Anti-biofilm surfaces based on the immobilization of a novel recombinant antimicrobial protein using SAMs

Xavier R. Rodríguez
Adriana Kyvik,[1] Ramon Roca[2], Marc Martínez[2], Marta Martos[1], Jaume Veciana[1], Judith Guasch[1], Elena García-Fruitós[2], Anna Arís[2], Imma Ratera[1]
[1]*Institute of Materials Science of Barcelona (ICMAB-CSIC)/CIBER-BBN, Campus UAB, 08193 Bellaterra, Spain.*
[2] *Department of Rumiant production, Institut de Recerca I Tecnologia Agroalimentàries (IRTA), 08140 Caldes de Montbui, Spain*
xrodriguez2@icmab.es

The increasing appearance of bacteria resistant (and in many cases multiresistant) to antibiotics has become a global health emergency [1]. Far from being a phenomenon that will decrease in the coming years, it is estimated that the emergence of new resistances and the number of pan-resistant microorganisms will continue to grow reaching an increase of 67% in 2030 [2]. The antibiotic resistance is even more complicated when bacteria form biofilms. One of the strategies recently used to provide antimicrobial properties to medical devices is the immobilization of antimicrobial peptides (AMPs) on surfaces. The use of self-assembled monolayers (SAM) strategy to anchor AMPs on surfaces has been shown to be one of the best strategies for a controlled design of antibiofilm surfaces to coat medical devices [3]. SAMs are based on well-organized molecules on surfaces which are easy to be prepared and functionalized and allow a fine control at the molecular level [4]. Here, JAMF1 Host Defense Peptide (HDP), with recently proved effective antimicrcrobial activity, [5] has been successfully anchored on a model gold surface using a mixed self-assembled monolayer (SAM) based on ((1-mercapto-11-undecyl)-(tetra(ethylene glycol)) terminated SAM (PEG-SH), and nitriloacetic acid (NTA) terminated EG4-SAM (NTA-PEG-SH). The immobilized novel antimicrobial protein in its soluble and insoluble (IBs) form [6][7] on S-NTA-Ni surfaces were characterized using a multi-technique approach (XPS, immunostaining, AFM,…). The biofilm assay against *E.Coli* and *Klebsiella Pneumoniae* showed that the antimicrobial protein in both soluble and IBs forms are able to significantly reduce the biofilm formation. This strategy opens up for new possibilities for controlled biomolecule immobilization for fundamental biological studies and biotechnology applications, at the interface of materials science and biology.

REFERENCES

[1] Richard Smith, Joanna Coast, *BMJ* 2013;346:f1493
[2] Van Boeckel et al. PNAS 112 18, (2015) 5649-5654
[3] Monteiro, C., Costa, F., Pirttilä, A.M. *et al. Sci Rep* **9,** 10753 (2019).
[4] Humblot, V. et al. Biomaterials 30, (2009) 3503–3512
[5] Roca-Pinilla R, López-Cano A, Saubi C, Garcia-Fruitós E, Arís A. *Microb Cell Fact*. 2020;19(1):122.
[6] Tatkiewicz, W. I., et al., *ACS Applied Materials & Interfaces*, 10 (2018) 25779
[7] Tatkiewicz, W. I., et al., *ACS Biomaterials Science & Engineering*, 5 (2019) 5470.

FIGURES

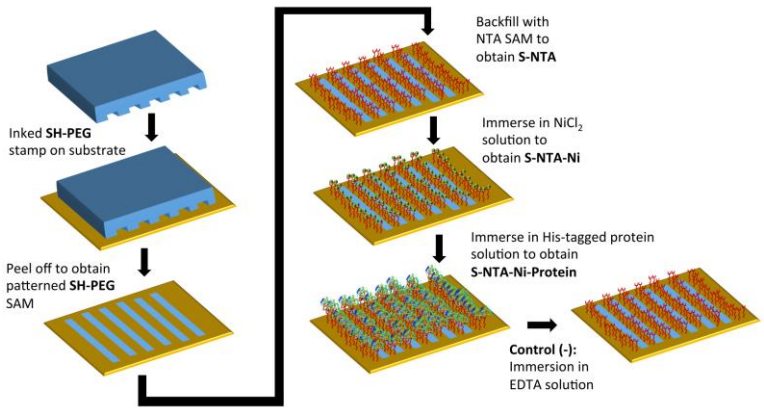

Figure 1: Schematic representation of the experimental procedure followed to prepare patterned SH-NTA and SH-PEG mixed SAMs, using the µCP technique. Subsequent protein immobilization via their His-tag termination led to the protein anchoring. Negative controls were prepared by immersing the substrates in to a EDTA solution (10 or 100 mM).