

FGF-2 binding to modified silk fibroin films mimicking the extracellular matrixE. Wenk¹, A.R. Murphy², L. Uebersax¹, D. L. Kaplan², H.P. Merkle¹, L. Meinel¹¹Institute of Pharmaceutical Sciences, ETH Zurich, Zurich, Switzerland. ²Department of Biomedical Engineering, Tufts University, Medford MA, USA.

INTRODUCTION: A suitable scaffold for tissue engineering should provide an extracellular matrix (ECM) mimicking environment that is able to support cell interactions and differentiation. The physiological ECM acts as a local depot for growth factors, releases them on demand, prevents their degradation and, in some cases, enhances binding to cell surface receptors. Fibroblast growth factor 2 (FGF-2) specifically interacts with the sulfated glycosaminoglycans heparin and heparan sulfate of the ECM¹.

In the present study, silk fibroin (SF) was modified through diazonium coupling chemistry in order to introduce sulfonic acid functional groups at different ratios. Adsorption of FGF-2 to unmodified and modified SF films and release profiles were analyzed. SF, a fibrous protein biopolymer, is currently explored for several biomedical applications, owing to its unique properties, such as aqueous processability, mechanical strength, biocompatibility and biodegradability². The present study represents the first step towards the development of a ECM-mimicking matrix for the storage and controlled delivery of growth factors for tissue repair.

METHODS: SF solution was obtained from cocoons of the silkworm *B. mori* as previously described³. Diazonium coupling reaction with SF solution was performed as described before³. The molar ratio of diazonium salt to tyrosine in the SF protein was tailored to produce different levels of modification. SF films were prepared and transformed into a β -sheet enriched, water-resistant form by exposing them to water vapor of 96% relative humidity at room temperature (RT) for 12 h. Adsorption of FGF-2 onto SF films was performed at RT and indirectly analyzed by measuring the amount of unbound FGF-2 by ELISA after 1, 2 and 4 h. FGF-2 release experiments were performed in PBS (pH 7.4) at 37°C for 6 d, and the samples analyzed for FGF-2 by ELISA.

RESULTS: Drug adsorption studies showed that after 4 h adsorption of FGF-2 to SF films ranged from 49% (unmodified silk) to 95% (80 sulfo groups per silk molecule) of the initial amount of FGF-2 (Fig. 1A). A linear correlation between sulfonation ratios and FGF-2 adsorption was

detected; higher sulfonation ratios led to increased FGF-2 binding (Fig. 1B). Drug release experiments showed that after 6 days, FGF-2 release from SF films ranged from 0.2% (80 sulfo groups per molecule) to 0.8% (unmodified silk) of the initial loading of FGF-2 (Fig. 1C). Again, a linear correlation between sulfonation ratio and FGF-2 adsorption was observed, with higher sulfonation ratios leading to decreased FGF-2 release (Fig. 1D).

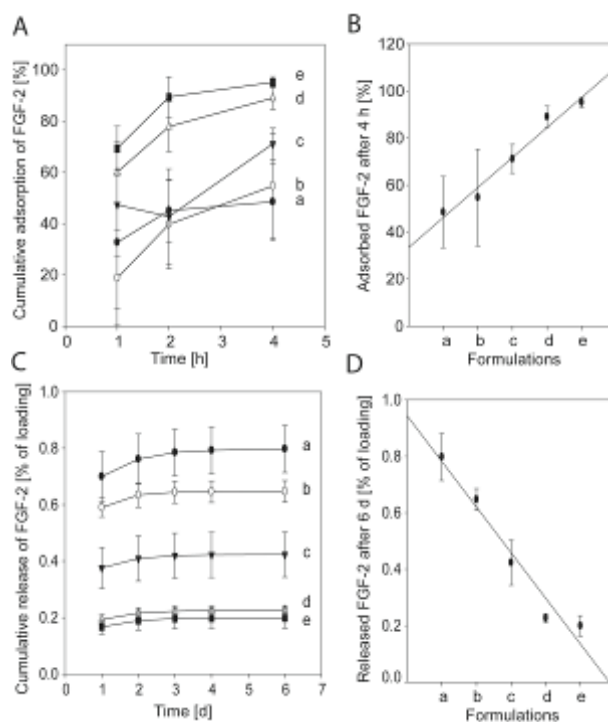


Fig. 1: Cumulative adsorption of FGF-2 to SF films (A), adsorbed FGF-2 after 4 h (B), cumulative release of FGF-2 from SF films (C), and released FGF-2 after 6 d (D); a = unmodified silk, b = 20 sulfo groups per silk molecule, c = 40 sulfo groups per silk molecule, d = 60 sulfo groups per silk molecule, e = 80 sulfo groups per silk molecule.

DISCUSSION & CONCLUSIONS: Modification of SF allowed us to obtain a system with adjustable growth factor binding and delivery. Future work is planned to investigate the impact of the findings on cell growth and differentiation.

REFERENCES: ¹L. Macri et al. (2007) *Adv. Drug Deliv. Rev.* **59**:1366-1381. ²G.H. Altman et al. (2003) *Biomaterials* **24**:401-416. ³A.R. Murphy et al. (2008) *Biomaterials*, in press.