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Development of an ex vivo assay for the characterization of a new elastin-like polymer with antimicrobial properties

A da Costa[1], R Machado[1], A Ribeiro[1,2], A Gomes[1], JC Rodriguez-Cabello[3,4] and M Casal[1]
[1]CBMA Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal; [2]Department of Biological Engineering, University of Minho, Braga, Portugal; [3]Bioforge Group for Advanced Materials and Nanobiotechnology, Centro I+D, Universidad de Valladolid, Valladolid, Spain; [4]Networking Research Centre on Bioengineering, Biomaterials and Nanomedicine CIBER-BBN, 47011 Valladolid, Spain

New treatment formulations for skin regeneration and wound infections have recently been the focus of research in the biomedical field, as they are one of the most common healthcare-associated infections. Antimicrobial peptides (AMPs) are a class of small molecules that can be used in the treatment of skin and wound infections as they occur as part of the innate defense mechanism in many organisms, even in microbes and virus, displaying immunomodulatory effects. With advances in protein engineering and recombinant DNA technology, it is now possible to reengineer protein-based materials with added functionality. Indeed, recombinant DNA technology allows combining in the same molecule distinct functionalities, leading to the production of a chimeric protein displaying the properties of each block of amino acids. With the aim of developing novel advanced materials and ultimately, the fabrication of advanced medical devices, hereby we describe the development, processing and characterization of a new recombinant protein-based-polymer (rPBP) with antimicrobial activity. The functional rPBP comprises a functional domain based on a synthetic cationic AMP, fused in frame with an elastin-like-polymer consisting of 200 repeats of VPAVG (A200), as structural unit.

The functional polymer, processed into free standing films by solvent cast, was analyzed for its secondary structure by circular dichroism and FT-IR and tested for their antimicrobial activity against different bacterial and fungal species, in both *ex vivo* and *in vitro* conditions. For *ex vivo* conditions, a new method was developed using pig skin as a model. When in contact with the infected skin, the functionalized polymer showed a good inhibition against the different microorganisms tested after 3 h of contact with skin, indicating that AMP::A200 films are promising candidates for application in skin treatment and wound infections. In addition, *in vitro* antimicrobial assays demonstrated that the chimeric AMP::A200 polymer is a potent antimicrobial material against a wide range of bacterial species, both gram positive and negative, as well as against yeast species. The antimicrobial activity was dependent on the time of exposition and remarkably, in some cases, almost 100% of microbial cell death was detected after 30 minutes contact with the cast films. Furthermore, the immunomodulatory effects of the AMP::A200 for wound healing and are currently underway.

Acknowledgments: This work was financially supported Portuguese funding from FEDER through POFC – COMPETE and PEst project C/BIA/UI4050/2011 (Portugal). AC and RM acknowledge FCT for SFRH/BD/75882/2011 and SFRH/BPD/86470/2012 grants, respectively.