed at 90 °. Surface spectra were collected over a range of 0-1200 eV to achieve high-resolution spectra of C(1s), O(1s), N(1s) and S(1s) regions. The coverage of BFg on the surface was calculated based on equation 1:

$$\Gamma_{BFg}=\frac{A_{mN}}{A_{pN}}\times 100\% \qquad (1)$$

where A_{mN} is the nitrogen atomic percentage on the SEBS surface (after protein adsorption) measured by XPS, and A_{pN} is the nitrogen atomic percentage of BFg under complete coverage of the membrane surface with BFg. A_{pN} is 10.09% based on the total atomic composition of C, O, N and S elements in pure BSA powder.

2.4 Platelet Adhesion

Fresh blood was extracted from a healthy rabbit in accordance with the guidelines issued by the Ethical Committee of the Chinese Academy of Sciences. A part of fresh blood was centrifuged at 1000 rpm for 15 min to obtain platelet rich plasma (PRP). All the samples were immersed in PBS (pH 7.4) at 37 °C for 2 h to equilibrate the surfaces. PRP was deposited onto each sample for 2 h at 37 °C, followed by rinsing (with PBS), fixation (with 2.5 wt% glutaraldehyde in PBS) and dehydration (with ethanol/water mixtures).[19] Platelet adhesion on the samples was sputter coated by gold and characterized by a field-emission scanning electron microscopy (SEM, Sirion-100, FEI, USA).

2.5 Antibacterial Tests

Escherichia coli (E. coli, ATCC 25922) were selected to evaluate the bacterial adhesion on SEBS surfaces. E. coli were grown in the LB broth medium at 37 ℃ overnight. E. coli suspension was obtained after centrifuged at 2500 rpm. The concentration of E. coli (~110 cells ml^{-1}) was determined by testing the absorbance of cell dispersions at 540 nm. Original and casted SEBS films were immersed in the bacterial suspension for 1 day at 37 ℃, follow by rinsing (with sterilized PBS), fixation (3 wt% sterilized paraformaldehyde in PBS) and dehydration (with ethanol/water mixtures). Bacterial on the samples was sputter coated by gold and characterized by SEM (Sirion-100, FEI, USA) based on the method described by Zhu.[22]

2.6 Whole Blood Clotting Time

The anticoagulants of different SEBS films were evaluated using fresh rabbit blood by clotting time method. Briefly, 30 μL fresh blood was dropped onto the samples, followed by incubation at 37 °C for 30 min. Then 3 mL deionized water was added to stop the reaction. The concentration of free haemoglobin in water was measured by TECAN absorbance reader (TECAN GENIOS, Austria) at 540 nm. The relative absorbency of 30 μL whole blood diluted by 3 mL distilled water was assumed to be 100. The blood clotting index (BCI) of biomaterial can be quantified by the following equation:[23]

$$\mathrm{BCI}(\%)=\frac{\text{Absorbency of sample solution}}{\text{Absorbency of fresh blood solution}}\times 100 \qquad (2)$$

2.7 Hemolysis

The fresh blood (0.1 mL) was added to samples for 120 min incubation, and then 2 mL normal saline was added to each sample for further incubation at 37 °C to stop the hemolysis completely. Positive and negative controls were produced by adding 0.1 mL blood to 2 mL distilled water and normal saline, respectively. After incubation, the blood cells were removed by centrifugation (3000 rpm, 3 min) and the supernatant was transferred to 96-well plates.[20] The hemolysis ratio was calculated based on the absorbance value at 540 nm using a TECAN absorbance reader (TECAN GENIOS, Austria). The hemolysis ratio was calculated according to the following formula:

$$HR(\%)=\frac{OD_{test}-OD_{neg}}{OD_{pos}-OD_{neg}}\times 100\% \quad (3)$$

where OD_{test} is absorbance value of test sample, OD_{pos}, and OD_{neg} are the positive (water) and negative (saline) control, respectively.

3 Result and discussion

Construction of a biocompatible and antibacterial surface is achieved by superhydrophobic coating of an elastomer, SEBS on different substrates (Fig. 1). SEBS is dissolved in the solvent mixtures of p-xylene and decanol, then casted onto different substrates under ambient atmosphere. After solvent evaporation, stable superhydrophobic surfaces with high biocompatibility and antibacterial properties are formed. Generally speaking, most of polymers that used to create superhydrophobic surfaces with conventional casting method are crystallic or plastic at room temperature,[11,24] the highly constrained motion of polymer chains can stabilize newly-formed micro- and nanostructure during solvent evaporation and polymer solidification. In contrast, elastomers are amorphous and soft at ambient temperature, the newly-formed micro- and nanoscale structures are more prone to collapse during solvent evaporation,[9] leading to failure in formation of superhydrophobic surfaces.

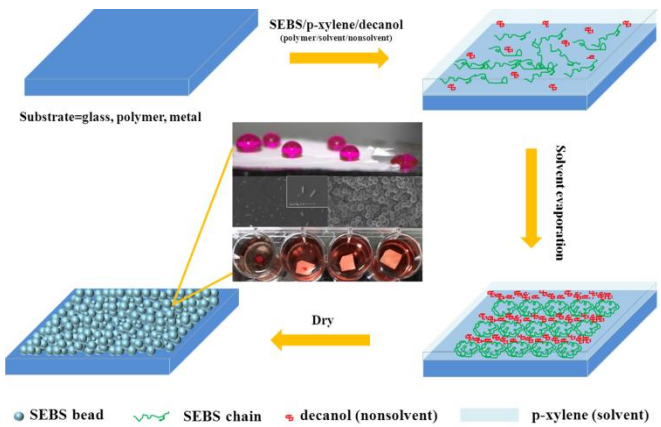

Fig. 1 Schematic of constructing biocompatible and antibacterial surfaces by superhydrophobic coating of an elastomer, SEBS on different substrates. Decanol acts as both a polymer precipitator to induce phase separation and a "liquid template" to stabilize superhydrophobic structure. The superhydrophobic surfaces are stable and durable with high biocompatibility and antibacterial properties.

To solve this problem, a less volatile solvent, decanol is used as a nonsolvent in this work. Decanol has higher boiling point (233 °C) than that of *p*-xylene (137 °C). During the evaporation of *p*-xylene, decanol tends to remain in the gel-like body of elastomer, which works as physical barriers to prevent the collapse of elastomer microstructure, similar as the "liquid template" in nanocasting techniques.[25] Thus, contrary to the role of nonsolvent on enhancing evaporation rate of solvent in conventional methods,[9] decanol acts as not only a polymer precipitator to induce microphase separation, but also a "liquid template" to stabilize the microstructure of SEBS during solvent evaporation, making a new approach to successfully create a superhydrophobic surface (Fig. 1).

Fig. 2 shows SEM images of SEBS coatings that cast from the mixtures containing 4 mL *p*-xylene and varied decanol contents. Decanol contents have substantial effects on the morphology and hydrophobicity of coatings. For clarify, SEBS coatings casted from the mixtures with 2, 3, 4 mL decanol are referred as "$SEBS_1$, $SEBS_2$, $SEBS_3$", respectively. Many irregular micropores that composed of intermingled sticks and beads, appear on the flat film of $SEBS_1$ (Fig. 2a), rendering the surface hydrophobic with the CA of 110° (Fig. 2d). The porous structure expands to the whole surface with increasing decanol to 3 mL (Fig. 2b). 4-6 μm beads dominate on the surface of $SEBS_2$, increasing the surface roughness with the CA of 163° (Fig. 2e, Supporting Information, Video S1). There is an increase in the inhomogeneity and size of the pores on the surface of $SEBS_3$, due to further enhancement of decanol content to 4 mL (Fig. 2c), resulting in reduced surface roughness and CA value (156°,) (Fig. 2f). The advancing (θ_{Adv}) and receding (θ_{Rec}) water contact angles of the $SEBS_3$ are 160.19° and 153.65°, respectively. As the water droplets tend to pin the porous surface in a wet-contact mode, and relatively high hysteresis (7°) is observed, Wenzel's model is applied to analyze the effect of surface roughness on the contact angle.

$$\cos\theta_w = r\cos\theta \quad (4)$$

where θ_W is apparent CA on a rough surface, θ is Young's CA on a flat surface, r is the roughness factor. Considering the water contact angle of SEBS is 96°,[19] high surface roughness results high contact angle. But when the content of decanol in the solvent mixture is larger than one half, the solid film cannot be formed. Therefore, the distribution and size of pores are dependent on the ratio of *p*-xylene to decanol.

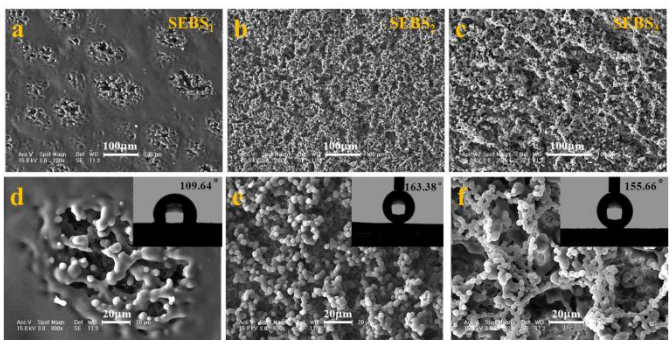

Fig. 2 SEM images of surface morphology of SEBS coatings prepared from the mixtures containing 4 mL of xylene with various decanol contents. a) $SEBS_1$ with 2 mL decanol; b) $SEBS_2$ with 3 mL decanol; c) $SEBS_3$ with 4 mL decanol; (d)-(f) enlarged images of (a)-(c) with CA values, respectively.

The morphology of coatings can be further controlled with mixed nonsolvents. Substitution decanol with cyclohexanol results in varied morphology of superhydrophobic surface (Fig. 3). Compared to large size of microbeads (6.8 μm) on the

surface casted from the mixtures with decanol as a single nonsolvent (Fig.3a), the 0.8 μm beads dominate on the porous surface when the ratio of cyclohexanol to decanol is 3 (Fig.3b). But increment of clyclohexanol content (the ratio of cyclohexanol to decanol is 4), the size of beads increases to about 2 μm (Fig. 3c).

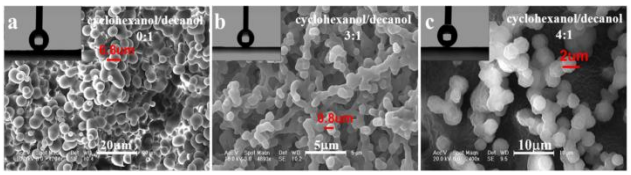

Fig. 3 SEM images of surface morphology of $SEBS_3$. a) decanol as single nonsolvent , b) cyclohexanol/decanol =3:1, c) cyclohexanol/decanol= 4:1.

The different boiling points of nonsolvent affect the rate of solvent evaporation, resulting in different morphology on the surface of casted film. Increasing the mixing time (xylene/decanol) from 2 h to 24 h, leads to the aggregation of microsticks. Elongation of mixing time decreases the size of SEBS beads, but has slight effects on surface morphology. Chloroform is one of good solvents for SEBS, but its volatilization rate is higher than that of *p*-xylene. Substitution of *p*-xylene with chloroform as solvent results in a chip-stacked morphology on casted film. The water contact angle on the casted coating (chloroform as solvent) is about 130 °. Our results demonstrate that appropriate choice of solvent /nonsolvent is critical for the formation of superhydrophobic coating of SEBS.

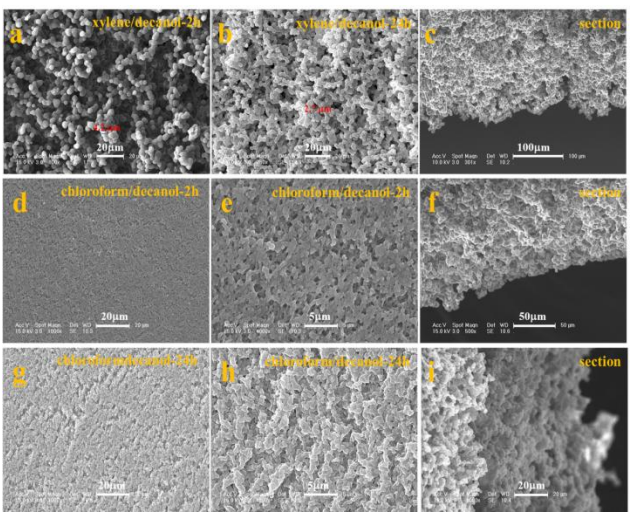

Fig. 4 SEM images of surface morphology of SEBS coating with different solvents and mixing times. a) *p*-xylene as solvent with 2 h mixing time, b) enlarged area of sample a, c) section of casted SEBS film, d) chloroform as solvent with 2 h mixing time, e) enlarged area of sample d, f) section of chloroform as solvent with 2 h mixing time, g) chloroform as solvent with 24 h mixing time, h) enlarged area of sample h, i) section of chloroform as solvent with 24 h mixing time.

Strongly adherent superhydrophobic coatings on different substrates and non-flat substrates are important for applications of blood-contacting biomaterials. An advantage of this method is that SEBS usually forms a strong bond with a substrate.[15-17] In addition, the adhesion to the underlying substrate can be enhanced by standard techniques.[24] Furthermore, due to the inherent bulk porosity of the polymer film, the obtained surfaces show relatively good durability and stability to scratching.[26]

Fig. 5 shows examples of different substrates coated with superhydrophobic porous SEBS. The substrates include non-flat SEBS, glass slides, aluminum foil, and non-flat polypropylene (Fig. 5a-d). Although the mixed solution may cause swelling issue to polymeric substrates, it has slight effect on solvent-resistant polymers, glass and metal substrates. Superhydrophobic coatings on different substrates demonstrate the convenience and versatility of this casting method. The coating of SEBS exhibits superhydrophobic after its surface was peeled with the adhesive tapes and remains stable for 3 months (Fig. S1, S2), convincing the potential application with long-term requirement.[26] The surface of porous SEBS film showed high uniformity, as demonstrated by SEM images (Fig. 2, 4) and the data of WCA on varied points of coating surface (158 ± 5°). In addition, the thickness has slight effect on surface topography, uniformity of porous SEBS film and corresponding superhydrophobic property (Fig. S5). Considering the stable adhesion of SEBS coating on the substrates, the thickness ranging from 200 μm to 400 μm is optimal.

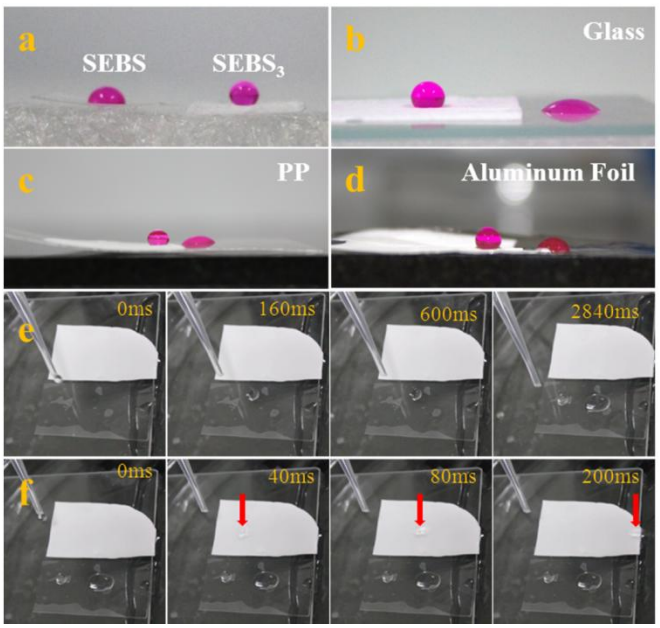

Fig. 5 Superhydrophobic coatings of SEBS on different substrates. a) non-flat SEBS; b) glass; c) non-flat polypropylene; d) aluminum foil; e) retention of water droplet on SEBS surface; f) rolling of water droplet on the $SEBS_3$ surface.

Rolling behaviour of water droplets on superhydrophobic surface and original SEBS surface is further investigated (Fig. 5e-f). When the water droplets are dropped on the surface of original SEBS, they do not roll down because of high hysteresis. In contrast, the droplets start to roll down once they contact the superhydrophobic surface (Video S2). The rolling behaviours

of water droplets confirm surface micro/nano structuring leads to extreme anti-wetting properties, which are key factors to construct biocompatible and antibacterial surfaces.[8]

Table 1. Atom percentages of elements C, O, N, and S on varied SEBS surfaces after BFg adsorption

Samples	Compositions (at. %)				Γ_{BFg} (%)
	C_{1s}	O_{1s}	N_{1s}	S_{1s}	
SEBS	77.19	15.62	6.71	0.48	66.50
$SEBS_1$	83.88	10.51	5.39	0.23	53.42
$SEBS_2$	82.39	12.40	4.91	0.30	48.66
$SEBS_3$	91.69	5.11	2.98	0.22	29.53
BFg[a]	77.19	15.62	6.71	0.48	100

a. The coverage of Pure BFg is assumed to be 100%

The superhydrophobic surface of SEBS coating exhibits high biocompatibility and antibacterial capability. Bfg adsorption on the surfaces of varied SEBS films is performed to evaluate anti-fouling capability of films. The coverage of BFg on the SEBS surface calculated by XPS is listed in Table 1. Compared to the BFg coverage on original SEBS, the coverage of BFg on casted films decreases substantially with increased hydrophobicity. And BFg adsorption on the surface of $SEBS_3$ is nearly one third of that on the surface of original SEBS, indicating high antifouling abilities.

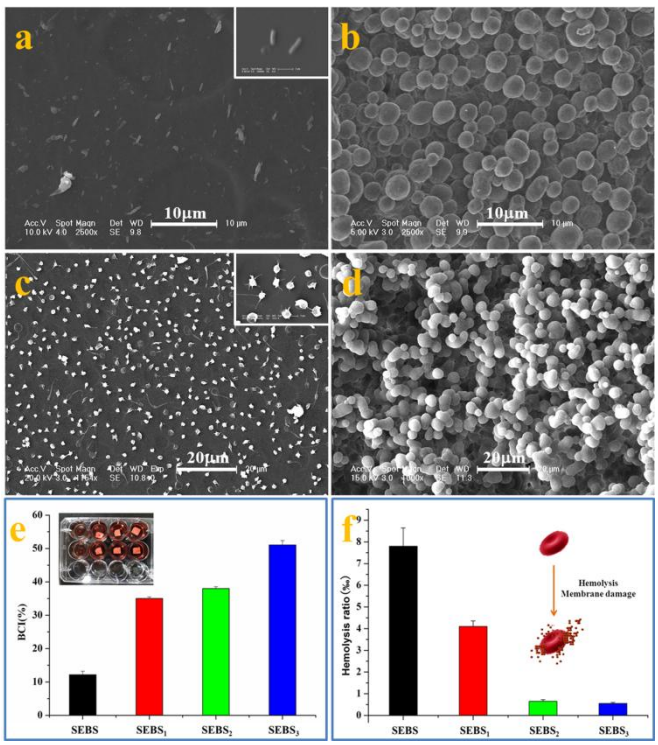

Fig. 6 Hemocompatibility of superhydrophobic coatings. (a-d) SEM images of platelet adhesion on original SEBS, $SEBS_1$, $SEBS_2$ and $SEBS_3$, respectively; (e) blood clotting time index (BCI) of samples; (f) hemolysis ratio of samples.

Escherichia coli are selected to evaluate the bacterial adhesion on SEBS surfaces. As shown in SEM images (Fig. 6a and 6b), there are many *Escherichia coli* on the surface of original SEBS, in contrast, no *Escherichia coli* are observed on the superhydrophobic surface of $SEBS_3$, confirming the high antibacterial surface has been obtained.[12-14]

The SEM images of platelet adhesion on the surface of original SEBS and superhydrophobic surface of SEBS are shown in Fig. 6c and 6d, respectively. The flat surface of original SEBS is covered with a large number of platelets and most of these platelets are highly activated, as evidenced by their spread-dendritic shape and presence of pseudopodia (Fig. 6c). No adhered platelets are observed on superhydrophobic surface of $SEBS_3$, indicating that the superhydrophobic coatings can effectively resist the adhesion and activation of platelets.

Whole blood clotting time on the surface of original SEBS and casted coatings is shown in Fig. 6e. Under the same condition, a larger BCI surface means improved anticoagulant property.[18] After 30 min incubation, BCIs on the superhydrophobic surface of $SEBS_2$ and $SEBS_3$ are 3-4 times that on the surface of original SEBS. Meanwhile, photographs of blood clots on the samples show that large blood clots appear on the surface of original SEBS, but no clots can be detected on the surface of $SEBS_2$ and $SEBS_3$ (inset of Fig. 6e, Fig. S2). Furthermore, hemolysis ratio on the surfaces of $SEBS_2$ and $SEBS_3$ is about one eighth of that on the surface of original SEBS (Fig. 6f), providing the direct evidence of enhanced biocompatibility.[21]

As SEBS can be substituted with other elastomers, such as poly(dimethylsiloxane) and polyurethane,[18,27] and interactions between superhydrophobic coatings and biological membranes of eukaryotic cells determine the antifouling and antibacterial properties of soft matter,[11,28] our work thus provides a universal method to develop antifouling, antibacterial and biocompatible surfaces on varied substrates.

Conclusions

We presented a facile method to construct a biocompatible and antibacterial surface on virtually any substrate under an ambient condition. The strategy was based on casting a highly adherent elastomer, SEBS, from solvent mixtures of decanol and xylene, where decanol acts as both a polymer precipitator to induce phase separation and a "liquid template" to stabilize superhydrophobic structure. Superhydrophobic surface was achieved by the formation of porous structure on the surface, which could be controlled by varying the decanol/xylene ratio. Additionally, the stable and durable superhydrophobic surface showed high resistance to bacterial adhesion, protein adsorption, platelet adhesion, elongation of blood clotting and reduced hemolysis. Our work thus established a universal principle to develop biocompatible and antibacterial surfaces on varied substrates.

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Notes and references

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Electronic Supplementary Information (ESI) available: [Figures S1-S5, and Movies S1-S2.]. See DOI: 10.1039/b000000x/

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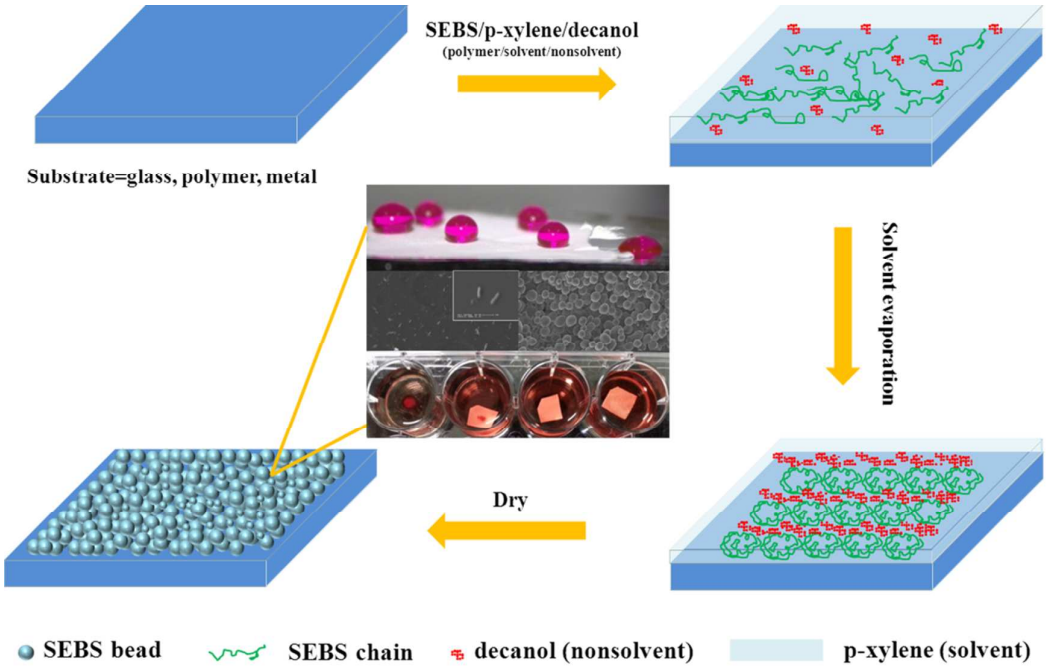
Table of Contents Entry



Casting elastomers with a liquid template was established to create superhydrophobic, biocompatible, antifouling and antibacterial surfaces on virtually any substrate.