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Engineered Nanostructured Surfaces for Antibacterial Applications in Biomedical Devices

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INTRODUCTION & AIM

The rise of antibiotic-resistant pathogens, including *Escherichia coli*, presents a pressing global health concern, demanding innovative, non-chemical strategies to combat microbial infections. Bacteria evolved several strategies to escape from antibiotic molecules, i.e. inactivation of antibiotics, efflux pumps etc. In this framework, nanotechnologies emerged as a powerful tool against antibiotic-resistant bacteria. Nanomaterials, such metal NPs, Liposome Nanoemulsions etc., can act through different mechanisms, ultimately leading to the death of the microorganism [1].

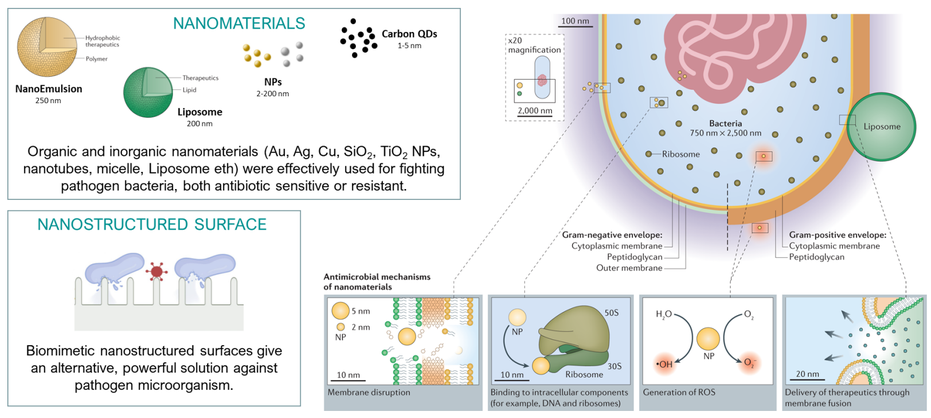

Fig.1 Nanomaterials and nanostructured surfaces as antibacterial strategies.Nanomaterials of various sizes and compositions interact with bacteria through mechanisms such as membrane disruption, targeting intracellular components, ROS generation, and therapeutic delivery. Nanostructured surfaces kill bacteria through physical deformation and membrane rupture, highlighting complementary nanoscale approaches to antimicrobial control [1].

Among these nanotechnology-based approaches, nanostructured surfaces inspired by natural bactericidal topographies offer a promising route

The proposed research aims to determine the morphological and physiological responses of nanopatterns on *E. coli* bacterial cells. In this perspective, we combined AFM analysis (both morphological and mechanical studies) with SEM for the investigation of the antimicrobial effect of synthetic polymeric nanopillars. We present a robust approach to fabricate highly controlled nanopatterns, specifically nanogratings and nanopillar arrays, on poly(methyl methacrylate) (PMMA) substrates using EBL, with pitch sizes varying from 160 to 200 nm [2].

METHOD

The fabrication and characterization workflow is summarized in the schematic illustration. Silicon substrates were thoroughly cleaned to ensure a contaminant-free surface suitable for polymer coating. A uniform film of PMMA resist was then deposited by spin coating, producing a smooth and consistent layer that serves as the active material for patterning.

Once coated, the samples were transferred to the electron beam lithography system, where the PMMA film was selectively exposed according to the desired nanoscale design. A single linear exposure produced nanograting structures, while two perpendicular exposures generated arrays of nanopillars. Following irradiation, the substrates were immersed in a developer solution to remove the exposed regions of PMMA, revealing well-defined nanofeatures that matched the programmed patterns.

The resulting nanostructured surfaces were examined using Atomic Force Microscopy to verify their geometry and surface quality. AFM scans provided high-resolution measurements of feature height, pitch, and uniformity, ensuring that both the nanopillars and nanogratings were fabricated correctly.

To evaluate the biological interaction with these structures, *E. coli* cells were deposited on the patterned substrates and incubated for adhesion. After fixation and drying, AFM was again employed—this time to investigate the bacterial response to the nanostructures. The AFM probe was used both for imaging cell morphology and for acquiring force–distance curves, enabling the assessment of mechanical properties such as stiffness and tip–cell adhesion forces. These measurements allowed visualization of bacterial deformation and damage occurring on the nanopillars and nanogratings.

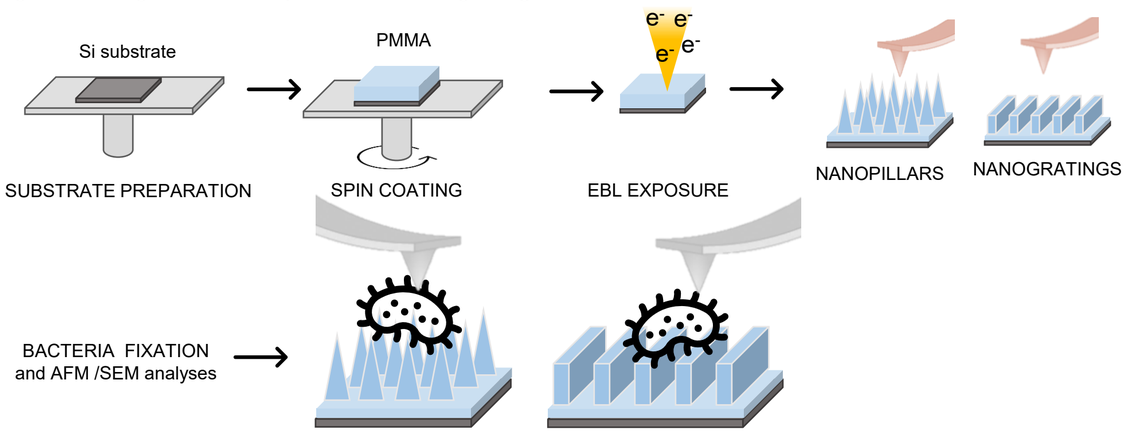

Fig.2: Schematic of the fabrication and analysis process for PMMA nanostructured surfaces. PMMA-coated silicon substrates are patterned by electron beam lithography to create nanopillars or nanogratings, then characterized by AFM. *E. coli* cells are introduced onto these surfaces, and their adhesion, morphology, and mechanical response are assessed using AFM imaging and force–spectroscopy, revealing structure-dependent bacterial deformation.

CONCLUSION

Nanostructured surfaces can physically disrupt bacteria, but their exact killing mechanisms remain unclear. To investigate this, PMMA nanogratings and nanopillars (160–200 nm pitch) were fabricated using EBL and characterized by AFM and UHR-SEM. When tested against *E. coli*, these surfaces caused clear morphological changes—altered shape, size, and increased surface roughness—along with a strong reduction in bacterial adhesion, particularly on nanopillars. Both types of nanostructures induced severe cellular damage. Mechanical measurements further showed reduced cell stiffness and higher adhesion forces with the AFM tip, suggesting that the nanostructures impose substantial mechanical stress on bacterial cells. Overall, the study provides new insight into how bacteria interact with nanoscale topographies and supports the development of more effective antibacterial materials.

RESULTS & DISCUSSION

Both PMMA nanopillar and nanogrooves arrays were fabricated by EBL, then characterized by AFM to verify the nanostructures' overall shape and morphological characteristics (Fig. 3). As shown in Fig.3, the nanostructures appear well fabricated and homogeneous, without relevant defects or damages. The analysis of the Fast Fourier Spectra confirmed the nanostructures pitches and Periodicity.

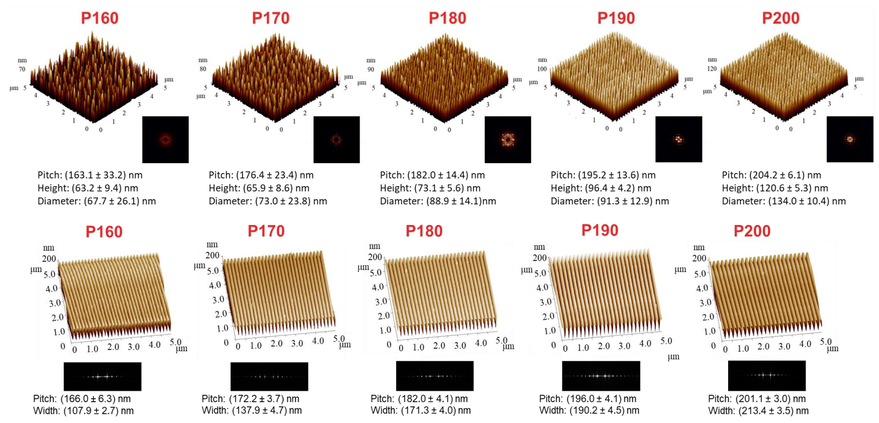

Figure 3: AFM characterization of PMMA nanograting and nanopillar arrays; the morphological characteristics are reported below each image.

E. coli number and cell morphology

Escherichia coli cells were cultured on nanopillars and nanogratings arrays with different pitches, and their morphology was *in-depth* investigated by AFM and SEM microscopy. The AFM and analysis revealed a drastic reduction in the number of cell on both Nanogratings and nanopillars array concerning the CTRL (*i.e.* flat PMMA); this phenomenon is more evident on the nanopillars array with a higher pitch (Fig.4). Moreover, the nanostructures induced damages of the bacterial cell, leading to the rupture of the cell's body and the release of the bacterial cytoplasm. This finding is more evident on cell growth on nanograting arrays with lower pitches and pillars with higher pitches (Fig. 5). Moreover, the nanostructures induced drastic variation in cell morphology (Fig.6) and E.coli surface roughness (Fig.7).

E. Coli number

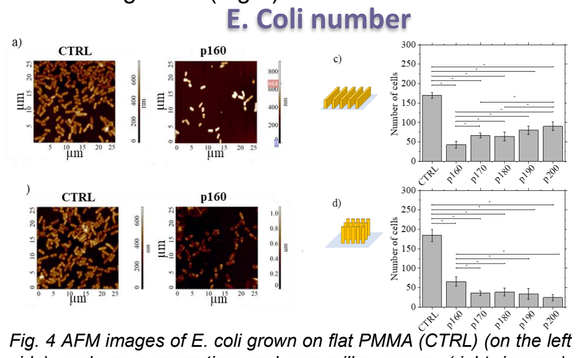

*Fig. 4 AFM images of E. coli grown on flat PMMA (CTRL) (on the left side) and on nanograting and nanopillar arrays (right images). Number of bacterial cells on c) nanograting and d) nanopillar array. The data were reported as mean value ± SD. Statistical significance is denoted by * $p < 0.05$.*

E. coli dead/damaged

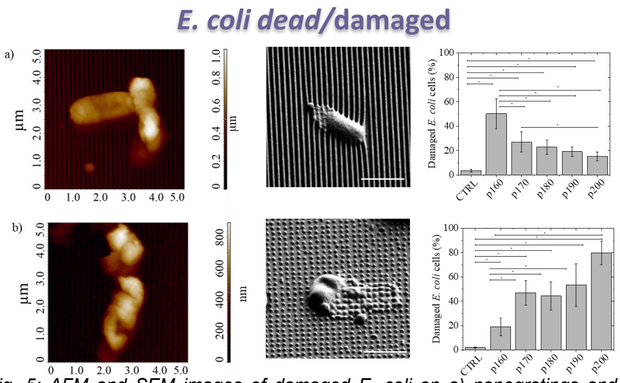

*Fig. 5: AFM and SEM images of damaged E. coli on a) nanogratings and b) nanopillars. The scale bars: 1 µm. Estimation of bactericidal activities of various nanostructured surfaces, of nanogratings and nanopillar arrays. The data were reported as mean value ±SD. Statistical significance * $p < 0.05$.*

Cell morphology

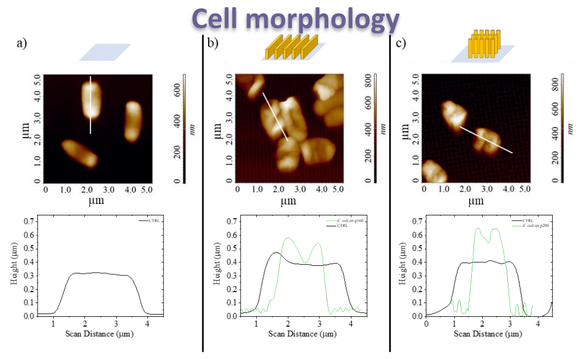

Fig.6: AFM images of *E. coli* on a) flat PMMA, b) nanogratings, and c) nanopillars. In the height profiles, black curves represent the flat PMMA control, while green curves show bacteria on the nanostructures.

E. coli Surface Roughness

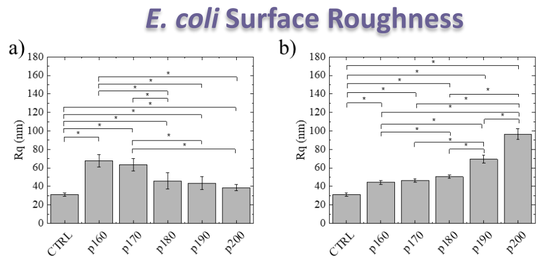

Fig. 7 Surface roughness (Rq) of *E. coli* cells measured on a) nanograting and b) nanopillar substrates. Values are presented as mean ± SD, with statistically significant differences indicated by $p < 0.05$.

NANOMECHANICS

E. coli grown on nanostructured PMMA surfaces showed major mechanical and chemical alterations compared to those on flat PMMA. Their stiffness (Young's modulus) dropped significantly, especially on nanopillars, indicating weakened cell walls and membrane damage (Fig. 7a and b). Adhesion forces between the AFM tip and bacterial surfaces increased dramatically on both nanogratings and nanopillars, a change commonly associated with disrupted membranes, loss of intracellular content, or altered surface biomolecule expression (Fig. 7c and d). Together with observed increases in surface roughness, these results suggest that nanostructured surfaces impose strong mechanical stress on bacterial cells, compromising their membrane integrity and contributing to cell death.

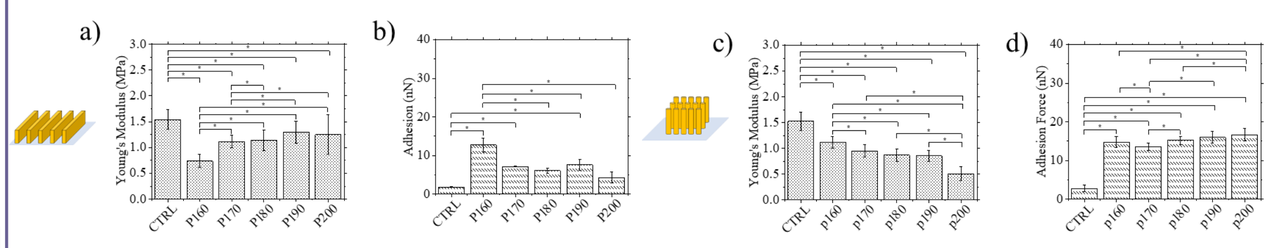

*Fig.8: AFM analysis measurements of cell elasticity and adhesion forces between AFM tip and bacteria on a) and b) nanograting; c) and d) nanopillars. The data were reported as mean value ± SD. Statistical significance is denoted by * $p < 0.05$.*

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