Controlling Microbial Infection by Submicron Textured Surfaces

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Introduction. Implant infection is one of the most frequent and severe complications associated with the use of biomaterials in medical devices, causing significant mortality and morbidity. Topographic surface modification with micro- or nano-sized features has been an effective approach to inhibit bacterial adhesion and biofilm formation, thereby reducing implant induced microbial infection. A variety of nano-/micro- structured surfaces have been created, however, the results are often conflicted in literature. This study designed and created a series of textured polyurethane surfaces with submicron pillars having diameter and spacing less than the dimensions of a single bacterium, ensuring a reduction in contact area for bacterial interaction and inhibiting bacterial adhesion (Fig. 1). Through a series of surface characterization and bacterial adhesion assessments, this study seeks to provide fundamental rationale for improved design of these type surfaces in controlling bacterial adhesion.

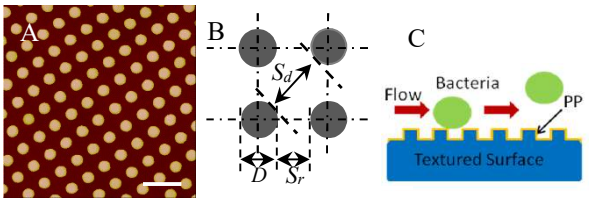

Fig. 1 Design of surface topography, (A) AFM topography image (scale bar = 2 um) of pattern 500/500/600 nm, (B) pillar distribution, S_r =distance at row direction, S_d = distance at diagonal direction, (C) schematic diagram for bacterial adhesion inhibition, PP= plasma proteins.

Materials and Methods. Pillar features on textured surfaces include shape, size, height, and separation distances (S_r and S_d in Fig. 1B). Two shapes of pillars with different dimensions were designed and created on Si wafers by e-beam lithograhy technique: 1) square pillars (500/300/500, 700/700/300, and 500/500/600 nm) (pillar side length/pillar distance between side to side/pillar height), 2) round pillars (400/400/600 and 500/500/600 nm). Polyurethane (PU) MS/0.4 was used as substrate and textured film surfaces were prepared using soft lithography two-stage replication molding technique.[1,2] Textured surfaces were characterized by atomic force microscopy (AFM) and Electron Microscopy (EM), and images were analyzed by Image J software. Surface wettability was measured as water contact angle. Polymer films were cut into round piectes for evaluations of bacterial adhesion using *Staphylococcal epidermidis* RP62A, was was measured at 37°C for 1 h in a petrie dishwith shaking at 90 rpm. Bacteria adhered on surfaces were fixed and stained, and examined by a fluorecent microscopy for counting bacterial cells adhered and looking at clustering.

Results and Discussion.

Characterization of textured PU surfaces. Surface topography of textured PU films was characterized by AFM and EM. Results showed the two-stage replication process faithfully created the textured patterns on PU surfaces with the dimensions of pillars in the range of sizes that were designed. All dimensions were less than 1000 nm including the spaces between pillars in all lateral directions, as expected. The surface texturing reduced the top surface contact area for bacterial interaction. The pattern 500/300/500 nm produced the highest pillar surface area fraction at 36.6% while the pattern 400/400/600 nm generated the smallest area fraction to 24.8% (Table 1). The PU smooth surface is hydrophobic with water contact angle about 92.8°. Surface texturing dramatically increased hydrophobicity due to the air trapped between pillars, with the pattern 400/400/600 nm having the highest hydrophobicity (Fig. 2).

Table 1. Pillar features of textured submicron patterns (mean ± SD).

Pattern (D/S_r/H) (nm) Pillar shape	Pillar height (nm)	Distance in row (S_r) (nm)	Distance in diagonal (S_d) (nm)	Pillar top surface area (μm^2)	Surface area fraction (%)
500/300/500 Square (S)	467.7 ±10.2	319.5 ±21.7	576.5 ±17.2	0.243 ±0.018	36.6
700/700/300 Square (S)	307.6 ±13.2	622.5 ±21.7	1012.7 ±25.9	0.478 ±0.045	29.1
500/500/600 Square (S)	565.8 ±21.1	481.6 ±23.5	799.6 ±32.5	0.291 ±0.029	28.9
400/400/600 Round (R)	**585.6 ±18.5**	**374.7 ±20.5**	**677.2 ±20.4**	**0.155 ±0.010**	**24.8**
500/500/600 Round (R)	601.8 ±9.8	453.8 ±22.2	850.6 ±37.2	0.279 ±0.030	26.7
Smooth	--	--	--	--	100.0

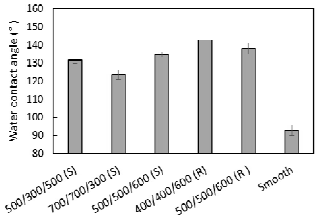

Fig. 2. Water contact angle of textured PU surfaces.

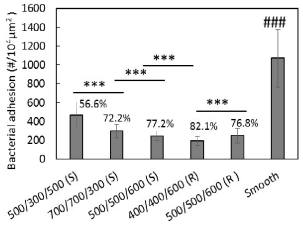

Fig. 3. S. epidermidis adhesion on textured PU surfaces, ***: p<0.001, comparing between two patterns; ###: p<0.001, comparing between smooth and all patterns.

Bacterial adhesion on textured PU surfaces. *S. epidermidis* adhesion was 1072±308 per 10^4 um^2 on smooth surfaces, significantly higher than all textured surfaces. 400/400/600 nm patterns had smallest pillar surface area fraction and highest hydrophobicity, producing lowest adhesion at 82.1% reduction, while the pattern 500/300/500 nm produced highest adhesion (56.6%), suggesting surface contact area and surface hydrophobicity are key parameters in inhibiting bacterial adhesion. Results suggest 2 primary parameters for design of textured surfaces for inhibition of bacterial adhesion; minimizing pillar top surface area fraction and maximize hydrophobicity. In addition, patterns of 500/500/600 nm that were either square or round had similar adhesion (p>0.05), suggesting pillar shape has no significant effect on bacterial adhesion. This will need to be confirmed with additional dimension pillars

Conclusion.

Submicron textured surfaces with reduced contact areas for bacterial interaction significantly inhibit bacterial adhesion. Minimizing pillar top surface area fraction while maximizing hydrophobicity appear to be 2 parameters for further design of textured surfaces to control microbial adhesion and infection.

Acknowledgement.

5 R21AI139706-02, 1R01HL153231-01.

References.

1. Xu & Siedlecki, Acta Biomaterialia. 2012, 8, 72-81.
2. Xu & Siedlecki, Biomedical Materials, 2014, 9(3), 035003.