Superhydrophobic and Superhydrophilic Nanocomposite Coatings for Preventing Microbial Adhesion in Liquid Food Flow Channel

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ABSTRACT

Many recent studies have shown that both superhydrophobic (SB) and superhydrophilic (SL) coatings can minimize microbial adhesion on solid substrates; however, a comprehensive investigation of both extreme surface characteristics is not available. Therefore, the aim of this paper is to test and evaluate the rate of microbial adhesion on liquid transport system by comparing the effects of SB and SL coatings. Water contact angles on control, SB and SL surfaces were 70°, 150° and 0°, respectively. For adhesion experiments, the suspension of 3×10^8 cells/ml *Escherichia coli* K-12 was pumped through the chamber at two flow rates of 0 and 200 ml/min. Increases in flow rate and hydrodynamic force significantly inhibited the bacterial adhesion on both developed surfaces. After running for an hour, the fluorescence intensities (FIs) of adhered bacteria on SB and SL surfaces were up to 80 and 65% lower than uncoated surface, depending upon the surface wetability.

Keywords: bacterial adhesion, *Escherichia coli*, nanocomposite coating, carbon nanotube, titanium dioxide

1 INTRODUCTION

Cell adhesion onto a metal substrate is highly sensitive to the wettability and the chemical nature of the surface. Biofilm or biofouling formation on the surfaces of food processing equipment has been recognized as a widespread problem. Since bacterial adhesion (Figure 1a) is a prerequisite condition for biofilm formation (Figure 1b), prevention of bacterial adhesion on a surface will have a major impact in inhibition of subsequent biofouling [1].

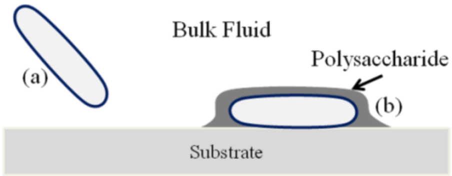

Figure 1: A schematic of bacterial adhesion onto the substrate; a) initial and reversible adhesion and b) irreversible adhesion.

There were many studies toward surfaces with extreme wettability, such as superhydrophilic (contact angle $\theta < 5°$) and superhydrophobic (contact angle $\theta > 150°$). Superhydrophobic surfaces have received more attention because of ‘lotus effect’. When the surface energy of the surface material is intrinsically low, the surface can repel any water drop that comes into contact with it. Carbon nanotubes (CNTs) provide excellent anti-fouling properties via superhydrophovicity as well as other desirable attributes; strength, flexibility, and conductivity. However, the antifouling effect of a superhydrophobic surface requires a constant presence of liquid flow, which is not always available for food thermal processor, especially when they are paused or stored [2]. On the other hand, ceramic coating such as TiO_2 nanoparticles shows the anti-fouling performance to show superhydrophilicity with water stretching reaction ready to formation of bacteria-free hydration layer around its surface. Therefore, the objective of this research was to test and evaluate the rate of microbial adhesion on liquid transport system by comparing the effects of superhydrophobic and superhydrophilic surface coating.

2 MATERIALS AND METHODS

2.1 Surface preparation

Stainless steel plates (food grade 316, 2”×5”×0.04”) were ultrasonically cleaned with acetone, ethanol and distilled water for 10 minutes, respectively. After dried in the air, plates were separated into three groups including control (uncoated steel), TiO_2 nanocomposite coating (superhydrophilic) and CNT-PTFE nanocomposite coating (superhydrophobic). The deposition of TiO_2 nanocomposites onto stainless steel surface was carried out using sol-gel method. Briefly, 20 ml ethanol was mixed with 1 ml ethyl acetoacetate at room temperature; 4 ml of tetra-n-butyl titanate was added to the mixture and kept stirring for an hour. 0.2 ml of deionized water was gently added to the solution for hydrolysis. After aging for 24 hours, prepared titanium dioxide solution was spin-coated on prepared steel plate. The wetted stainless steel surface

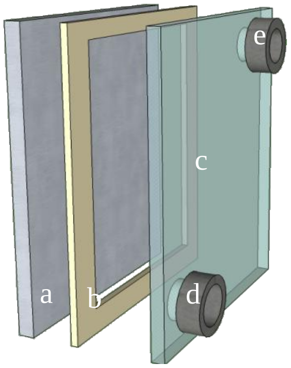

Figure 2: A schematic diagram of test rectangular flow channel; a) test plate b) rubber gasket c) front acrylic panel, d) inlet port and e) outlet port.

was heated at 150°C for 15 min to evaporate water from the surface. Dried steel plate was incubated inside a high temperature furnace at 360°C for 1 hour where the superhydrophilic feature of stainless steel plate was achieved by the oxidation process. The plate was cooled down in the air until reached room temperature before being rinsed with deionized water to wash away excessive TiO_2 particles from surface. The water contact angle (WCA) of stainless steel decreased from 70° to 0° (superhydrophilic). For superhydrophobic surface, 20% w/w of CNT-PTFE solution was prepared by ultrasonicating COOH functionalized multi-walled CNT with water-based PTFE suspension (PTFE30) for 1 hour. Mixture was then spin-coated on pre-cleaned steel plate. CNT-PTFE nanocomposites were also kept inside furnace at 360°C for 1 hour for completed annealing. The WCA value of CNT-PTFE coated stainless steel was closed to 150° (superhydrophobic). Field emission electron microscopic (FESEM) technique was used to visualize the micro/nano-scale structure of developed surface coatings.

2.2 Bacterial adhesion test

The initial concentration of *E. coli* K-12 in tryptic soy broth (TSB) mixture was 3×10^9 CFU/ml. The prepared bacteria solution was then diluted to 3×10^8 CFU/ml using phosphate buffer solution (PBS). Bacterial adhesion tests were carried out on 316 stainless steel (control), TiO_2 and CNT-PTFE treated plates. Plates were placed individually in a test parallel flow channel (Figure 2), and then 250 ml of *E. coli* solution was pumped through the test unit at the flow rates of 0 (stagnant) and 200 ml/min (continuous), separately. Adhesion times for both runs were controlled to be an hour. Subsequently, six adhered plates were gently rinsed with 1 ml of sterile deionized water in order to remove loosely attached *E. coli* from the surfaces. After plates were dried in the air, 1 drop of fluorescence stain namely 4',6-diamidino-2-phenylindole (DAPI) and a glass cover slip were placed on the surfaces in order to make *E. coli* easily observed under fluorescence microscope (Olympus BX 51, 100x, Biological Electron Microscope Facility, University of Hawaii). Corresponding fluorescence intensity of each surface was determined by ImageJ software with EdgeRatio macro plug-in.

3 RESULTS AND DISCUSSION

3.1 Surface morphology

The morphologies and micro/nanostructures of CNT-PTFE and TiO_2 nanocomposite coated surfaces were illustrated by FESEM. A FESEM image of CNT-PTFE coating (Figure 3a) insures that well-distributed CNT nanoparticles were partially anchored by PTFE layer leaving some parts uncovered by the polymer matrix. The PTFE-merging parts of CNTs significantly improved the overall wear resistance of CNT nanocomposite coating while the unconcealed parts increased the nanoscale surface roughness to characterize superhydrophobicity. However, the pore size of these voids was too small (< 0.5 microns) to accommodate *E. coli* within the entangled nanocomposite structure. For TiO_2 coated surface, it can be seen that nanocomposite coating was dense and highly uniform. Average particle sizes of TiO_2 composites were less than 0.1 microns, resulting in occurrence of many tiny voids around TiO_2 micropapilla. Therefore, these three-dimensional structures accommodated water to instantly spread onto TiO_2 coated surface due to the water surface tension and capillary effects [3].

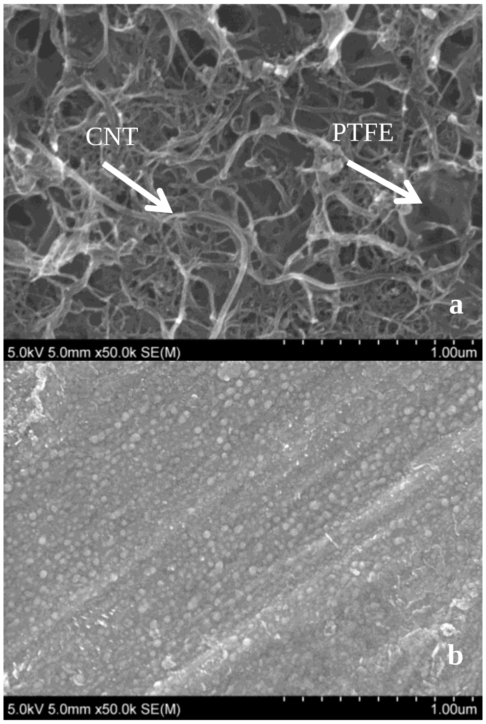

Figure 3: FESEM images of a) superhydrophobic and b) superhydrophilic surfaces.

3.2 Fluorescence images of adhered *E. coli* K-12 on steel surfaces

Figure 4 shows the number of adhered bacteria on six different surfaces including control (a and b), superhydrophilic (c and d) and superhydrophobic (e and f) surfaces. The shape and morphology of bacteria adhered on each surface were clearly seen under DAPI staining. This dye is well known as DNA binding agent on both live and death cells having the excitation and emission wavelengths in the ultraviolet range which are 358 and 461 nm, respectively. It can be noted that an increase in the flow rate significantly decreased the numbers of bacteria adhering on all surfaces. This was due to the changes in wall shear rates in the flow channel. Wall shear rate ($\dot{\gamma}$, s^{-1}) of stagnant and flow at 200 ml/min for rectangular liquid flow channel i.e., duct or parallel plates were calculated from Equation 1 as follow;

$$\dot{\gamma} = \frac{3Q}{2(h_0/2)^2 w_0} \tag{1}$$

where Q is the volumetric flow rate of bacteria suspension in m^3/s, h_0 and w_0 are height and width of cross-sectional flow path in m.

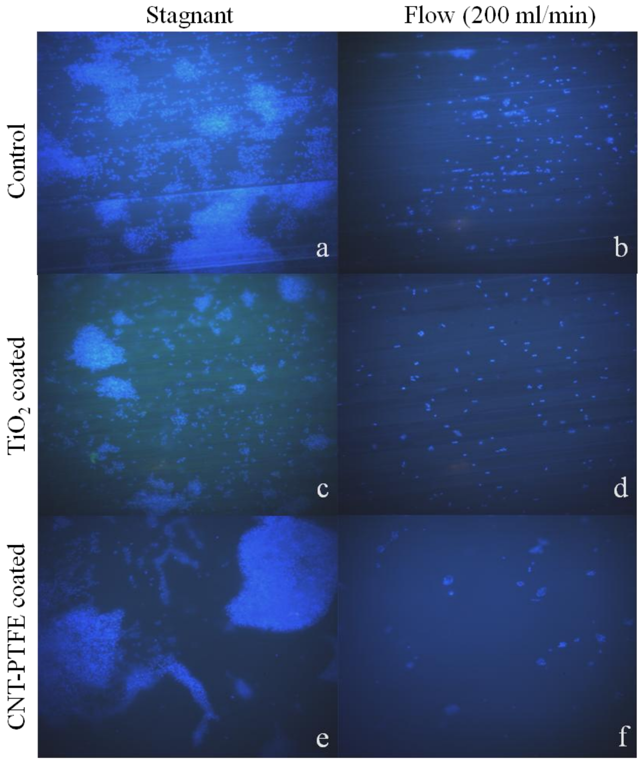

Figure 4: Microscopic images of six surfaces adhered by bacteria using fluorescent stain.

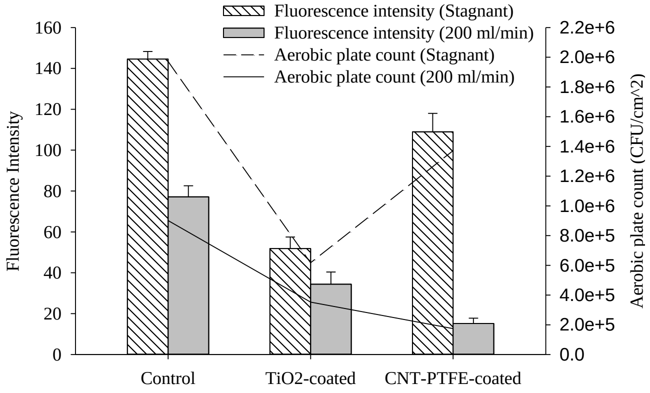

Figure 5: Fluorescence intensity and aerobic plate count results on all surfaces.

The calculated shear rates in stagnant and flow environments were 0 and 16000, respectively. Figure 5 shows the fluorescence intensity values of all adhered surfaces which seemed in a good agreement with aerobic plate count as well as the visualized results shown in Figure 4. Both figures illustrate that anti-adhesion mechanisms on superhydrophilic and superhydrophobic surfaces were significantly different from each other. According to [4], the existence of a hydration layer/shield around superhydrpphilic surface in an aqueous solution would limit the surface from interacting with other materials. Therefore, TiO_2 plate had the lowest FI value among three surfaces in the stagnant or dry environment (65% lower than control). on the other hand, the FI value for CNT-PTFE surface was 80% lower than control in a continuous mode due to little surface energy and air voids in coating matrix promoting water droplet to roll over without being absorbed.

4 CONCLUSION

The comparisons of *E. coli* layers adhered on three different rectangular liquid flow surfaces were determined in this experiment. It is clearly observed that microbial adhesion rates on metal substrate were affected by wall shear rates and surface chemistry. This study leaded our intension to development of superhydrophilic-superhydrophobic hybrid surface. This could exhibit anti-adhesion attribute in both stagnant and continuous flow environments. Especially when viscous liquid i.e., syrup or starch slurry were being transported through piping system in which an increase in flow rate is limited by nature of viscous food. In terms of consumer safety, the numbers nanotoxicology study on both CNT and TiO_2 are very limited and unclear. However, CNT-PTFE nanocomposite was developed in order to exhibit superhydrophobic attribute as well as wear resistance. Thus, CNT nanocomposite was designed to be strongly conjugated with PTFE matrix. TiO_2 nanocomposite coating has been widely utilized as protective ceramic shield on metal substrates for decades. In addition, the coating layer of TiO_2 was micronthin containing very low amounts of the nanocomposite which might be removed by human body through gastrointestinal tract without causing any toxicological concerns even if consumed [5].

REFERENCES

[1] Liu, Y. and Zhao, Q. 2005. Influence of surface energy of modified surfaces on bacterial adhesion. Biophysical Chem. 117, 39-45.
[2] Patel, P., Choi, C.K., and Meng, D.D. 2010. Superhydrophillic surfaces for antifogging and antifouling microfluidic devices. Journal of the Association for Laboratory Automation. 2, 114-119.
[3] Song, S., Jing, L., Li, S., Fu, H., Luan, Y. 2008. Superhydrophilic anatase TiO2 film with the micro- and nano-scale hierarchical surface structure. Materials Letters. 62, 3503-3505.
[4] Peng, C., Song, S., and Fort, T. 2006. Study of hydration layers near a hydrophilic surface in water through AFM imaging. Surface and Interface Analysis. 38, 975-980.
[5] Oberdörster, G., Oberdörster, E., and Oberdörster, J. 2005. Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. Environmental Health Perspectives.113, 823 – 839.