Peptide block copolymers to improve silk biomaterial/hard-tissue interfaces

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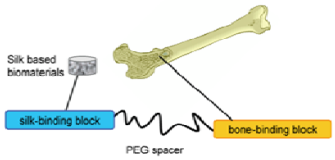
Statement of Purpose:

Silk fibroin from *Bombyx mori* is an intriguing natural fibrous protein with unique properties for a use as a biomaterial for tissue engineering and regenerative medicine [1]. The effectiveness of an implanted biomaterial depends on its integration within the body, and often selective anchoring to native tissues requires the optimization of the interface. Particularly challenging is the improvement of interface between a biomaterial and hard tissues to provide stabilization to promote integration.

The aim of this work was to develop new molecular tools for interfaces between silk biomaterials and native hard tissues. The design and self-assembly of silk-binding peptides (SBPs) with bifunctional features was exploited for non covalent interaction with bone. Novel bifunctional peptides consisting of two main domains, a silk binding domain and collagen or hydroxyapatite (HA) binding domain, separated by a PEGn linker were synthesized (Figure 1).

Figure 1. Bi-functional peptides consisting of one domain with high affinity for silk (SBP), and a bone-binding peptide (BBP) domain.



SBP-PEG-BBP diblock copolymer

Methods:

Silk fibroin was extracted by degumming *B. mori* cocoons (Tajima Shoji Co., Japan), as previously described [2]. Peptides were obtained from Biomatik, Wilmington, Delaware, USA.

Synthesis of SBP-PEG-Biotin conjugates. SBP1 was reacted with NHS-PEG_{12}-Biotin (Pierce, USA) in a 20% DMSO/borate buffer 50 mM pH 8.5 solution, overnight at room temperature (molar ratio SBP1/NHS-PEG_{12}-Biotin = 1.4). SBP2 was reacted with Biotin-PEG_{11}-Amine (Pierce, USA) in MES buffer at pH 4.7, via EDC coupling, overnight at room temperature (molar ratio EDC/SBP2 = 1; molar ratio Biotin-PEG_{11}-Amine/SBP2 = 2). The products were purified by NAP-25 desalting columns and the solutions obtained were lyophilized.

Syntheses of the SBP-PEG-BBP conjugates.

SBP1 was reacted with NHS-PEG-maleimide (Pierce, USA) in DMSO in 25% DMSO/PBS solution (NHS/NH_2molar ratio= 2) for 2 h at room temperature. SBP2 was reacted with the NH_2-PEG-Maleimide (Nanocs Inc., USA) in MES buffer at pH 4.7 via EDC coupling, for 3 h at room temperature. The intermediate SBP-PEG-maleimide products were purified by NAP-25 desalting columns pre-equilibrated with PBS/EDTA buffer, and reacted with $CGG(POG)_X$ (X = 7 or 8) or HABP in PBS/10 mM EDTA buffer pH 7.2 (theoretical thiol/maleimide molar ratio =1.5). The reactions involving ColBPs were carried out for 1 hour at 60°C, and the products were purified by dialysis against water at 65 °C for 48 hours. The reactions involving HABP were carried out for 2 hour at room temperature and the products obtained were purified by dialysis against water at room temperature 4 days.

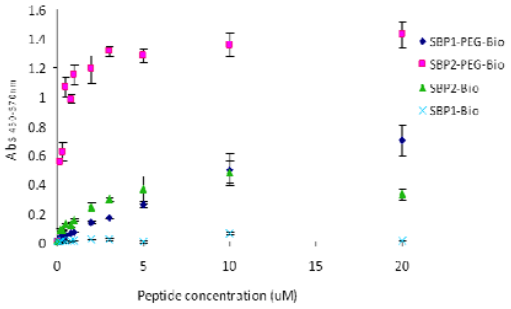
Studies of binding to silk fibroin films.

The binding of the synthesized conjugates onto silk fibroin solid surfaces was investigated, by spectrophotometric analysis. Silk films, obtained by casting 2% w/v silk aqueous solution, and methanol treatment to induce beta-sheet formation, were incubated with solutions of each SBP-PEG-Biotin or SBP-Biotin conjugates in PBST (PBS 0.1 M pH 7.4, 0.1% Tween 20), for 2 hours at 37°C. The binding was assessed as previously reported [3].

Results:

We report the synthesis of bi-functional peptides with a silk binding domain, containing the sequences GAGAGSGAGAGS (SBP1) or GLHVMHKVAPPR (SBP2), and a bone binding domain, with the sequence $CGG(POG)_x$ specific for collagen [4] or CMLPHHGAC, specific for HA binding [5]. The selected sequences bind to silk with strong affinity, with a K_D in the range 0.4 – 3.5 µM. Moreover, the conjugation with PEG improved binding to silk (Figure 2).

Figure 2. Concentration dependent binding assay of SBP-PEG-Biotin and SBP-Biotin conjugates on silk films



Conclusions:

Bifunctional peptides for simple non-covalent modification of silk materials with bone-binding peptides were synthesized. The approach described is a new approach to improve silk biomaterial-hard tissue interfaces.

References:

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