Porous Silk Fibroin Hydrogels with Tunable Stiffness for Vascular Tissue Engineering

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Statement of Purpose: Naturally derived polymers have frequently been utilized as matrices for tissue engineering applications as often their macromolecular structure share components or properties similar to natural ECMs. Silk fibroin, the structural protein of *Bombyx Mori* silk fibers, is a natural fibrous protein that has been studied extensively for tissue engineering applications due to its biocompatibility, controllable degradation rate and excellent mechanical performance. Silk proteins self-assemble into strong intra- and intermolecular β-sheet structures and complexes providing several attractive physical properties such as controlled degradation and mechanical stiffness. Previously we developed a method to produce porous silk hydrogels using high pressure carbon dioxide (CO_2) as a volatile acid (Floren, 2012). This simple technique requires no additional solvents and can induce silk protein gelation in short times, less than 2 hours, resulting in hydrogels with enhanced porosity and improved physical properties. Due to its tunable elasticity approaching vascular tissues (E = 10-30 kPa), combined with clean fabrication, we further explore its use as an instructive platform for differentiating mesenchymal stem cells (MSCs) toward vascular phenotypes. In this communication, silk hydrogels with tunable stiffness are prepared and evaluated for their capacity to support MSC differentiation into vascular phenotypes.

Methods: *Preparation of Silk Hydrogels from High Pressure CO_2:* Preparation of silk fibroin aqueous solution and hydrogel fabrication from high pressure CO_2 have been described previously (Floren, 2012). *Material Characterization:* Silk hydrogel stiffness and structural properties are evaluated using compression tests and FTIR, respectively. Fourier self deconvolution (FSD) is employed to determine the percentage of different secondary structures obtained in the silk specimens. *Cell Culture:* hMSCs with passages 2–6 are cultured. To seed cells on the top of the hydrogel constructs, a concentrated cell drip of 50,000 cells per scaffold are utilized. *Biological Evaluation of hMSCs on Silk Hydrogels in Serum Free Conditions:* Silk hydrogels of varying elastic moduli (E = 15-60 kPa) are used to culture MSCs using several serum conditions ranging from 0-10% serum content. The presence of serum in cell culture media is known to influence attachment and spreading of MSCs in the absence of cellular attachment motifs. Cultures are evaluated at 1,3 and 7 days for viability using by observing cell morphology for cell nuclei (DAPI) and cell cytoskeleton after sample fixation. *Analysis of SMC Phenotype and Elastogenesis Silk Scaffolds:* We study mRNA and protein expressions of markers specific to mature SMC phenotype including calponin, SM22 and MHC as well as elastin. Marker analyses are performed after 1, 3 and 7 days culture. Additionally, elastin expression and cell contraction is analyzed respectively by Griess assay and contractility assay.

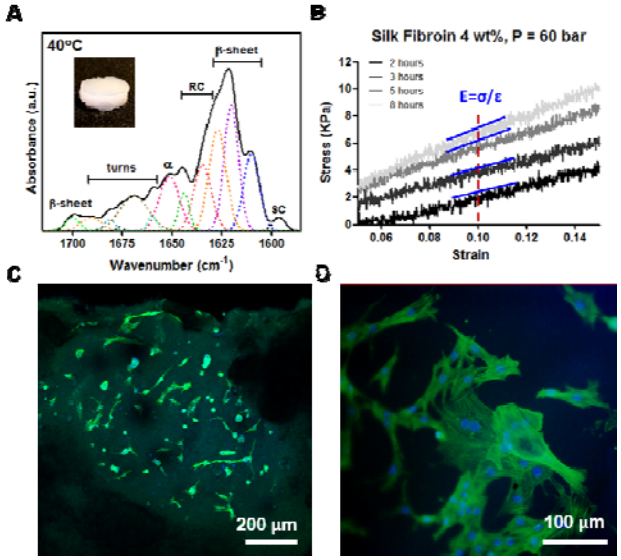

Figure 1. (A) FTIR spectrum of silk hydrogel (inset) with FSD algorithm. (B) Stress/strain curves obtained for various silk hydrogel preparations under high pressure CO_2. (C-D) Confocal and fluorescent images of cell cytoskeleton (green) and nuclei (blue) of MSCs seeded on silk hydrogel for 7 days.

Results: We have fabricated silk hydrogel using our previous protocol utilizing high pressure CO_2. FTIR and FSD analysis were performed on these specimens (Figure 1A) to determine the level of β-sheet formation at approximately 53%. Mechanical properties of different silk hydrogel preparations were studied with compression tests and stress-strain results are represented in Figure 1B. It shows that silk hydrogel stiffness increases with increased CO_2 processing time. Biological evaluation of the silk hydrogels was performed using a serum-starved seeding protocol, and then stained and imaged for cell cytoskeleton and nuclei (Figure 1C-1D). MSC attachment and growth was found after 7 days serum-deprived cell culture, indicating a robust cellular response to the silk hydrogel matrices.

Conclusions: We developed novel silk hydrogels using high pressure CO_2 technique and evaluated their performance to induce vascular differentiation of MSCs. Our preliminary results indicate that these silk hydrogels represent ideal substrates for MSC attachment and growth with great potential to act as tunable platforms for MSC-based vascular tissue engineering.

References: Floren M., Spilimbergo S., Motta A., Migliaresi C. *Biomacromolecules* 2012, 13, 2060-2072