

Enzymatic phosphorylation of silk fibroins: a platform for the production of biocompatible, cell-static, materials

Vadim Volkov¹, Andreia Vasconcelos¹, Marisa P Sárria^{1,2}, Andreia C Gomes², Artur Cavaco-Paulo^{1*}

¹ Centro de Engenharia Biológica (CEB), Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

² Centro de Biologia Molecular e Ambiental (CBMA), Departamento de Biologia, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Contact e-mail: vadimbiu@gmail.com

Abstract

Silks are natural protein polymers produced by insects¹. Silk heavy chain of *B.mori* is primarily composed of hydrophobic, $-(\text{Ala-Gly})_n-$ β -sheet crystalline domains³. Based on silk biocompatibility, biodegradability and strength, different materials were developed^{4, 5}. Silk offers a stabilizing environment for incorporated proteins and molecules⁶. Silk properties can be controlled via structure manipulation^{8,9}, by coupling molecules^{11,12} of biological significance; its Tyr and Ser residues can be modified^{13,14}. Once incorporated into a protein, the phosphate group establishes hydrogen bonds that affect intra- and inter-molecular interactions¹⁶. Phosphorylation is stable under physiological conditions¹⁷, thus directing the formation and reorganization of protein networks. Curiously, using phosphorylation for protein functionalization is largely unexplored¹⁴. Significant research is devoted to bio-inspired materials with various cell-differentiating²⁰ and cell-supporting^{21,22} features. However, little attention is paid to develop cell-static bio-materials. Such materials do not promote cell growth. That can be achieved by lowering the probability of cell attachment to the material, via creation of negatively charged material surface²³.

The goal of this study was to produce bio-compatible materials with the cell-static properties by phosphorylation. Silk solutions were made to cast films of variable pH and phosphorylated content. Obtained materials were tested and a dependency between amount of phosphorylation and bio-chemical properties confirmed.