Effect of hydrophobicity of metal-based surfaces on bacterial adhesion

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The presence of bacteria on metals is considered a serious source of potential contamination for domestic and industrial environments. The possible contributing factors to the bio-film formation are related to the surface properties of materials used such as surface topography, surface chemistry and thus hydrophobicity. Hydrophobicity will be the main focus in this investigation, which is referred to the contact angle of water between the substrate surface and water surface. It is mainly dominated by two aspects; chemical modification and surface roughness. In this paper, efforts have been focused on the surface topography (roughness factor) and its coating to investigate their effects on the contact angle and bacterial attachments. Copper and other metal substrates were fabricated (mechanical machined and chemically etched) to provide different surface finish and some were deposited with a certain coating. These specimens were characterized by contact angle measurement, to investigate the relationship between surface parameters such as roughness, skewness and kurtosis and the surface hydrophobicity. The contact angle measurement on copper substrates shows a higher contact angle up to 152°. E.coli has been used to investigate the inhibition of bacteria on these substrate surfaces. Preliminary results on the effects of surface materials and their micro topographic features on the contact angle and bacterial attachment will be discussed in this paper.

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1. Introduction

The presence of bacteria on a surface is a common phenomenon in our daily life. In most cases, these bacteria are harmless and part of our natural environment. But in other cases, they are the source of contamination and food poisoning. Many R&D efforts have been made in order to make a surface anti-bacterial or self-cleaning over the past decades. In general there are two type of self-cleaning surfaces. One type is to make a surface super-hydrophobic in analogy to Lotus leave effect, as reviewed by Barthlott & Neinhuis in 1997 [1], and the other is to apply a photocatalytic coating to a surface, which was first discovered by Dr. Fujishima Akira[2]. Both types clean th emselves through the action of water, the former by rolling droplets (hydrophobic) and the latter by sheeting water (hydrophilic) that carries away dirt. The super-hydrophilic surfaces provide true meani ng of self-cleaning. For instance, coatings containing TiO_2 can abs orb UV light and react with oxygen and moisture in the air to for m activated oxygen. This helps oxidize and decompose organic de bris and gases, and kill bacteria [3]. Superhydrophobic surfaces have considerable technological potential for various applications due to their extreme water-repellent properties without the need for sunlight. In recent years, the importance of understanding and controlling the topography of surfaces for their functionality has been recognized globally [4-6]. However, it is still not very clear whether microstructures, nanostructures, or certain combinations on the surface, are required for superhydrophobicity.

Numerous studies have attempted to explain bacterial attachment as a function of bacterial properties such as cellular surface charge, hydrophobicity and outer membrane proteins amongst others [7, 8]. The effect of surface topography on bacterial attachment varies, indicating the complexity in understanding of the issues. For the time being, we consider the following two factors of the critical surface properties for materials: non-wetting (hydrophobicity) and *"surface attachment point"* (organisms smaller than the scale of the surface microstructure) [7]. Surface attachment point is mainly governed by surface roughness and the mechanical properties of the concerned surface. On the other hand, by controlling the roughness, the number of contact points can be manipulated in order to reduce the actual contact [9, 10].

Hydrophobicity is generally characterized by the static contact angle between a water droplet and an inert surface. A surface is hydrophilic if the value of the contact angle is less than 90° and the surface is hydrophobic if the value of the contact angle is greater than 90°. Surfaces with the contact angle between 150° and 180° are characterized as superhydrophobic. The contact angle is affected by several factors such as surface energy, roughness and surface cleanliness and detailed analyses can be found in above references.

In this paper, we will investigate the effect of surface topography of some common metals on the contact angle and then the bacterial attachment. Instead going down to nanometer scales, most of surfaces were made with micro features by using just ordinary machine tools. The purpose of the studies is simply to see how an ordinary metal surface used in domestic and hospital situations could be improved for their hygiene conditions. The material choice is based on the wide range industrial and bioengineering applications.

2. Materials and characterization

2.1 Substrate Preparation

In order to analyze the effect of surface topography, sample materials were prepared from sheets of metals. Copper, stainless steel (316L), titanium and aluminium samples were fabricated by cutting sheet metals into a size of 20×20×2 mm. The primary method employed for modifying the surface of the metallic samples was through the use of a mechanical milling machine.

A simple superhydrophobic copper surface was also made as a reference by reducing silver ions from a silver nitrate solution onto a copper surface through a galvanic exchange reaction [11]. Copper plates with a size of 76×26×1 mm were first polished by sandpaper with a grit of 1200. The substrate surface was then rinsed with acetone and deionized water. Silver nitrate of 0.33 g was dissolved in 100ml deionized water. The substrates were dipped into the as-prepared solution (0.020 mol/dm^3) for 10s, 30s and 60s. The substrate was then washed with a great deal of deionized water and dried in air. Finally, the substrates were dipped into ethanol solution of n-octadecanethiol (0.010 mol/dm^3) for 5 min in order to form a self-assembled film of the low surface energy material. The plates were dried in a flow of nitrogen. It is in fact this film that renders the surface of the copper sample hydrophobic. Indeed, the *thiol* head groups of the 1-octadecanethiol molecules will bond with the newly created silver nano particle surface, thus making the film stable. However, because the tail of the *thiol* groups is highly hydrophobic, this will confer similar properties to the overall coated surface.

2.2 Contact Angle Measurement

Contact angle measurements were obtained using a sessile drop method with a Drop Shape Analyzer (DSA100) manufacture by KRUSS. This apparatus consists of a sample table with up to three mobile axes or a rotating wafer table, a video system with camera, optical system, prism, light source and aperture and manual software-controlled dosing system. This instrument has a CCD video camera with a resolution of 768×576 pixels and a rate up to 50 images/s, a multiple dosing/micro-syringe (500μl) and a temperature controlled environmental chamber. The drop shape image was processed by an image analysis system, which calculated both the left- and right contact angles from the shape of the drop with an accuracy of ±0.1°. Distilled water was used as test liquid and the data for the contact angle measurement of the test liquids are given in Table 1. All measurements were made at 24℃.

2.3 Surface Topography Measurement

Surface topography measurement on the samples was carried out by using both a stylus instrument (Form TalySurf) and an interferometric profiler (WYKO NT2000). Specimens were also analyzed under a SEM for a large range inspection. 3D surface parameters are mainly obtained from WYKO NT2000. Average roughness and root-mean square roughness values are the main parameters for correlation analysis. Furthermore, two additional height distribution parameters of skewness (R_{sk}) and kurtosis (R_{ku}), are also used to provide further characteristics. Skewness (R_{sk}) is a measure of the symmetry of the curve describing the height distribution and it can discriminate between wide valleys with narrow sharp peaks and high plateaus with sharp deep valleys. Negative skewness values, often specified between -1.6 to -2.0, are used as a criterion for a good bearing surface, indicating the presence of comparatively few spikes which will wear away quickly. Meanwhile, kurtosis (R_{ku}) represents a key feature of the height distribution; the 'peakedness' of the profile. A surface with a Gaussian height distribution has a kurtosis value of 3; a surface with a narrow height distribution has a kurtosis value greater than 3; while a surface that has a well spread out height distribution has a kurtosis value of less than 3.

2.4 Bacterial Culture

The bacteria selected for the experiments is non-harmful strain of E.coli (EC100) obtained from School of Life Sciences, University of Warwick, The bacteria were sub-cultured and preserved in 15% glycerol as frozen stock at -80℃. For all adhesion tests, a single colony were streaked out with a loop from the frozen stock and grown statistically overnight in 20ml LB Broth media at 37℃ and 250 rpm. The culture was grown to mid-exponential phase since cells harvested in this state showed a better adhesion to inert surface. Then, the bacteria were harvested by centrifugation at 4000 rpm for 12 min at 22℃.

2.5 Bacterial Adhesion Test

In order to evaluate adhesion for a large number of bacteria on substrates, the concentration of bacteria attached to a surface for a fixed time period have been studied in this experiment. All experimental variables except the substrates were held constant, including cells concentration, exposure time, shaking speed, container size, and solution volume. The substrates were put in a glass container with 20ml bacterial suspension (10^8 CFU/ml) and 250ml phosphate buffered saline (PBS), and incubated at 37℃ for 4h under a gentle stirring at 90 rpm in a shaker incubator. Substrates were then removed and rinsed twice with PBS solution to remove unbounded bacteria. Each of the substrates was examined under microscopic conditions to determine the amount of attached bacterial. The next step involved preparing a 1/1000 dilution of the dye. For this, 2μl of SYTO 9 (3.34mM) dye was dissolved in 2mL of de-ionized water. The mixture was done in an eppendorf and placed on a stirrer to ensure solution homogeneity. SYTO 9 is a nucleic acid stain that can penetrate all cell walls and can be visualized with a Narrow Blue (NB) excitation93 that induces fluorescence. Eight spots of 10μl drops of dissolved dye solution were placed on top of each sample along with a glass slide to spread the liquid. Each sample was then examined under microscopic conditions and resulting images processed using the *Open Lab 4.0.2* software. The level of magnification used for observations was ×1000 as a result of combining a ×100 objective lens with ×10 ocular lens.

3. Result and Discussion

3.1 Characterization of specimen surfaces

The results of the metal-based surface topography with mechanical milling and chemical etching approach are listed in Table 1. The topography images from WYKO NT2000 are shown in Figs.1 and 2, where surface parameters of central line average R_a, root-mean-squares R_q, skewness R_{sk} and Kurtosis R_{ku} are labeled on each image. The face milled specimens have clear patterns of lines and crossed lines. From Table 1, it can be seen that the average roughness values have been increased from as-received substrates to modified substrates for those mechanical modified surfaces. For silver nitrate coated copper specimens, the coating has made a slightly smoothing effect on the surface.

In general, the mechanically modified surfaces have shown a clear change in skewness parameter; from a negative value for as-received to a positive value, while the kurtosis value has been decreased to a value around 3. Take aluminium sample for instance, Rsk changes from -1.59 to +0.43 and Rku from 8.02 to 2.17 respectively. This shows that the profiles of as-received susbtrates change from compressed profile into more protrusive profiles, which means more spikes on top of the surface after machining. Titanium, steel and copper all show this pattern for R_{sk} and R_{ku}.

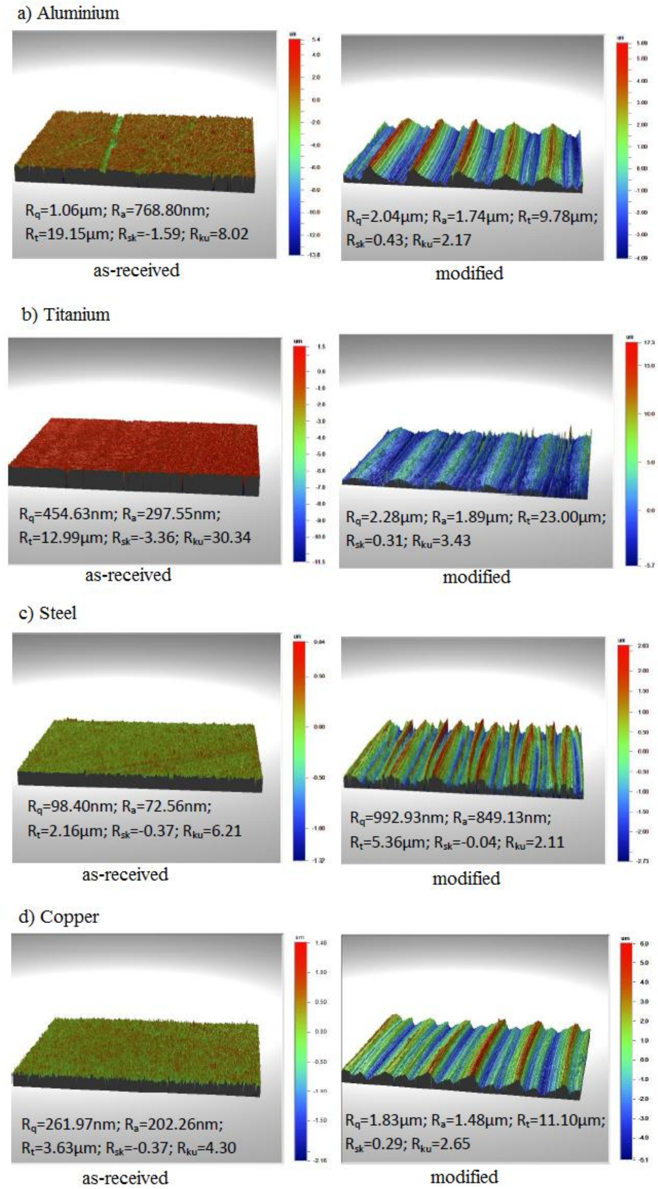


Fig. 1 Surface topography of as received and mechanically milled surfaces.

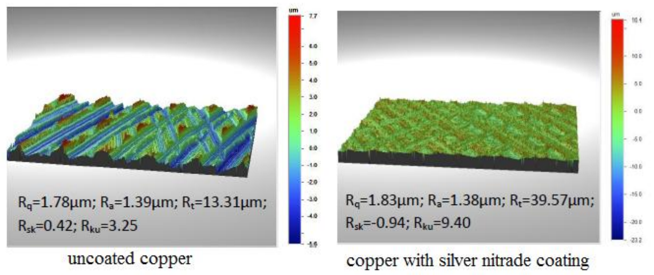
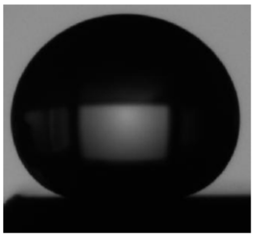


Fig. 2 Surface topography of uncoated and coated copper surfaces.

3.2 Contact Angle Measurement

The contact angle for selected materials is in an order from low to high of steel, titanium, aluminium and copper in a range of 45.7° to 75.4°. The modified surfaces all give a significant increase in contact angle with a rate 22% to 68%. This is understandable as the nature of the patterned surfaces allowed for air bubbles to get trapped underneath the water droplet like in the case of the naturally occurring lotus leaf topography.

It shows that the copper substrates with silver nitrate coating are superhydrophobic, with a water contact angle of 154.5°. The value varies slightly with the different deposition film thickness, but it is in general higher than 140°. For the contact angle, it is clearly showing the effect of higher roughness with higher contact angle either with milling machine or chemical etching techniques. Fig. 3 shows a typical image obtained from the contact angle measurement.



Left CA value: 154.7 degrees

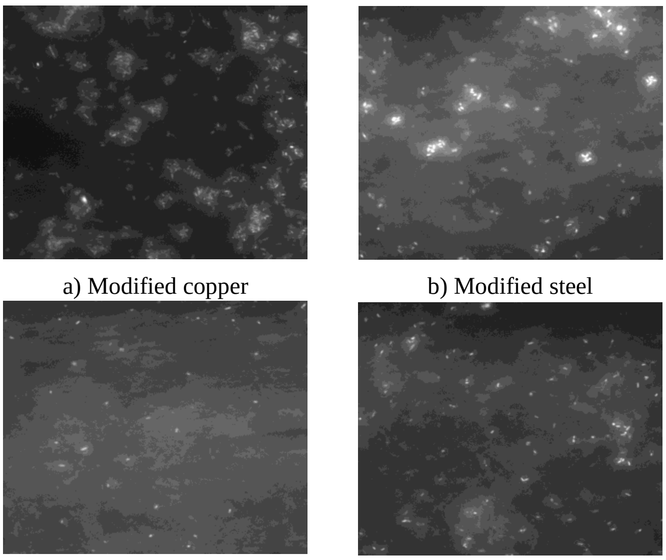
Right CA value: 154.3 degrees

Mean CA value: 154.5 ± 0.2 degrees

Fig 3 Image of contact angle measurement on coated copper.

3.3 Bacterial adhesion

Several images of both the as-received substrates and modified substrates were taken from different spots so as to possibly identify the presence of bacteria. Fig. 4 shows a few selected images that reveal interesting results. As-expected, bacteria spread out randomly across the as-received substrates. The attachment patterns of E.coli were examined by EC Plan Neufluar (Carl Zeiss). In summary, the number of cells attached to the modified surfaces was lower than those of as-received samples, indicating the effect of improved hydrophobicity. However, the copper samples, no matter coated or not, have a tendency to attract more bacteria. At the moment, we have only tested one type of bacteria, E Coli, which has a size much smaller than the topography features provided by these specimens. The patterned grooves may become niche places for bacteria to stick to.



a) Modified copper b) Modified steel

c) Modified titanium d) Modified aluminium

Fig. 4 Images of bacterial colonization on different substrate surfaces.

Substrate		Rq (root mean square) µm	Average Roughness (R_a) µm	Skewness (R_{sk})	Kurtosis (R_{ku})	Contact Angle (°)	Cells attached ×10^4 (number of cells per mm^2)
Aluminium	as-recieved	1.06	0.769	-1.59	8.02	74.9°	4.48
	modified	2.04	1.74	0.43	2.17	91.5°	2.28
Titanium	as-recieved	0.455	0.297	-3.36	30.34	59.9°	34.8
	modified	2.28	1.89	0.31	3.43	84.1°	4.8
Steel	as-received	0.098	0.073	-0.37	6.21	45.7°	38.8
	modified	0.993	0.849	-0.04	2.11	74.2°	21.2
Copper (mechanical milling)	as-received	0.262	0.202	-0.37	4.30	75.4°	54.4
	modified	1.83	1.48	0.29	2.65	92°	74.8
Copper (chemical etching)	uncoated	1.83	1.48	0.29	2.65	92°	388.4
	silver nitrate coated	1.77	1.44	0.20	5.21	154.5°	226

Table 1 Contact angle measurement, average roughness and cell attachment on substrates.

4. Conclusions

The results presented here suggest that modified surface topography even at conventional scales can affect the hydrophobicity of the surface. When analyzing surface measurement results, the more sensitive parameters are surface skewness and kurtosis. In general, to enhance the water contact angle, a surface with a negative skewness and a kurtosis less than 3 should be preferred. It is evident that the influence of large contact angle and surface topographic features on bacterial adhesion is far more complex. There needs a further investigation because it is not clear which parameter will influence bacterial adhesion on the surface at the moment. The suggestion that bacteria may be susceptible to higher contact angle and nano-scale surface roughness casts serious doubt on the conventional wisdom that smoother surfaces represent a more repellent environment to bacteria.

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REFERENCES

1. W. Barthlott and C. Neinhuis, Purity of the sacred lotus or escape from contamination in biological surfaces, *Planta*, vol. 202, no. 1, p. 1-8, 1997.
2. Fujishima, Akira and Honda, Kenichi, "Electrochemical Photolysis of Water at a Semiconductor Electrode". *Nature* 238: 37–38, 1972.
3. Y. Paz, Z. Luo, L. Rabenberg, and A Heller, "Photooxidative self-cleaning transparent titanium dioxide films on glass", J. Mater. Res., 10, 2842-2848, 1995.
4. X. Zhang, F. Shi, J. Niu, Y. Jiang and Z. Wang, Superhydrophobic surfaces: from structural control to functional application, *J. Mater. Chem.*, vol. 18, no. 6, p. 621-633, 2008.
5. D. Spori, T. Drobek, S. Zürcher, M. Ochsner, C. Sprecher, A. Mühlebach and N. Spencer, Beyond the Lotus Effect: Roughness Influences on Wetting over a Wide Surface-Energy Range, *Langmuir*, vol. 24, no. 10, p. 5411-5417, 2008.
6. M.J. Dalby, N. Gadegaard, and C.D.W. Wilkinson, The response of fibroblasts to hexagonal nanotopography fabricated by electron beam lithography, *Journal of Biomedical Materials Research Part A*, vol. 84A, no. 4, p. 973-979, 2008.
7. Mitik-Dineva N, Wang J, Truong VK, Stoddart P, Malherbe F, Crawford RJ, Ivanova EP, "Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus attachment patterns on glass surfaces with nanoscale roughness", Curr Microbiol. 58(3):268-73, 2009
8. Goulter RM, Gentle IR, Dykes GA, "Issues in determining factors influencing bacterial attachment: a review using the attachment of Escherichia coli to abiotic surfaces as an example", Lett. Appl. Microbiol. 49(1):1-7, 2009
9. B. Bhushan and Y. C. Jung, "Wetting, adhesion and friction of superhydrophobic and hydrophilic leaves and fabricated micro/nanopatterned surfaces," J. Phys.: Condens. Matter **20** (2008) 225010 (24pp).
10. B. Bhushan, "Nanotribology and nanomechanics in nano/biotechnology," Phil. Trans. R. Soc. A, vol. 366, pp. 1499 – 1537, 2008.
11. Xianghui Xu, Zhaohua Zhang and Jin Yang, "Fabrication of Bio mimetic Superhydrophobic Surface on Engineering Materials by a Simple Electroless Galvanic Deposition Method," Langmuir, 26(5), pp 3654–3658, 2010.