## A first new look into the interaction of *Staphylococcus epidermidis* biofilm-released cells with the host immune system

A. França<sup>1,2</sup>, B. Pérez-Cabezas<sup>3</sup>, V. Carvalhais<sup>1</sup>, A.I. Freitas<sup>1</sup>, G. Pier<sup>2</sup>, M. Vilanova<sup>3</sup> and Cerca N<sup>1\*</sup>

<sup>1</sup> Institute of Biotechnology and Bioengineering - CEB-IBB - University of Minho, Braga, Portugal

<sup>2</sup> Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

<sup>3</sup>ICBAS – Institute of Biomedical Sciences Abel Salazar, Porto, Portugal.

\* Corresponding author: <u>nunocerca@ceb.uminho.pt</u>

The widespread application of indwelling medical devices in the clinical setting, together with the remarkable ability of the commensal *Staphylococcus epidermidis* to adhere to these surfaces and form biofilms, has given to this bacterium the recognition of being a leading causative agent of nosocomial infections [1]. Biofilms lifecycle is currently divided into 4 main steps: initial adhesion, accumulation, maturation and, disassembly [2]. Biofilm disassembly, the release of the cells within the biofilm into the involving environment, is the less understood of all steps despite its involvement in the development of several serious acute infections such as endocarditis, bacteremia and pneumonia [3]. Hence, due to its important consequences in human health and disease, the study of the cells released from *S. epidermidis* biofilms is crucial to create effective therapeutic strategies against these serious infections.

For that reason, in order to better characterize *S. epidermidis* biofilm-released cells, we assessed their cell properties by determining 1) the expression of key genes involved in initial adhesion, biofilm regulation and disassembly, 2) the total protein profile, 3) the susceptibility to routinely used antibiotics for the treatment of staphylococcal infection, and 4) the adhesion ability to coated and uncoated surfaces. Additionally, 5) the interaction of these cells with the host immune system was also assessed using an intravenous mouse infection model. Planktonic and biofilm cells were also used for comparison purposes.

Our results revealed that *S. epidermidis* biofilm-released cells share some particular features with planktonic cells, such as expression of  $psm\beta$ , but at the same time share some features similar to biofilms, such as high antibiotic tolerance. Moreover, although these shared features, the cells released from the *S. epidermidis* biofilms produced a unique protein that is not detected in the other assessed phenotypes. Additionally, *S. epidermidis* biofilm-released cells elicit a different *in vivo* response than their planktonic counterparts, by stimulating a lower production of inflammatory chemokines KC and MCP-1, and interleukine-6 as well.

Altogether, these results indicate that this particular phenotype may present some advantageous features allowing a more effective host colonization and infection spreading. Thus, the targeting of the particular properties of the biofilm-released cells could present new opportunities to more effectively prevent the pathologic events associated with dissemination of cells from a biofilm to more distant sites.

This work was funded by Fundação para a Ciência e a Tecnologia (The Foundation for Science and Technology: FCT) and COMPETE (Programa Operacional Factores de Competitividade) grants PTDC/BIA-MIC/113450/2009 and FCOMP-01-0124-FEDER-014309. AF and VC were funded by FCT fellowship SFRH/BD/62359/2009 and SFRH/BD/78235/2011, respectively.

Keywords: S. epidermidis, biofilm-related infectins, biofilm-released cells, Immune evasion

## References

- [1] Otto M. Nat.Rev.Microbiol. 2009; 7: 555-567.
- [2] Fey PD, Olson ME. Future.Microbiol. 201 ; 5: 917-933.
- [3] Boles BR, Horswill AR. Trends Microbiol. 2011 ; 19: 449-455.

7