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Highly porous silk scaffolds for bone defect repair

L. Uebersax[1], S.Hofmann[1], H.Hagenmüller[1,2], R. Müller[2], H.P.Merkle[1], L.Meinel[1,3,4]
[1] Drug Formulation and Delivery, ETH, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich.
[2] Institute for Biomedical Engineering, ETH, Moussonstrasse 18, CH-8044 Zürich
[3] Harvard – M.I.T. Division of Health Sciences and Technology, M.I.T., Cambridge, MA
[4] Department For Bioengineering, Tufts University, Medford, MA

INTRODUCTION: The distinguishing features of silk fibroin (SF) matrices as compared to other protein biomaterials revolve around their excellent biocompatibility together with their mechanical properties rivalling even synthetic high performance fibers such as Kevlar. We developed a novel production process for silk scaffolds that is starting off from aqueous SF solutions, whereas earlier methods used organic solutions. Here, we describe the fabrication of SF implants, with a precise control over scaffold porosity and interconnectivity.

METHODS: Aqueous silk fibroin solutions were prepared from *Bombyx mori* cocoons. Briefly, cocoons were washed and dissolved in 9M LiBr and dialyzed under osmotic pressure resulting in 20% (w/w) SF solutions. This solution was transferred into a mold, filled with sieved paraffine globules (porogens). Pore sizes were controlled by globule diameter and interconnectivity by heat treatment of the spheres (prior to adding the SF solution), resulting in a controlled melting of the contacting areas of the paraffin (Heat treatment at 24, 37 and 45°C for 50minutes each). Scaffolds were assessed by SEM and mechanically tested (Zwick 1456). To evaluate cellular responses, SF scaffolds were seeded with human mesenchymal stem cells (MSC). Proliferation was assessed using a Pico Green assay (DNA) and histologically (H&E staining) in control medium (10% FBS in DMEM) and osteogenic responses by µ-computed tomography (mineralization and histological evaluation will follow), in osteogenic medium (10% FBS, 10mM µ-glycerolephosphate, 50 µg/ml ascorbic acid-2-phosphate, and 1 µg/ml BMP2.

RESULTS: The mechanical properties of the scaffdolds were markedly influenced by implant porosity and pore interconnectivity (**Fig. 1A**). The elastic modulus (2% strain) was higher for scaffolds treated 37°C as compared to 25°C or 45°C. The impact of the pore diameter on mechanical properties was less pronounced with the exception of scaffolds treated at 25°C, when pore sizes between 300 – 400 µm resulted in a considerably higher elastic modulus as compared to the other pore sizes (**Fig.1B**). Cell proliferation on the scaffolds was similar as compared to SF scaffolds prepared form organic SF solutions [1,2], and MSC deposited a network of calcified clusters, more pronounced at the scaffold rim as compared to the scaffold center.

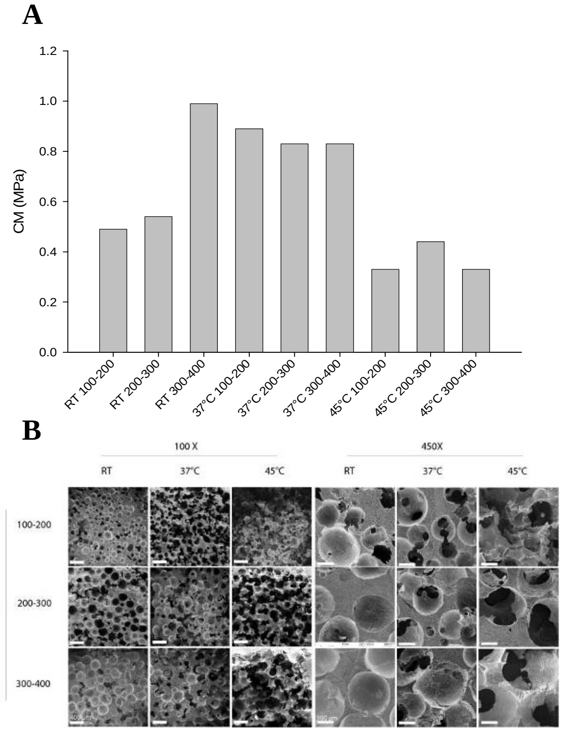

Fig. 1(A): Influence of pore size and interconnectivity on the young's module of silk scaffolds. (B) SEM images taken from scaffolds with different pore sizes and interconnectivity

DISCUSSION & CONCLUSIONS: This study describes a novel process for the fabrication of silk implants starting off from aqueous SF solutions, with a precise control over porosity and interconnectivity. Future directions of our research using highly concentrated and aqueous SF solutions direct at expanding the biomedical applications of silks, with a focus on drug formulation and delivery.

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