Genetically engineered silk-based composite biomaterials functionalized with fibronectin type-II that promote cell adhesion

Ana Margarida Pereira[1,2,3¥*], Raul Machado1[*¥], André da Costa[1], Artur Ribeiro[1], Tony Collins[1], Andreia C. Gomes[1], Isabel Leonor[2,3], David L. Kaplan[4], Rui L. Reis[2,3], Margarida Casal[1]

¥ These authors contributed equally to this work
[1]CBMA (Centre of Molecular and Environmental Biology), Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[2]3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal
[3]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
[4]Departments of Biomedical Engineering, Chemistry and Physics, Tufts University, Medford, Massachusetts, 02155, USA

*ana.pereira@bio.uminho.pt

Recombinant protein-based polymers (rPBPs) are an emerging class of biopolymers inspired by Nature and produced by synthetic protein biotechnology approaches. Due to their exceptional physical-chemical and biological characteristics, as well as their ability to be customized for specific applications, rPBPs have been explored for the development of advanced biomaterials [1]. Within rPBPs, silk-like polymers (SLP) are being utilized in a range of studies in materials science [2]. Furthermore, advances in molecular genetics tools and recombinant protein engineering and biotechnology allow the design new bioactive rPBPs by combining active peptides/domains from different natural proteins in the same fusion protein, with precise control of their composition, polymer size and structure [2]. In this work, fully genetically engineered silk-based composites were produced by combining a functionalized spider-silk block as functional module and a silk-elastin-like protein as structural matrix. The chimeric protein 6mer+FNII, composed of a spider-silk block and fibronectin type II domain from human matrix metalloproteinase-2, was combined with a silk-elastin-like protein to produce free-standing films and assessed for their biological performance. The biological performance of the silk-based composites was significantly improved in a 6mer+FNII concentration-dependent behaviour. Our results outline the formulation of a novel class of biopolymer composites with cell adhesion properties able to support cell proliferation, and highlight the potential of using genetically engineered protein-based polymers for the development of new customized biomaterials.

[1] N. Dinjaski and D.L. Kaplan, Recombinant protein blends: silk beyond natural design. Current Opinion in Biotechnology 39, 2016, p. 1-7.
[2] Machado, R., et al., High level expression and facile purification of recombinant silk-elastin-like polymers in auto induction shake flask cultures. AMB Express, 2013. 3: p. 1-15.

ACKNOWLEDGEMENTS
This work was supported by FCT Funded Project "Chimera" (PTDC/EBB-EBI/109093/2008), by FCT/MEC through Portuguese funds (PIDDAC) – PEst-OE/BIA/UI4050/2014, by the strategic programme UID/BIA/04050/2013 (POCI-01-0145-FEDER-007569) funded by national funds through the FCT I.P. and by the ERDF through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI). TC is thankful to the FCT for its support through Investigador FCT 2015. ARibeiro thanks FCT for the SFRH\BPD\98388\2013 grant. RMachado and AdaCosta acknowledge FCT for SFRH-BPD/86470/2012 and SFRH/BD/75882/2011 grants, respectively.