ANTIBACTERIAL SURFACE WITH CYLINDRICAL NANOSHEEL ARRAY

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ABSTRACT

In this paper, a novel nanostructure that shows excellent antibacterial property against living microorganisms is presented. A fabrication process of the novel nanostructure termed nanoshell array using spacer lithography is introduced. The proposed structure has extremely small solid/liquid area interface compared to other conventional well-ordered micro or nanostructures such as pillar-like arrays. The structural property of nanoshell array to have intrinsic low solid/liquid area interface reduces the bacteria binding sites, hence preventing the bacteria cells from forming a colony. As a result, nanoshell array showed superior antibacterial property against *Escherichia coli* (*E.coli*) cells compared to the smooth surface.

KEYWORDS: Spacer Lithography, Nanoshell Array, Antibacterial Surface, Escherichia Coli

INTRODUCTION

Prevention of bacteria colonization and sterilization on a surface is important in several fields, e.g. food manufacturing utilities, laboratories dealing with human cell cultures, and biomedical devices. Studies have been made to physically modify surfaces by forming micro [1] or nanostructures [2], hence influence the interactions between the surface and the bacteria that come into contact with it. However, these structured surfaces are not optimized in reducing the bacteria binding sites, which is critical in antibacterial surface [3].

In this paper, we report a novel nanostructure, termed cylindrical nanoshell array, which by its geometrical merit shows excellent antibacterial property. The experiment was carried on with *E.coli* BL21 cells, where nanoshell array surface showed superior bacteria repellent behavior compared to the smooth surface.

EXPERIMENTAL

The fabrication process of the cylindrical poly-Si nanoshell array, which uses sub-lithographic patterning technique known as "spacer lithography", is schematically shown in Figure 1. First, an array of oxide pillars was delineated on the silicon wafer by conventional photolithography and reactive ion etching (RIE). Then, 80nm of poly-Si was deposited by low-pressure chemical vapor deposition (LPCVD) on the oxide pillar array. The poly-Si was then etched by RIE with a target of 80nm to reveal the top surface of the oxide pillars. Finally, the oxide pillars left inside the poly-Si nanoshell array were removed by HF (or buffered oxide etchant) so as to obtain nanoshell array as shown in Figure 2. The height, diameter, thickness and pitch of the fabricated nanoshell array were 2μ m, 1μ m, 30nm, and 3μ m respectively. It is worth-while to note that the poly-Si sidewall thickness can be controlled with a nanometer scale precisely by virtue of the aforementioned LPCVD process.



Figure 1. Fabrication process flow of cylindrical nanoshell array. (a) Formation of oxide pillars, (b) deposition of poly-Si, (c) RIE of poly-Si, and (d) removal of oxide pillars by wet etching.



Figure 2. SEM image of nanoshell array from the (a) tilted view, (b) top view, and (c) magnified top view.

For the preparation of bacteria suspension, *E.coli* were grown in LB medium for 12 hours. By the serial dilution method, cell concentration was measured as 4.1×10^8 CFU/mL. Nanoshell array surface and smooth surface samples were immersed in the *E.coli* suspension of 20mL and then incubated at 37°C for 90 minutes, as illustrated in Figure 3. Thereafter, it was rinsed with phosphate buffer solutions (PBS) and then was soaked in a mixture of 2.5% glutaraldehyde and PBS solution for 12 hours to fix bacteria on the surface.



Figure 3. Schematic showing experimental procedure of E.coli incubation on smooth surface and nanoshell array surface.

RESULTS AND DISCUSSION

As shown in Figure 4, each *E.coli* cell is more likely to exist as a form of group on a smooth surface than on nanoshell array surface, where *E.coli* cells exist individually. In Figure 4(a), it is clear that adjacent *E.coli* cells are intertwined together. On the other hand, as in Figure 4(b), *E.coli* cells on nanoshell array surface are sparsely located from one another. Due to the reduction in bacteria binding sites, nanoshell array surface showed better immunity against the aggregation of *E.coli* than the smooth surface. It indicates that certain surface morphology can be harsh for settlement and proliferation of *E.coli* colony on the surface. Considering that nanoshell has extremely small binding sites (due to its nanoscale thickness), it is certainly not a favorable surface for any bacteria to proliferate on it.



Figure 4. SEM images of (a) smooth surface and (b) nanoshell array surface after immersed in the *E.coli* suspension for 90 minutes.

(a) Before immersion Smooth surface Nanoshell array surface 2mm (b) After immersion



Figure 5. Photograph of smooth surface and nanoshell array (a) before and (b) after immersed in bacteria suspension. Less visible spots of E.coli colony on nanoshell array surface than on smooth surface indicates bacteria repellency of nanoshell array.

As shown in Figure 5, after immersed in *E.coli* suspension, spots of *E.coli* colony were visible on the smooth surface, whereas those spots were not so much visible on nanoshell array surface. Nevertheless, nanoshell array was shown to be susceptible to the settlement of single *E.coli* cell on the surface because of its pitch size being larger than *E.coli* cell size. However, this immunity to bacteria can be enhanced if nanoshell array is properly scaled down. This work gives an insight for a possible candidate of nanostructure in realizing antibacterial surface.

CONCLUSION

The cell proliferation behavior of *E.coli* cells on nanoshell array surface was studied in comparison with the smooth surface. As a result, nanoshell array surface showed superior antibacterial property than the smooth surface. The structural advantage of nanoshell array to have reduced bacteria binding sites provides an insight for optimized structure for antibacterial surface.

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