Electrical monitoring of bacterial virulence factors using nanoporous platforms

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Nanopores and nanochannels-based platforms stand out from the variety of nanostructured materials used for biosensing applications. The emerging use of arrays of solid-state nanochannels has opened the way to different and versatile sensing systems ranging from electrical to optical detection devices [1].

In this talk, a general overview on electrical biosensing systems based on the use of nanoporous membranes will be given, focusing on recent approaches for the monitoring of bacterial virulence factors secreted by bacterial pathogens [2]. Bacterial hyaluronidase (HYAL), produced by several invasive Gram-positive bacteria, is selected as a model virulence factor [3]. The analytical method is based on the electrical monitoring of the steric/electrostatic nanochannels blocking upon formation of an antibody−HYAL immunocomplex.

The developed electrochemical setup takes advantage of the flat surface of indium tin oxide/poly(ethylene terephthalate) (ITO/PET) electrodes for their assembly with nanoporous alumina membranes. The inert surface of the ITO/PET electrodes together with the anti-biofilm properties of the 20 nm pore-sized alumina membranes allows for culturing the bacteria, capturing the secreted enzymes inside the nanochannels, and removing the cells before the electrochemical measurement. Secreted HYAL at levels of 1000 UI/mL (270 U/mg) are estimated in Gram-positive *Staphylococcus aureus* cultures, whereas low levels are detected for Gram-negative *Pseudomonas aeruginosa* (used as a negative control).

The proposed method is of particular interest for the evaluation of the HYAL secretion inhibition by antimicrobial/antivirulence agents. The proof-of-concept of such application was provided by detecting *in situ* the reduced HYAL secretion upon incubation with the known anti-infective RNAIII-inhibiting peptide.

This method holds great potential for the screening of novel antimicrobial agents able to regulate secretion of HYAL and may be extended to other extracellularly produced proteins/enzymes as well. We envisage future applications for monitoring of bacterial virulence/invasion as well as for testing of novel antimicrobial/antivirulence agents.

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References

[1] A. de la Escosura-Muñiz, A. Merkoçi, (a) *ACS Nano* 6(9) (2012) 7556-7583; (b) *TRAC* 79 (2016) 134-150.
[2] W.L. Hynes, S.L. Walton, *FEMS Microbiology Letters* 183 (2000) 201-207.
[3] A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov, *ACS Appl. Mater. Interfaces* 11 (2019) 13140-13146.

Figures

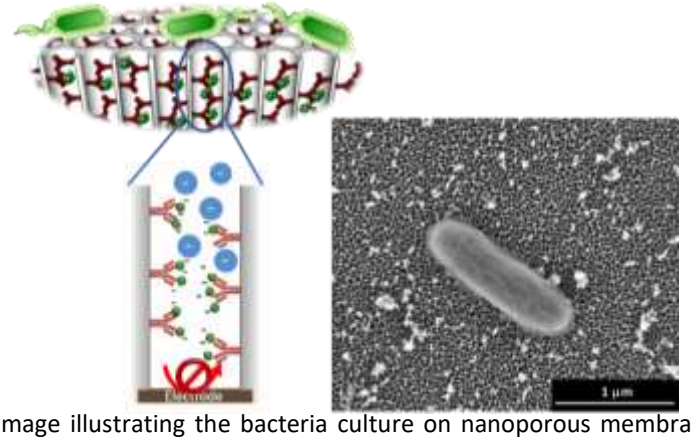

Figure 1. Scheme and HRSEM image illustrating the bacteria culture on nanoporous membranes and the continuous capturing of secreted virulence factor by antibodies inside the nanochannels, leading to their steric/electrostatic blocking.