

NUCLEOTIDE NANOSTRUCTURED SURFACES TO STUDY BACTERIAL ADHESION AND BIOFILM GROWTH

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INTRODUCTION: The understanding of how surface properties influence bacterial adhesion and biofilm growth is of great importance to eventually develop materials which inhibit or stimulate bacterial colonisation. To study the fundamental aspects of biofilm growth, model surfaces are needed, with different but controlled chemical and topographical properties. Using an amphiphilic nucleotide-based diblock copolymer, we developed nanostructured surfaces. A twelve nucleotide-long sequence composed of dCMP cytosine, C, covalently bond to polybutadiene, self-assembles into vesicular structures in dilute aqueous solution. Immobilization of those nucleo-vesicles on a surface modified with the same nucleotide sequence enables the preparation of surfaces with similar chemistry but varied topographies. Bacterial biofilm growth on both flat and nanostructured surfaces was compared, in terms of total number of adherent bacteria and adherent bacteria expressing curli, a proteinic organelle of adhesion.

METHODS: Smooth surfaces were made by grafting DNA sequences onto silicon wafers and characterized as described in another poster [1]. Rough surfaces were obtained by depositing 230 nm large PB-(dCMP)₁₂ nucleo-vesicles self-assembled in PBS buffer (described and characterised in a specific work [2]) on the smooth surface obtained as described above. The properties of the rough surface were characterized by AFM and water wettability measurements.

Microbial experiments were conducted with a wild strain (PHL818) and two mutants of *E. Coli*, which does not produce curli (PHL 847) or expresses GFP when curli are produced (PHL 1273). After incubation between 1 and 168 hours in a selective M63G medium, biofilms were studied by fluorescence microscopy, using Syto9[®] (Molecular Probes) to label PHL 818 and PHL 1273 bacteria and images were analyzed statistically.

RESULTS: Characterization of the smooth surface shows that the nucleotide sequences were grafted with a brush-like conformation and formed a smooth stable layer in liquid media. Rough surfaces were also stable in aqueous medium, with

homogeneous surface chemistry and a surface density of 1 nucleo-vesicle/ μm^2 (fig.1a).

Adherent PHL 818 and PHL 847 bacteria number increased with time and were similar on smooth, rough and control (glass) surfaces. On the contrary, bacteria number expressing curli was significantly higher on nucleotide-modified surfaces (smooth and rough) compared to control surfaces (fig.1b).

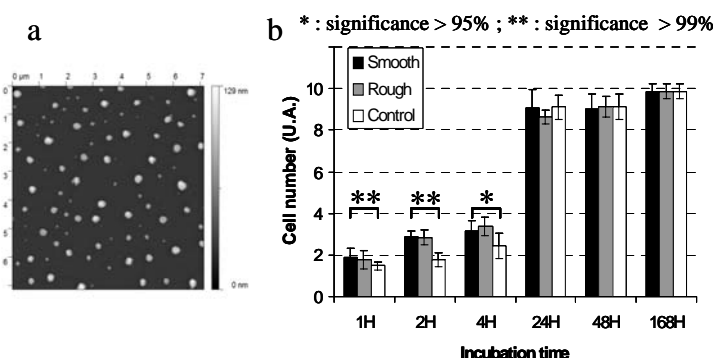


Fig. 1: a) AFM topographic image of 1 vesicle/ μm^2 PB-(dCMP)₁₂ rough surface; b) Quantity of adherent *E. Coli* PHL 1273 expressing curli.

DISCUSSION & CONCLUSIONS: This work demonstrates the relevance of PB-(dCMP)₁₂ modified surfaces to study bacterial adhesion and biofilm growth. PB-(dCMP)₁₂-based surfaces are not cytotoxic for *Escherichia Coli*. Moreover, the results show that grafted nucleotide molecules influence the expression of curli even if the topography of 1 nucleo-vesicle/ μm^2 has no impact on bacterial adhesion and biofilm growth. However, the topography can be varied by modifying the vesicle-size and density. To control the surface chemistry, the possibilities of combining the four natural nucleotides are unlimited. Eventually, specific nucleotide sequences are expected to lead to specific interactions with some bacterial components.

REFERENCES: ¹see Razumovitch et al. ² see Teixeira et al.