Anti-biofilm peptide combinations against *Pseudomonas aeruginosa* and *Staphylococcus aureus*

P. Jorge[1], Daria Grzywacz[2], Wojciech Kamysz[2,3], A. Lourenço[1,4] and **M. O. Pereira[1]**

[1]CEB - Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]Lipopharm.pl, Zblewo, Poland
[3]Faculty of Pharmacy, Medical University of Gdansk, Gdansk, Poland
[4]ESEI - Escuela Superior de Ingeniería Informática, Edificio Politécnico, Universidad de Vigo, Campus Universitario As Lagoas s/n, 32004 Ourense, Spain

Today, we are facing a major challenge regarding the development of new strategies and the discovery of new compounds with effective antimicrobial outcomes. The emergence of resistance is a preoccupant health threat and conventional antibiotics are being rendered ineffective [1]. Specifically, biofilm related infections are becoming a serious threat, being highly related to chronic infections but also nosocomial and biomaterial related infections, and they are considered the major cause of dissemination of antibiotic resistance in the nosocomial scenario [2].

Researchers are now focusing in alternatives, such as the discovery of new antimicrobials with different modes of action, and the combination of agents potentiating their efficacy. AMPs are an example of new antimicrobials with promising applications, since they have different and sometimes unspecific mechanisms of action compared to traditional antibiotics, reducing the chance of acquired resistance, and are showing promising results in the biofilm area [3].

A growing interest has been emerging for the use of antimicrobial combinations as a strategy to increase the antimicrobial spectrum, prevent the emergence of resistance, reduce toxicity and side effects and provide synergistic activity. Because of this, in this work we analyse AMP combinations against major pathogenic bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, currently great contributors for resistance development and responsible for chronic infections, such as cystic fibrosis pneumonia.

We present a screening of combinations of the AMP antibiotic colistin with the AMPs temporin A, citropin 1.1 and tachyplesin I against these pathogens, including references and clinical isolated strains. Planktonic and biofilm mode of growth were implemented and results show that most combinations have addictive and synergetic activities, including total inhibition of biofilm formation for some of the combinations tested. This means that AMP combinations should be a viable way for the development of new antimicrobial treatments, thus reducing their toxicity and side effects, while maintaining efficacy.

Keywords: antimicrobial peptide combinations; synergism; *Pseudomonas aeruginosa*; *Staphylococcus aureus*

References

[1] E. K. Jagusztyn-Krynicka, A. Wyszynska, and A. Wyszyńska, "The decline of antibiotic era--new approaches for antibacterial drug discovery," *Pol J Microbiol*, vol. 57, no. 2, pp. 91–98, Jan. 2008.
[2] N. Høiby, O. Ciofu, H. K. Johansen, Z. Song, C. Moser, P. Ø. O. Jensen, S. S. Molin, M. Givskov, T. Tolker-Nielsen, T. Bjarnsholt, and N. Hoiby, "The clinical impact of bacterial biofilms," *Int J Oral Sci*, vol. 3, no. 2, pp. 55–65, Apr. 2011.
[3] P. Jorge, A. Lourenço, and M. O. Pereira, "New trends in peptide-based anti-biofilm strategies : a review of recent achievements and bioinformatic approaches," *Biofouling*, vol. 28, no. November, pp. 1033–1061, 2012.

Acknowledgements: The authors thank the project PTDC/SAU-ESA/646091/2006/FCOMP-01-0124-FEDER-007480FCT, the Strategic Project PEst-OE/EQB/LA0023/2013, the Project "BioHealth - Biotechnology and Bioengineering approaches to improve health quality", NORTE-07-0124-FEDER-000027, co-funded by the Programa Operacional Regional do Norte (ON.2 – O Novo Norte), QREN, FEDER, the project "RECI/BBB-EBI/0179/2012 - Consolidating Research Expertise and Resources on Cellular and Molecular Biotechnology at CEB/IBB", FCOMP-01-0124-FEDER-027462, and the Agrupamento INBIOMED from DXPCTSUG-FEDER unha maneira de facer Europa (2012/273). The research leading to these results has received funding from the European Union's Seventh Framework Programme FP7/REGPOT-2012-2013.1 under grant agreement n° 316265, BIOCAPS. This document reflects only the author's views and the European Union is not liable for any use that may be made of the information contained herein. The authors also acknowledge the PhD Grant of Paula Jorge, Ref. SFRH/BD/88192/2012.