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Chitosan surface enhances the mobility, cytoplasm spreading, and phagocytosis of macrophages

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ABSTRACT

A chitosan micropattern was prepared on glass by inkjet printing to visualize and compare in real-time macrophage developments on chitosan versus glass during microfluidic culture. The mobility of macrophages on chitosan was significantly higher, since the cells on glass were anchored by the development of podosomes whereas those on chitosan did not form podosomes. The phagocytosis of bacteria by macrophages was considerably more effective on chitosan because of: (1) the macrophages' higher mobility to scavenge nearby bacteria and (2) their cytoplasm's ability to spread, re-distribute, and recover more freely to engulf the bacteria. Consequently, bacteria growth on chitosan surface was significantly reduced in the presence of macrophages in comparison to that on glass surface, as measured by surface bacteria density and effluent bacteria concentration. These findings suggest the synergistic effect of chitosan as a potential coating material on biomedical implants in promoting macrophage response upon the arrival of opportunistic bacteria.

1. Introduction

Chitosan is a non-toxic, biocompatible and biodegradable polymer derived from deacetylation of chitin. Coatings, nanofiber meshes, and porous sponge-like scaffolds made of chitosan and chitosan composites have been investigated for their ability to promote cell growth and tissue formation in tissue regeneration applications [1–3]. It is known that both the degradation rate of chitosan and the adhesion behavior of tissue cells on chitosan surface can be controlled by the degree of acetylation [4]. Also, drug-loading properties of chitosan-based materials have been explored for applications including growth factor delivery, controlled release of antibiotics, and cancer therapy. In comparison to other synthetic polymer materials, cross-linked chitosan is known to exhibit steady drug elution behavior [5].

Another important application of chitosan involves its ability to kill bacteria when it is dissolved in solutions [6,7]. One possible mechanism for the bactericidal effect has been postulated to be electrostatic attraction between positively charged chitosan polymer chains and negatively charged bacterial cell membrane, which can cause the leakage of intracellular constituents of bacteria [7]. However, a recent study [8] suggests that chitosan in an immobilized form does not exhibit the bactericidal effect based on the following observations: (1) the surface of a chitosan thin-film, prepared by solution-casting, does not inhibit the growth of *Staphylococcus epidermidis* and *Staphylococcus aureus* and (2) the same chitosan dissolved in an acetic acid solution inhibits the growth of the bacteria by more than 90%. Although chitosan surface in its immobilized form may not be intrinsically antibacterial, we postulate that there may be another important ability of chitosan to function as the surface of biomedical implants to enhance host immune response and therefore help prevent bacterial infection of the implants. The present study is particularly motivated by the possibility of chitosan surface to promote macrophage response and development upon the arrival of opportunistic bacteria at the surface.

It has been well established that early stage bacterial infection of biomedical implants occurs because a small number of bacteria such as *S. aureus* and *S. epidermidis* adhere to the implant surface and form antibiotic-resistant biofilm colonies [9,10]. Macrophages, as the most abundant immune cells in our body, rapidly respond and infiltrate to wound sites and therefore to the surface of implanted biomaterials to clean dead tissue debris and engulf pathogens such as bacteria via phagocytosis [11–13]. The engulfed bacteria are not necessarily killed by the macrophages, but are further attacked by coordinated actions with other immune cells and antibodies for complete killing [14]. Effective phagocytosis requires: (1) macrophage mobility to scavenge nearby bacteria

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and (2) fast spreading and re-distribution of macrophage cytoplasm to engulf bacteria [15]. Previously, Fernández et al. [16] has shown that, in a 2-h culture period, the migration of and phagocytosis by macrophages were significantly enhanced on poly(ethylene)glycol (PEG)-based coating in comparison to those observed on normal glass surface. The enhanced mobility was attributed to the lack of adhesion between macrophages and PEG surface.

In this paper, we report for the first time, to our best knowledge, that chitosan thin-film can significantly enhance macrophage mobility, cytoplasm spreading and retraction, and phagocytosis in comparison to normal glass surface. The overall approach of the investigation was to prepare a chitosan micropattern on glass surface by inkjet printing, as a means of directly visualizing and comparing difference in macrophage response on chitosan versus glass using the previously established microfluidic culture method (Fig. 1a and b) [17–19]. The inkjet printing was used to create sharp boundaries between the chitosan and glass surfaces with a lateral spatial resolution of about 50 μm (Fig. 1c).

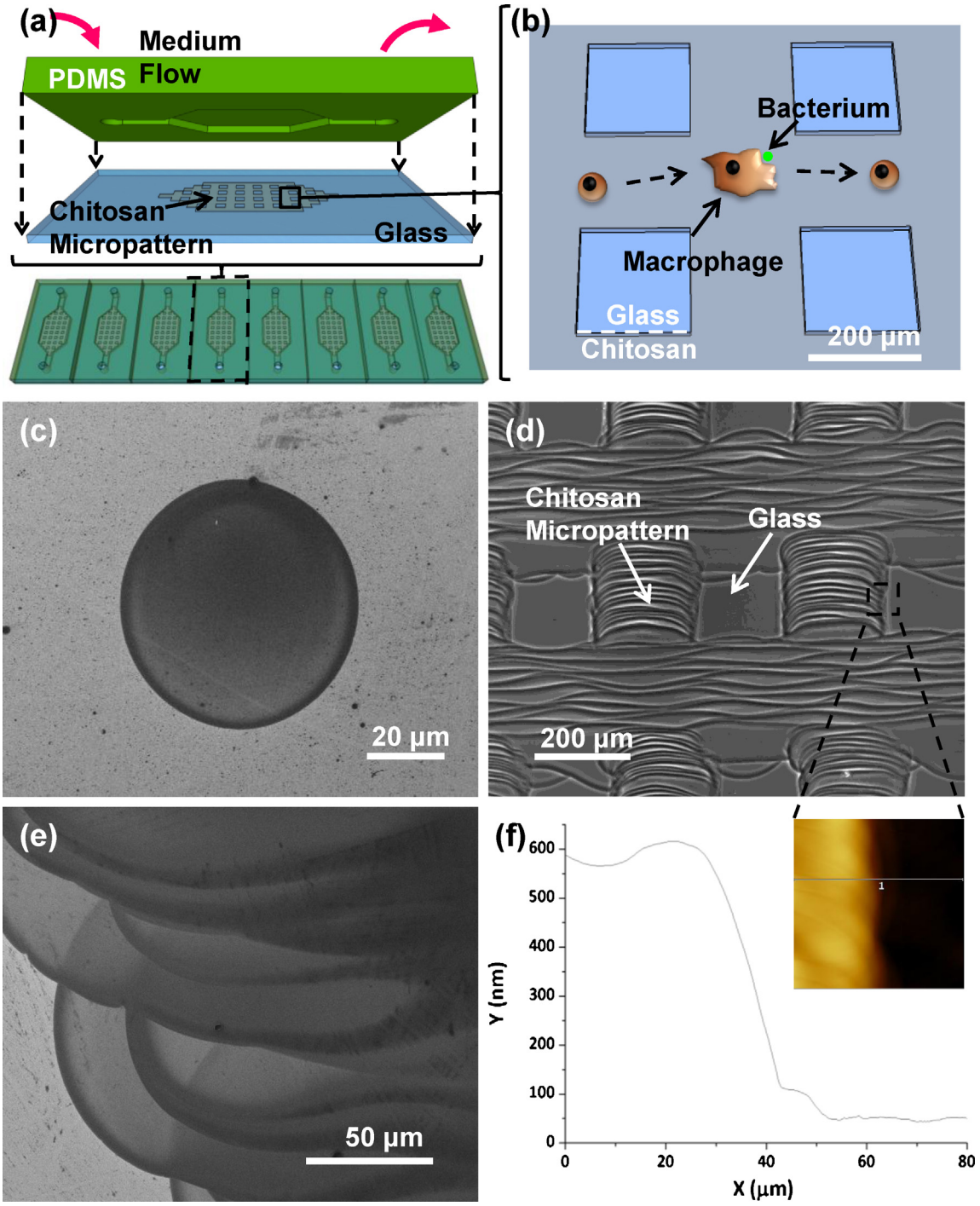

Fig. 1. Schematic illustrations of (a) the microfluidic culture device integrated with chitosan micropattern inkjet-printed on glass and (b) enhanced macrophage mobility, cytoplasm spreading, and phagocytosis on chitosan vs. glass; (c) SEM image of a single dried ink droplet; (d) optical microscope image of chitosan micropattern; (e) SEM image of micropattern showing lined morphology; and (f) profile graph from an AFM image (inset), showing the thickness of the micropattern.