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Purification and cytotoxicity of tag-free bioengineered spider silk proteins

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Abstract: Bioengineered spider silk-like proteins can serve as biomaterials for various biomedical applications. These proteins can be assembled in several morphological forms such as films, microcapsules, spheres, fibers, gels, and scaffolds. However, crucial points for recombinant spider silks for human use are toxicity and immunogenicity. To assess this issue, two bioengineered spider silk proteins composed of different numbers of repetitive motifs of the consensus repeats from *spidroin-1* from *Nephila clavipes* (15X and 6X) were cloned and expressed in *Escherichia coli*. The proteins were free of tag sequence and were purified using two methods based on (1) thermal and (2) organic acid resistance of the spider silks. The soluble spider silk proteins were not cytotoxic and did not activate macrophages over a wide range of concentrations, except when present at the highest concentration. Films made of the different silk variants supported the growth of the cells. Based on these data, and as the biodegradation rate of silk is very slow, the bioengineered spider silks are presumed safe biomaterials for biomedical applications. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 101A: 456–464, 2013.

Key Words: spider silk, bioengineering, tag-free purification, cytotoxicity, immunogenicity

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INTRODUCTION

The unique properties of silks such as mechanical strength, toughness, biocompatibility, and biodegradability make them excellent materials for biomedical applications.[1] In nature, silks are generated in fiber form, whereas after processing *in vitro* they can be assembled into several morphological forms such as films, hydrogels, fibers, scaffolds, microcapsules, microspheres, and nanospheres.[2,3] Tissue engineering and regeneration, controlled drug release, medical imaging, and biochemical sensors are examples of potential applications for silks in medicine. The textile industry has provided silkworm silk as an accessible biomaterial. In contrast, spider silks are limited in supply mainly owing to difficulties in breeding (short lifespan, territoriality, and cannibalism of spiders) and complexity in collecting pure silk (as multiple silk fibers are produced from spinnerets). The identification of partial cDNA sequences encoding spider silk proteins triggered efforts to generate recombinant silk production.[4,5] Initially, the expression of fragments of native cDNA did not result in satisfactory yield. However, recent development with synthetic spider silk gene designs opened up new horizons to explore spider silks for medical needs.[4,6]

The sequences of bioengineered spider silks are based on the consensus motifs of the corresponding natural equivalents. The consensus motif is reversely transcribed into oligonucleotides compatible to the host organism codon usage. Codon optimization renders higher protein expression in the host. Next, the double-stranded oligonucleotides are repeatedly ligated to generate larger sized genetic constructs. Using synthetic oligonucleotides, various DNA modules can be generated and their combinatorial ligation allows for the design of genes encoding bioengineered spider silk proteins with different properties. Moreover, the bioengineered silk proteins may be further modified to gain new functions. This strategy of hybrid protein construction at the DNA level combines the sequence of bioengineered spider silk, which is responsible for biomaterial structure, with sequences of polypeptides for functionalization. The cell-adhesive sequence of the fibronectin (RGD—Arg-Gly-Asp), silica formation sequence of silaffin (R5 peptide),

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