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## **Preparation of Microstructures and Their Integration into a Microfluidic Platform for Biofilm Assays**

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Bacterial biofilms represent complex structures formed by bacteria. Within this specific arrangement, cells communicate much more effectively and, most importantly, they exhibit significantly higher resistance to antimicrobial agents compared to their planktonic form. For this reason, it is essential to explore biofilm formation and its resistance to environmental conditions such as shear rate, as well as the antimicrobial effect of different agents. It is well established that the topography of the surface represents a key factor influencing bacterial behavior, as it can fundamentally modify processes such as cellular adhesion or bacterial morphology.

This work focuses on the design and fabrication of microstructured surfaces using laser lithography. Specifically, the research addresses the creation of two-layer structures. These structures primarily serve as a master mold for subsequent surface replication via soft lithography, which represents a crucial step toward the successful fabrication and assembly of a functional microfluidic platform.

A substantial part of the entire research is dedicated to the detailed optimization of the fabrication process parameters of microstructures. These parameters, such as exposure dose or focus correction, play a critical role, as they directly influence the final morphology, dimensional accuracy and overall quality of the prepared microstructures, which are essential for ensuring the reliability and reproducibility of future experiments.

In the initial phase, the fabricated microstructured surfaces will be subjected to testing under static conditions to evaluate their primary effect on bacterial cell behavior. The main objective of this step is to determine whether the specific surface topography inhibits the formation of microcolonies. Once these fundamental interactions between bacteria and the surface are characterized, the experimental design will advance to the dynamic phase. The microfluidic platform will be connected to syringe pumps and will be operated in a continuous flow regime. This will simulate physiological and environmental conditions more accurately, including the effect of hydrodynamic shear stress. In this setup, the dynamics of biofilm development will be monitored and evaluated in detail, with the results from the structured chip being compared against reference data from a control chip with a smooth, unstructured surface.

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