Integrating Biomimetic Nanotopography and Plasmonic Photothermia for Bimodal Antibacterial Polymeric Surfaces

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Antibacterial surfaces have emerged as a promising technology in materials science and healthcare, driven by the pressing need to combat microbial infections and antibiotic resistance. As surfaces play a critical role in hosting and transmitting bacteria, the development of materials with inherent antibacterial properties has become a paramount goal. These surfaces are designed to kill or inhibit the growth of bacteria, and find applications in medical implants, food processing, and public spaces, offering a multifaceted approach for minimizing the risk of infections and improving overall hygiene [1]. In broad terms, various strategies have been employed to develop effective and durable antibacterial surfaces, with a focus on nanostructured surfaces (nanotopographies), chemical functionalization, and superwettable surfaces. Nanotextured surfaces incorporate micro- and nano-scale features inspired by nature (e.g., lotus leaf, shark skin, or cicada wing) that prevent microbial adhesion (anti-biofouling) and/or exhibit bactericidal activity. In contrast, chemical approaches involve surface modification with compounds or materials possessing contact-killing capacity (biocidal) or the ability to generate heat or reactive oxygen species upon light exposure (photothermal or photodynamic agents). Photothermia exploits the unique properties of plasmonic materials (e.g., gold or silver nanomaterials) or other light-absorbing compounds to convert light into heat. When exposed to visible or near-infrared (NIR) light, these materials generate localized heat, leading to bacterial cell damage or death, providing a targeted and precise way of killing bacteria within human tissues (in vivo) [1,2]. Increasingly, researchers are exploring the integration of multiple technologies to create synergistic antibacterial surfaces. In recent years, there has been a trend towards incorporating chemical and photothermal agents into bimodal surfaces [3,4]. On the other hand, combining nanotopographies with photothermia may offer great benefits by exploiting both physical disruption and thermal damage to enhance antibacterial efficacy, targeting bacteria through multiple mechanisms and reducing the likelihood of resistance development.

Herein, we explore a novel bimodal antibacterial polymeric surface that integrates biomimetic nanotopography and plasmonic photothermia. For this purpose, we utilized off-stoichiometry thiol-ene (OSTE), which is a very promising polymer due to its excellent bonding to metallic gold (due the presence of surface thiol groups), scalability, and biocompatibility [5]. A biomimetic cicada wing-inspired OSTE nanostructured surface was manufactured by UV-nanoimprint lithography (UV-NIL). Firstly, a silicon mold was created through a cost-effective nanofabrication approach based on colloidal lithography, which was then replicated in OSTE polymer via UV-NIL (**Figure 1a**). This process resulted in densely-packed arrays of OSTE nanocones, measuring ~500 nm in height, with base and cap diameters of ~400 nm and ~80 nm, respectively, and spacing between nanocones of ~300 nm. To assess the antibacterial efficacy of the nanotextured surface, we employed the Gram-negative bacterium *Pseudomonas aeruginosa*, a well-known biofilm former and common colonizer of medical devices. Scanning electron microscopy (SEM) imaging clearly showed that surface-attached bacterial cells appearing to be "sinking" between the nanocones and undergoing mechanical deformation after 30 minutes of incubation (**Figure 1b**). Subsequently, after 60 minutes, most bacteria seemed to sink further and became increasingly deformed, consistent with previous observations on other nanotextured surfaces [6,7]. Besides, confocal laser scanning microscopy (CLSM) analysis of live/dead stained cells revealed an almost continuous monolayer of live *Pseudomonas aeruginosa* cells on the flat OSTE surfaces (**Figure 1c**). In contrast, the nanostructured surfaces exhibited a lower surface cell density and a notably higher proportion of dead cells. Overall, these findings indicate that the OSTE nanotextured surface presented bactericidal properties against Gram-negative bacteria..

Furthermore, the nanostructured surface was endowed with photothermal capability. Owing to the presence of surface thiol groups, the OSTE polymer is able to reduce redox compounds, enabling the in-situ growth of gold nanostructures directly on the surface without the need for external reducing agents under mild conditions (**Figure 2a**). SEM imaging revealed the presence of gold nanostructures dispersed between the nanocones, which increased in size with the incubation time in $HAuCl_4$ solution, ranging from ~60 nm (after 2 hours) to ~150 nm (6 hours), until ~600 nm after 24 hours (**Figure 2 b and c**). Subsequently, the photothermal activity was assessed utilizing NIR laser irradiation (808 nm) and a thermographic camera. As depicted in **Figure 2d**, all surfaces exhibited photothermal response. However, the temperature increase depended on the incubation time. Surfaces subjected to shorter incubation times (2 hours) demonstrated relatively low surface heating, while the sample incubated for 6 hours exhibited the maximum temperature increase (~6°C), which is probably linked to the distinct resonance frequency of the plasmonic nanostructures as a function of their size and shape..

Following steps involve further characterization (and optimization) of the optical and photothermal properties of the plasmonic surfaces, testing the bactericidal efficacy of the photothermal surfaces , evaluating the stability of the gold nanostructures in liquid medium, as well as assessing its effectiveness against other microorganisms with diverse cell size and shape, including Gram-positive bacteria, e.g., *Staphylococcus aureus*. Lastly, chemical functionalization using cationic polyelectrolytes or other biocidal molecules will be investigated towards developing multimodal surfaces.

References

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Figures

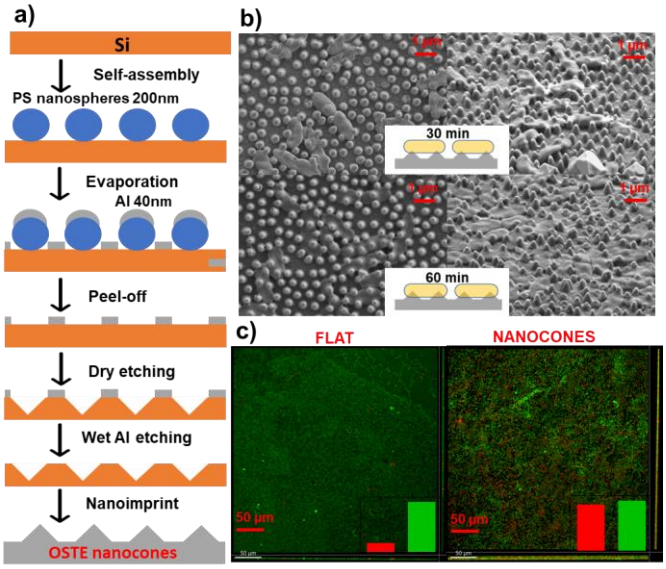

Figure 1. a) Colloidal lithography-based approach for manufacturing the OSTE nanotopography (nanocones). b) Top-view and side-view SEM images of *Pseudomonas aeruginosa* cells on the nanostructured surface after 30 and 60 min of incubation (in PBS buffer, ~5*10^8 cells/mL). c) CLSM images of *Pseudomonas aeruginosa* cells adhered to flat and nanostructured OSTE surfaces after 1 hour of incubation (in PBS buffer, ~5*10^8 cells/mL). Live cells are stained with SYTO 9 (green), while dead cells are stained with propidium iodide (red).

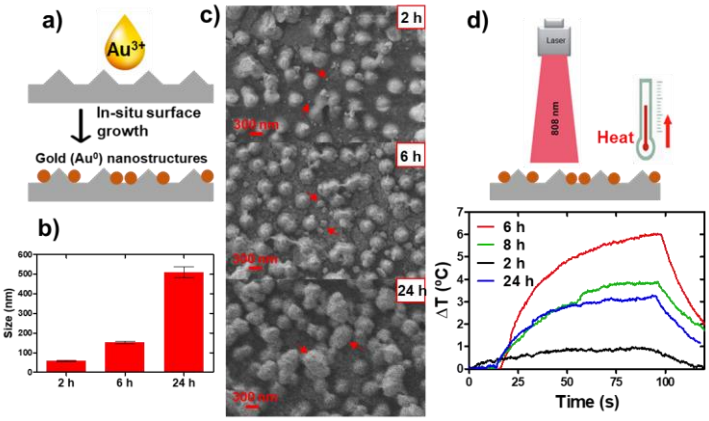

Figure 2. a) In-situ surface growth of gold nanostructures on the OSTE nanostructured surface. b) Variation in gold nanostructure sizes with incubation time at room temperature (n=20). c) Top-view SEM images of the gold nanostructures. d) Photothermal light-to-heat conversion by the plasmonic nanostructured surfaces under NIR laser irradiation (808 nm, 200 mW/cm^2, 90 s).