Silk fibroin/gelatin blended materials as osteogenic scaffolds for tissue engineering

KA Luetchford[1], JB Chaudhuri[2], PA De Bank[1]
[1]*Centre for Regenerative Medicine, Department of Pharmacy & Pharmacology, University of Bath, U.K.*
[2]*School of Engineering and Informatics, University of Bradford, U.K.*

INTRODUCTION: Silk fibroin (SF) is a biocompatible, light-weight, and strong material that can be processed into many formats [1]. While there are reports of successful cell adhesion and proliferation on SF materials, there are a number of instances where SF has been blended with other biopolymers such as collagen and gelatin to improve its properties, specifically cell adhesion [2]. This work investigates SF/gelatin (SF/G) blends as osteogenic cell scaffolds.

METHODS: An aqueous solution of SF was generated following extraction from the cocoons of *Bombyx mori* silkworms. Following lyophilisation, SF was dissolved in HFIP at 2% w/v, and blended with porcine Type A gelatin (2% w/v, in HFIP) at ratios of 75:25, 50:50 and 25:75. 2D films were cast from these solutions and cross-linked with 50 mM EDC in methanol. SF/G blended microparticles were created from aqueous solutions of SF/G using microfluidic flow focussing and cross-linked the same way. Mesenchymal stem cells (rMSCs), harvested from the bone marrow of juvenile Wistar rats, were maintained in MEM with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C in a 5% CO_2 atmosphere. Osteogenic differentiation medium (ODM) consisted of basal medium supplemented with 0.1 µM dexamethasone, 0.2 µM ascorbic acid 2-phosphate, and 10 mM glycerol 2-phosphate. Cells were cultured in either basal medium or ODM for a minimum of 14 days. Osteogenic differentiation was confirmed by positive alkaline phosphatase (ALP) activity (BCIP/NBT assay), and osteocalcin and osteopontin expression.

RESULTS: Cell attachment to the films was improved by the inclusion of gelatin, as determined by the MTS assay three days after seeding. This was reflected in 3D by increased seeding efficiencies of cells on microparticles. Osteogenic differentiation on the SF/G films was confirmed by ALP activity, osteocalcin expression and osteopontin expression (Fig. 1). All SF/G blends supported osteodifferentiation at a level equivalent to that on tissue culture plastic (TCP). Interestingly, SF alone appeared to support a higher degree of differentiation, suggesting that where cells do adhere, the surface is highly osteogenic. However, this is hindered in real terms by the reduced cell adhesion in comparison to SF/G blends. Differentiation of rMSCs on microparticles was confirmed initially by ALP activity (Fig. 2)

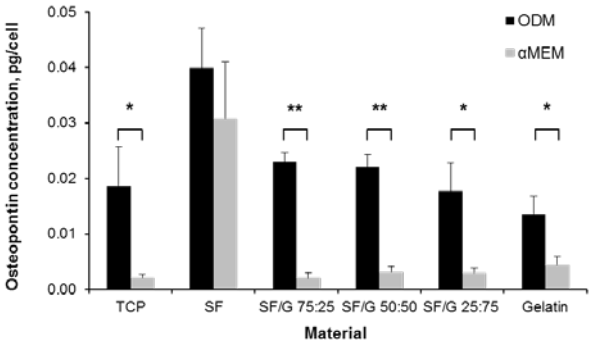

*Fig. 1: Osteopontin expression of rMSCs cultured on SF, SF/G, and gelatin films for 14 days. Data shown represents mean + standard error, n=3. ** p<0.001; * p<0.05.*

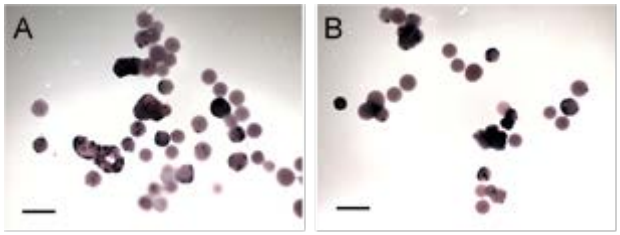

Fig. 2: Positive purple staining for ALP activity in rMSCs cultured on SF/G 25:75 microparticles in basal media (A) or ODM (B). Scale bar = 500 µm.

DISCUSSION & CONCLUSIONS: SF/G blends are shown to support cell attachment in 2- and 3D at higher levels than SF alone. The blends of materials are also shown to support osteodifferentiation, suggesting these biomaterials could be useful scaffolds for bone tissue engineering or repair.

REFERENCES: [1] Wang, Y., et al., Biomaterials, 2006. 27(36): p. 6064-6082. [2] Chomchalao, P., et al., Biomed Eng Online, 2013. 12: p. 28

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