

Engineered RGD-Silk Scaffold for Improved Cell Adhesion

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INTRODUCTION: Silk is an alanine- and glycine-rich protein consisting of repeating crystalline and amorphous regions. These features give silk its outstanding mechanical properties, which are complemented with good biocompatibility and biodegradability. Together these properties make silk a suitable scaffold material for tissue engineering purposes. The objective of this work is to produce a genetically engineered spider silk-based protein with the cell signaling amino acid sequence RGD (arginine-glycine-aspartic acid) directly incorporated into the primary sequence. The hypothesis is that the engineered RGD-silk will promote receptor-mediated cell adhesion and spreading, features crucial for a functional bioactive scaffold.

METHODS:

GRG₂LG₂QGAGA₅G₂AGQG₂YG₂LGSQG is the consensus sequence of the repetitive unit derived from the major ampullate spidroin 1 (MaSp1) of the spider *Nephila clavipes*. A DNA sequence coding for 15 repeats of this consensus sequence was constructed (15mer) [1] and also an analogue flanked by DNA coding for the amino acid sequence VTGRGDSPA derived from fibronectin domain III₁₀ was assembled (RGD-15mer) [this work]. For affinity purification and quantification terminal His- and S-tags were attached. The two engineered silks were produced by fed-batch fermentation using a bacterial expression system, and then purified and cast into films with a surface density of 1 mg/cm². The films were seeded with DiI stained human mesenchymal stem cells (hMSC) at a density of 5'000 cells/cm², and cell adhesion was studied with time-lapse microscopy.

RESULTS: The final concentration of the engineered silks after fermentation was approximately 1'000 mg/L, and 25% of this could be recovered after purification to a purity of >95%. Films made of the engineered silks with the fibronectin derived RGD sequence (RGD-15mer) and without the RGD sequence (15mer) were cast and seeded with hMSCs. On the RGD-15mer silk the cells started to polarize and migrate, something that could not be observed on the 15mer silk over the duration of the time-lapse experiment, see Fig. 1. As a positive control fibronectin-coated 15mer silk (5 µg/cm²) was used. All experiments were performed with serum free medium.

Analysis of the mechanical properties of the engineered silks, and long term cell culture studies to investigate proliferation and differentiation remain to be performed.

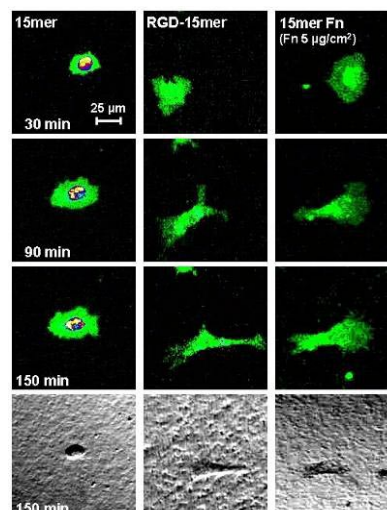


Fig. 1: Micrographs of human mesenchymal stem cells on 15mer, RGD-15mer and fibronectin-coated 15mer silks 30, 90 and 150 minutes post-seeding. Top 3 rows: fluorescence images, bottom row: differential interference contrast images.

DISCUSSION & CONCLUSIONS: We have set up a production system for tailor-made silk materials for tissue engineering applications. Furthermore we have integrated the cell signaling amino acid sequence RGD into one of the engineered silks, and we could detect an effect in adhesion of human mesenchymal stem cells on films made of this RGD-silk. These results show that this novel biomaterial has the potential to be used as a cell adhesion promoting scaffold material in the field of tissue engineering.

REFERENCES: ¹ E. Bini, C.W. Foo, J. Huang, V. Karageorgiou, B. Kitchel, D.L. Kaplan (2006) *Biomacromolecules* 7:3139-45.

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