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Hierarchically imprinted polymer substrates for enhanced attachment of *Escherichia coli*

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ABSTRACT

Escherichia coli (*E. coli*) detection is important for ensuring human health and public security. One critical step in most detection methods is to have the *E. coli* cells attach to the substrate or transducer of a biosensor before they can be detected and/or identified. In this context, a chemical or physical enhancement effect arising from the substrate will help to achieve a high sensitivity of bacterial detection. This work makes use of hierarchically imprinted surface structures to demonstrate such effect using quartz crystal microbalance (QCM). Specifically, hierarchical structures are imprinted on polystyrene coated resonance crystals of QCM; such crystals, after incubation in an *E. coli* suspension of reduced concentration (1×10^4 colony forming units/mL), exhibit improved resonance frequency shifts, which are 1–2 orders of magnitude higher than those without the hierarchical structures. The enhancement effect is attributed to the enlarged surface area of the substrate and the way it immobilizes the bacteria. As revealed by scanning electron microscopy, the hierarchical substrates immobilize the *E. coli* cells by both trapping them in the micro-trenches and having them adhere to the nano-protrusions, while the single-level imprinted structures accommodate the cells mainly in the trenches or over the protrusions, instead of both.

1. Introduction

Escherichia coli (*E. coli*) are Gram-negative, pathogenic bacteria that can cause various diseases such as urinary tract infections, inflammations and peritonitis. These diseases are often correlated with direct or indirect pollution of drinking water and food products by *E. coli* Therefore, monitoring and detection of this bacterium in water and food is important for ensuring public health. *E. coli* detection can be achieved through a number of methods such as culture and colony counting [1], polymerase chain reaction [2,3], and different sensing devices including quartz crystal microbalance (QCM) [4,5], electrochemical sensors [6–8], acoustic wave sensors [9,10], and optical biosensors based on surface plasmon resonance [11,12], quantum dots [13,14] and conjugated polymer [15–17].

In a typical process of *E. coli* detection using QCM, the bacteria are first separated and pre-concentrated from a sample of drinking water or milk (1–10 colony forming units per 100 mL, or CFU/mL). After being enriched to a higher concentration (normally above 10^6 CFU/mL), the bacteria suspension is injected into the sample cell of a QCM whose resonator crystal has been surface treated with an antibody. Once the bacteria bind to the resonator surface through the antibody, a shift in the resonance frequency of QCM will be observed, and this is referred to as in situ measurement. Alternatively, one can also adopt an ex situ route, i.e. to measure the frequency of the bare QCM crystal, incubate it in the bacterial suspension, measure the frequency again after incubation and drying, and then make a comparison between the two frequencies. In both cases, bacterial affinity of the QCM crystal will influence the resulting frequency shifts. A poor affinity may result in an insignificant frequency shift that is hardly distinguishable from control or noise, since factors other than mass adsorption, including variation of surface viscoelasticity and solution density and viscosity can all result in a frequency change of the quartz crystal [18]. With these considerations, an effective strategy to improve the bacterial affinity of a biosensor substrate, or to enhance bacterial attachment to the substrate, is necessary for detection of bacteria with a high sensitivity.

Attachment of cells, either eukaryotes or prokaryotes (including *E. coli*), to a substrate like the QCM crystal involves both chemical and physical interactions between the cell and the substrate. Currently, most bacterial attachment is achieved by the use of antibodies, avidins or peptides, which are adsorbed or grafted on surface-functionalized substrates or transducers [19–21,9]. For the purpose of enhancing *E. coli* attachment, combined immbolization of avidin and biotinylated antibody has been adopted on a polyurethane film, which resulted in 5-fold increase in the number of *E. coli* attached in comparison with the blank polyurethane [22]. While topographical features of a substrate surface have been reported to influence attachment or immobilization of eukaryote

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cells and some bacterial species [23–25], there is rather limited work on attachment and/or detection of *E. coli* using patterned substrates. The present work will explore this aspect, and investigate the role of surface patterns in *E. coli* attachment to a biosensor substrate. Previously, we have reported a sequential nanoimprinting technique for fabrication of three-dimensional complex structures on different polymer surfaces and the applications of such structures in surface wettability tailoring [26–28]. In this work, we make use of the sequentially imprinted structures to achieve enhanced attachment of *E. coli* to polystyrene substrates. When the hierarchical structures are incorporated on the QCM crystals, the mass of bacteria immobilized is improved by 1–2 orders of magnitude as compared with the case where there is no hierarchical structure on the crystal. We also analyzed the way of *E. coli* attachment on different substrates (with or without hierarchical structures) with the aid of scanning electron microscopy (SEM).

2. Experiments

2.1. Polystyrene imprinting

Polystyrene (PS, Mw = 45,000 g/mol) films were prepared by spin coating on well cleaned, oxygen-plasma-treated glass slides, followed by baking at 150 °C for 5–10 min; the resultant films were about 2.2 μm in thickness. Two types of silicon molds (supplied by Institute of Microelectronics Singapore) were used for imprinting: 2 μm grating (1:1 duty cycle, 2 μm depth) and 250 nm grating (1:1 duty cycle, 250 nm depth). Prior to imprinting, the molds were ultrasonicated in isopropyl alcohol (IPA, 90–100%), rinsed with IPA, treated with oxygen plasma (10 sccm, 100 W, 250 m Torr for 10 min) and subsequently treated with perfluorodecyltrichlorosilane (FDTS, 96%) vapor in a desiccator under reduced pressure. Such treated molds then underwent one dummy imprinting on polycarbonate films (160 °C, 40 bar for 5 min) to get rid of any residual physisorbed FDTS molecules so that these molecules will not transfer to PS surface during imprinting and affect the subsequent bacteria experiment. Imprinting of single-level 2 μm and 250 nm gratings were carried out at 130 °C, 40 bar for 5 min. The hierarchical patterns were obtained by imprinting of 250 nm gratings at 60 °C, 30 bar for 10 min on the pre-imprinted 2 μm gratings with different alignments.

2.2. Bacteria culture and attachment

E. coli strain *DH5-α* was cultured aerobically in Luria Bertani (LB) broth overnight at 37 °C. The glass-supported, imprinted PS films were sterilized by UV irradiation for 15 min before use. Bacterial attachment experiments were performed by impregnating different PS films with the *E. coli* cultures (1×10^4 colony forming units/mL, CFU/mL) in a rocking incubator (rocking speed 100 rpm) and kept at 37 °C. The use of rocking incubator prevented sedimentation of the bacteria over time. After incubation for 4 h, the films were taken out, rinsed rigorously and copiously with phosphate buffered saline (PBS), and dried with nitrogen. The *E. coli* cells on the films were then stained by a combination dye (LIVE/DEAD *Bac*light bacteria viability kits, Molecular Probes, L13152) for fluorescence study.

2.3. Quartz crystal microbalance (QCM) study

QCM studies were performed using a MAXTEK PM 710 Plating Monitor coupled with a MPS-550 Sensor probe. The MAXTEK quartz resonators were made from AT-cut quartz crystals covered by evaporated gold on both faces. The resonators have a 5 MHz resonance frequency and a surface area of 0.316 cm^2. They were spin coated with PS films, which were subsequently imprinted with different grating patterns. In the next step, the resonators, which carry imprinted surface coatings, underwent bacterial attachment following the same procedure as that for glass-supported PS. The QCM frequencies of each resonator with nanoimprinted patterns were measured (5–10 min for frequency stabilization) before and after *E. coli* immobilization.

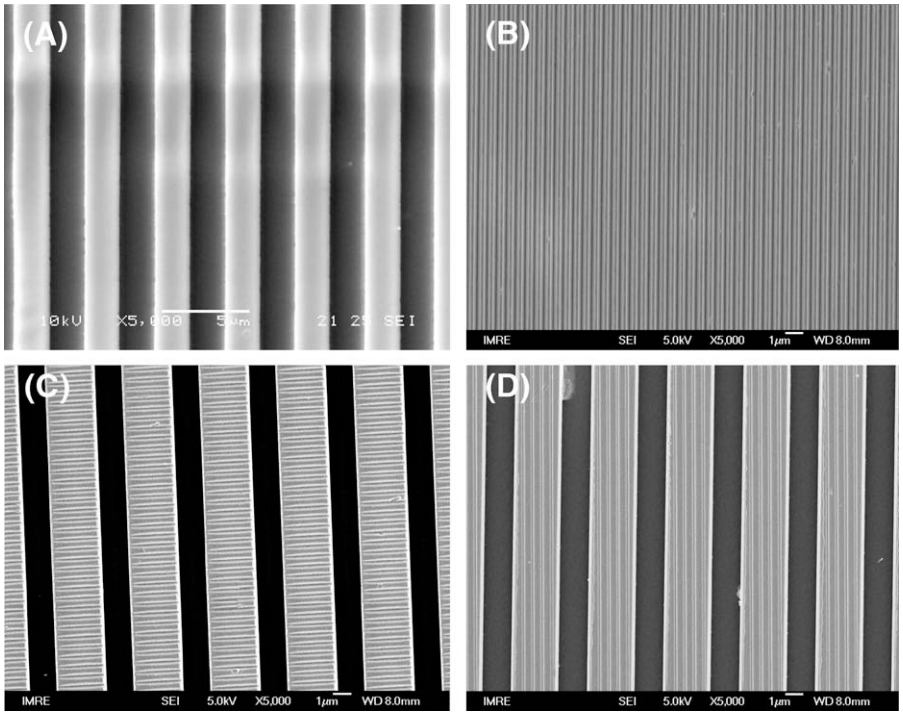

Fig. 1. Surface patterns created on the PS film by nanoimprinting: (A) 2 μm grating, (B) 250 nm grating, (C) 250 nm on 2 μm grating with perpendicular alignment (or 2 μm ⊥ 250 nm), (D) 250 nm on 2 μm grating with parallel alignment (or 2 μm ∥ 250 nm).