Anti-biofouling, blood-repellent and biocompatible coatings for medical equipment interfaces

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***Abstract*— Thrombogenesis and infections due to pathogen transmission caused by medical equipment interfaces are one of the biggest health problems that threaten human life. One of the recent approaches to overcome these problems is superhydrophobic surfaces with low surface energies and micro/nano surface roughness. Here, medical equipment interfaces such as gloves, lancets, surgical drapes and gowns have been made superhydrophobic. These superhydrophobic surfaces exhibited high blood repellency with a static contact angle of 172° against blood, allowing continuous blood flow without leaving any residue on the surface. Furthermore, these coatings perfectly suppressed the biofilm formation by preventing the adhesion of the most common *S. aureus* and *E. coli* bacterial species to the surfaces. Superhydrophobic material, which has over 100% biocompatibility in vitro, also exhibited high hemocompatibility, giving hope for its applicability to a number of in-body medical device interfaces such as catheters, artificial vessels, and implants.**

Keywords—antibiofouling; blood repellent; biocompatible; superhydrophobic; coatings.

I. INTRODUCTION

When medical device interfaces come into contact with blood, they pose a threat by triggering platelet adhesion and aggregation or undesirable activation of other bioactive components such as coagulation factors [1]. This reaction can lead to blockages in devices directly related to blood, such as catheters, artificial vessels, blood transfusion devices. More importantly, early or late blood clots that may occur in the body due to medical devices can cause embolization, inflammation, serious tissue damage and even death [2]. Anticoagulants such as heparin are often used to prevent this, but unfortunately, heparin can also cause mortality by triggering disorders such as thrombocytopenia and hyperkalemia [3]. For this reason, devices and materials that come into contact with blood are always a serious concern for patient health.

Another major concern with medical devices is pathogens. In his study, Dolan clearly demonstrated the danger by revealing biofilms originating from *Candida albicans*, coagulase-negative *Staphylococci*, *Enterococcus spp.*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* pathogens on medical devices used for health such as prostheses, central venous catheters, and heart valves [4]. These pathogens cause morbidity and mortality by transmitting into the body with the use of medical devices. For example, a contamination from the catheter first causes micro-organisms to colonize along the transcutaneous portion of the tunnel and cause skin infection. Subsequently, contamination of the intraluminal surface results in bloodstream infection, clinical sepsis, or even death if untreated [5]. Considering that infections caused by medical devices constitute approximately 20% of all infections, it is very important to protect medical equipment interfaces from pathogens.

One of the emerging approaches to overcome these problems recently is the use of superhydrophobic surfaces [6]–[9]. Superhydrophobic surfaces can delay coagulation by minimizing the contact of blood proteins and cells with their low surface energies and micro/nano structure surface roughness, and also prevent biofilm formation by preventing the adhesion of pathogens. Çelik et al. developed superhydrophobic surfaces from biocompatible materials in a study they conducted and showed that this surface is repellent (contact angle of 169° and sliding angle of 3.0°) against all blood components such as whole blood, platelet suspension, erythrocyte concentrate and fresh plasma [10]. In another study by Sahin et al., they immersed the superhydrophobic surface they developed in gram-negative *E. coli* and gram-positive *S. aureus* bacterial suspensions for 36 hours, and then presented that bacterial adhesion was prevented by electron microscope images [11]. These studies demonstrate the potential of superhydrophobic surfaces in biomedical applications.

In this study, the application of superhydrophobic coatings for medical equipment interfaces is demonstrated. Surfaces such as gloves, lancets, surgical drapes and gowns have been coated with a superhydrophobic coating, and the blood repellency and anti-biofouling properties of these surfaces have been tested in detail. The medical applicability, biocompatibility and hemocompatibility of the presented approach were demonstrated.

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II. MATERIALS AND METHODS

A. Preparition of Superhydrophobic Surface

Superhydrophobic surfaces were developed with the approach reported by Çelik et al.[12]. In order to reduce the surface energy, hydrophobic silica nanoparticles were obtained by sol-gel process. 4 g of hydrophilic silica nanoparticles (SiO_2 , Sigma-Aldrich) were dispersed in 80 mL of toluene (Sigma-Aldrich) and mixed in a magnetic stirrer for 5-6 minutes. After obtaining a dense honey-like solution, 2 mL of dodecyl trichlorosilane (Gelest Inc.) was added dropwise. Then, the dispersion, which became fluid from a honey-like viscosity, was centrifuged for 15 minutes and the gel-like layer was precipitated. After removing the supernatant, hydrophobic nanoparticles were obtained by keeping the gel-like structure in the oven for 12 hours. Afterwards, hydrophobic silica nanoparticles were dispersed in 2% ethanol (Merck). These dispersions were coated on various surfaces such as glove, lancet and paper by a spray gun with a nozzle diameter of 0.35 mm at a pressure of 4 bar from a distance of ~20 cm. After drying, the blood repellency of the superhydrophobic surfaces was characterized by measuring the contact angle and sliding angle with a goniometer (Attension, Theta Lite).

B. Anti-biofouling Test

Bacterial adhesion to surfaces was tested using gram positive *S. aureus* bacteria and gram-negative *E. coli*. 1 cm^2 of superhydrophobic material coated and untreated glass slide was immersed in 0.5 McFarland turbid bacterial suspensions (in 5 mL of Muller-Hinton broth). After 24 hours of incubation at 37 °C with 150 rpm shaking, the surfaces were retrieved. Bacterial colonization's formed on the surface were visualized with scanning electron microscope (SEM, Zeiss EVO LS10) and examined.

C. Cytotoxicity and Hemolysis Test

The cytotoxicity of the superhydrophobic material was assessed by MTT assay (following by Sezer et al.) [13] using the L929 cell line. 1 mL of complete medium was added to 10 mg, 1 mg and 0.1 mg hydrophobic silica nanoparticles, which were sterilized under UV light for 45 minutes. After vortexing for 10 seconds to increase the interaction of nanoparticles with the medium, the dispersion was incubated at 37 °C in 5% CO_2 for 24 hours. After incubation, the complete medium was filtered through a filter (0.20 µm pore diameter). Cells were incubated with this obtained extraction medium (at a density of 105 cells/well) for 24 hours. Then, 10 µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide solution was added and incubated for 3 hours. Formazan crystals were dissolved in dimethyl sulfoxide and cell viability was calculated by measuring the absorbance values (OD, optic density) at 560 nm with the following equation:

$$Cell\ Viability\ \% = ODsample/ODcontrol \times 100 \qquad (1)$$

For the hemolysis test, 100 mg of hydrophobic silica nanoparticles were added into 10 mL of PBS containing 2% erythrocyte suspension. After 1 hour of incubation at 37°C, samples were centrifuged at 4000 rpm for 3 minutes to stop hemolysis. By measuring the absorbance values of the supernatants at 545 nm, the hemolysis rate was calculated with the following equation. 2% erythrocyte-deionized water suspension was used as positive control (PC) and 2% erythrocyte-PBS suspension was used as negative control (NC).

$$Hemolysis\ Rate\ \% = (Sample - NC) / (PC - NC) \times 100 \qquad (2)$$

III. RESULT AND DISCUSSION

In this study, we evaluate the blood repellency, biofouling prevention, and cytotoxicity of superhydrophobic coatings applied to medical equipment interfaces via silane-modified silica nanoparticles. Firstly, the morphological and chemical structure of the surface produced by applying superhydrophobic coating on gloves, which is one of the most frequently used medical equipment, was evaluated by SEM and EDX (energy-dispersive X-ray spectroscopy). According to the SEM images of the surface, it is seen that the surface is covered with spherical nanostructures of 42 ± 13 nm size, quite densely (Fig. 1a-b.). This silica deposition on the surface was confirmed by elemental analysis. Detailed examination of EDX has shown that the surface is composed entirely of 46% Si and 54% O atomically, (glove)(Fig. 1c). Elemental mapping of the coating at macro scale (2000x mag.) also provided the information that the surface was composed of very dense silicon and oxygen, layer by layer (Fig. 1d). These results are an indication of good applicability of the superhydrophobic coating to the surface. The coated glove is extremely superhydrophobic with a static contact angle of 172° and a sliding angle of less than 3°(Fig. 1e). Fig. 1f shows the blood repellency of these coatings applied to medical equipment interfaces such as lancets as well as gloves (Supporting Video S1). This example demonstrates that coatings can be applied to different products for protection against blood components and pathogens.

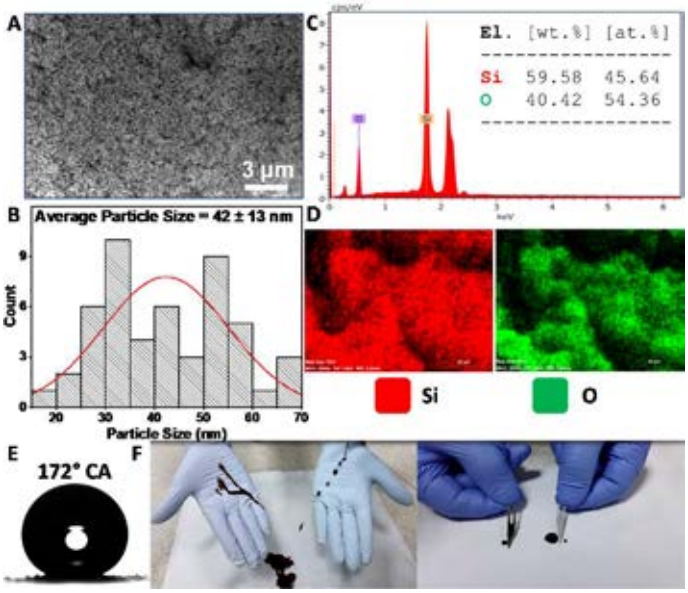

Fig. 1. Analysis of the superhydrophobic surface properties. A) SEM image of superhydrophobic glove surface. B) Particle size distribution obtained from SEM images. C) EDX elemental analysis of the surface. D) EDX mapping of silicon and oxygen on the surface. E) An image of a 5 µL water droplet on of superhydrophobic glove surface with a contact angle of 172°. F) Blood repellency of superhydrophobic coatings applied on glove and lancet. In the

pictures, the untreated surfaces on the left are contaminated with blood, while the coated surfaces on the right repel blood.

To demonstrate the usability of superhydrophobic coatings as blood repellent in various healthcare applications, we investigated their blood repellency and coagulation reduction properties by applying them on widely used materials such as paper, polystyrene, fabric and glass. Here, regardless of the substrate, the surface exhibited extreme repellency against whole blood with a 172° static contact angle (Fig. 2a). Moreover, even when the surface is held at an angle of less than 3°, blood moves away from the surface rapidly. Compared to the untreated glass surface, a drop of blood glides within 0.17 seconds on a superhydrophobic surface, while it does not slip even after 300 seconds on a normal glass surface and begins to coagulate (tilt angle 30°)(Fig. 2b). Even with continuous blood flow, the superhydrophobic glass allows flow without leaving any blood residue, while on an untreated glass surface this flow results in blood adhesion (Fig. 2c). In addition, the blood (12 µl) dropped on the surface stops after 4-5 jumping movements on the surface. More importantly, this drop of blood fixed on the superhydrophobic surface was able to remain uncoagulated for an hour at room conditions, probably as a result of not triggering coagulation activation by platelet adhesion. These results mean reducing the risk of coagulation and infection in applications involving medical devices such as artificial vessels, blood transfusion device tubing.

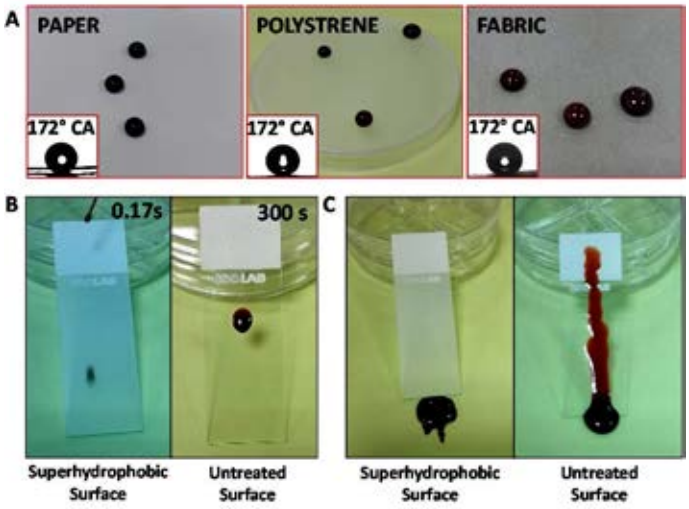

Fig. 2. Demonstration of blood repellency of the superhydrophobic surfaces. A) Picture of droplets of the whole blood on the superhydrophobic paper, polystrene and fabric surface. Comparison of superhydrophobic glass slide and untreated glass slide's B) removal rate of a drop of blood from the surface, C) self-cleaning properties against blood.

One of the advantages of superhydrophobic surfaces is the prevention of biofouling as a result of inhibition of bacterial adhesion with low surface energies. To assess antifouling capability, we immersed a superhydrophobic coated slide and uncoated slide into suspensions of gram-positive *S. aureus* and gram-negative *E. coli*. According to the SEM images at the end of 24 hours, the superhydrophobic surface does not show any bacterial adhesion and colony. It is clearly seen that especially *E. coli* bacteria form an aggressive biofilm on the uncoated surface (Fig. 3). Consequently, superhydrophobic surfaces hold great potential for suppressing bacterial adhesion, which is essential for cell and tissue integration, especially in implantation applications. This approach will prevent the attachment of pathogens that may originate from medical staff, medical equipment, the environment or even the body itself, thus increasing implant cell integration as well as minimizing post-implantation infections.

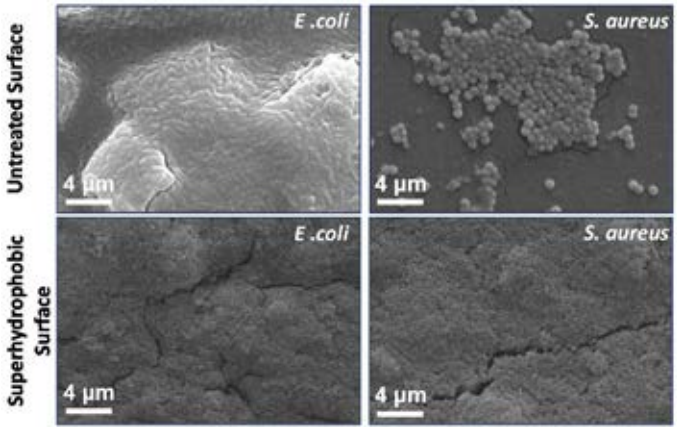

Fig. 3. Characterization of the anti-biofouling property of the surface by SEM images of the bacteria adhering to the surface. The top line shows bacteria that adhere to the untreated surface and form colonies, while the bottom line shows that the superhydrophobic surface completely inhibits bacterial adhesion.

The excellent blood-repellent and bacterial adhesion-inhibiting performance of the superhydrophobic coating makes it important for medical equipment interfaces. However, the applicability of a material is directly related to its biocompatibility and this needs to be evaluated. To this end, we evaluated the cytotoxicity of the material via the in vitro MTT assay, an expression of mitochondrial NADPH activation using the L929 cell line. Cells cultured with 10 mg, 1 mg, and 0.1 mg hydrophobic silica nanoparticle medium extraction media had cell viability of 108 ± 8 (%), 108 ± 5 (%) and 110 ± 8 (%), respectively, compared to cells cultured with complete medium without nanoparticles under the same conditions. According to the results, the material was completely biocompatible, even at high concentrations, and even triggered cell proliferation. Moreover, we evaluated the compatibility of the material with blood by the hemolysis test. Materials that are incompatible with blood cause the erythrocytes to break down and therefore hemoglobin to be released. Information about the blood compatibility of the material can be obtained by measuring the 541 nm and 576 nm peaks corresponding to the oxygen and Q band of hemoglobin [14] with Uv/Vis spectrophotometer. According to these absorbance curves seen in Figure 4b, the material has a hemocompatibility of 96 ± 0.9 at 541 nm wavelength and 95.8 ± 1.1 at 576 nm wavelength with blood.

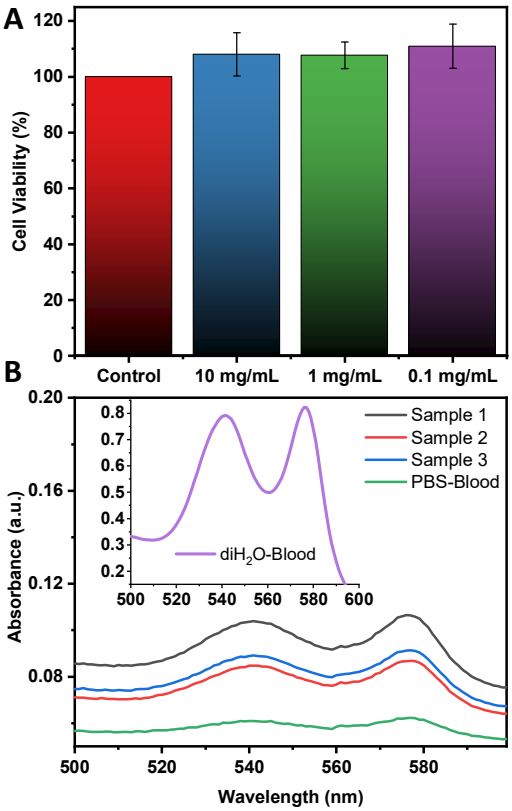

Fig. 4. Cytotoxicity and hemocompatibility characterization of the superhydrophobic coating. A. MTT results of 3 replicates of hydrophobic silica nanoparticles at 3 different concentrations, B. UV/Vis absorbance spectra of hemoglobin showing hemolysis triggered by superhydrophobic coating. Inset: Absorbance curve of the water-blood mixture positive control causing hemolysis

IV. Conclusion

This work presented practical superhydrophobic coatings for the prevention of blood clots and infections caused by medical equipment. The superhydrophobic material is prepared by modifying the hydrophilic silica nanoparticle and applied to various material interfaces via a spray gun. These superhydrophobic coated surfaces not only exhibit high blood repellency, but also exhibit anti-biofouling properties by preventing bacteria from adhering to the surface. The high biocompatibility exhibited by the material also contributes to the proliferation of cells. This makes it ideal for implant interfaces requiring cell-tissue integration. In addition, its compatibility with blood does not cause hemolysis and it is promising for the elimination of thrombogenesis and infection problems by being applied to the interfaces of intra-body medical equipment such as artificial vessels and catheters.

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