Synthetic Protein Biotechnology approaches for the creation of antimicrobial biopolymers

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The spread of antimicrobials resistant microorganisms has triggered the search for new ways to treat infections. In the present work we explored the ABP-CM4 peptide properties from *Bombyx mori* for the creation of biopolymers with broad antimicrobial activity. An antimicrobial recombinant protein-based polymer (rPBP) was designed by cloning the DNA sequence coding for ABP-CM4 in frame with the N-terminus of the elastin-like recombinamer consisting of 200 repetitions of the pentamer VPAVG, here named A200. The new rPBP, named CM4-A200, was purified via a simplified nonchromatographic method, making use of the thermoresponsive behavior of the A200 polymer. ABP-CM4 peptide was also purified through the incorporation of a formic acid cleavage site between the peptide and the A200 sequence. In soluble state the antimicrobial activity of both CM4-A200 polymer and ABP-CM4 peptide was poorly effective. However, when the CM4-A200 polymer was processed into free-standing films high antimicrobial activity against Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi was observed. The antimicrobial activity of CM4-A200 was dependent on the physical contact of cells with the film surface. Furthermore, CM4- A200 films did not reveal a cytotoxic effect against both normal human skin fibroblasts and human keratinocytes. Finally, we have developed an optimized ex vivo assay with pig skin demonstrating the antimicrobial properties of the CM4-A200 cast films for skin applications.

Acknowledgments
This work was supported by FEDER through POFC – COMPETE by FCT through the project PEst-OE/BIA/UI4050/2014. By the Spanish Minister of Economy and Competitiveness (MAT2012-38043-C02-01) and Junta de Castilla y León-JCyL (VA152A12-2 and VA155A12-2), Spain. AC and RM, acknowledge FCT for SFRH/BD/75882/2011 and SFRH-BPD/86470/2012 grants, respectively.