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Development of fibrous porous silk scaffolds

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INTRODUCTION: Pore structure is very important when considering scaffolds for tissue engineering. High porosity and well connected pores create good mass transfer properties increasing cell viability. Pore size helps to determine cell differentiation[1]. Previous work with silk scaffolds with defined pore sizes has made scaffolds with small pores with limited connections[2], or large (>500µm), well connected pores[3]. This work has developed a technique for making silk scaffolds with small, well connected pores. These scaffolds have an unusual fibrous structure.

METHODS: Silk scaffolds were fabricated from freeze dried silk fibroin (Hobbycraft) *(prepared as in the literature[2]).* The silk fibroin was dissolved at either 10% (w/v) or 7.5% (w/v) in 20% (v/v) formic acid. It was then added to a circular mould (15mm diameter) either before or after NaCl was added as a porogen. Scaffolds were then left covered in the mould for 24 hours, when the cover was removed. After another 24 hours the bottom plate of the mould was removed. After a final 24 hours the scaffolds were removed from the mould and either placed in methanol or propanol (Fisher) for 30minutes or transferred immediately for salt leaching. Scaffolds for cell work were cut to 2mm height after 30 minutes in methanol. The scaffolds were then salt leached by placing into water, which was changed six times over 24 hours. Scaffolds for cell proliferation measurement were autoclaved, and then soaked in media (DMEM + 10% FCS, 100mM ascorbate-2-phosphate) overnight. Scaffolds were then seeded with 800,000 P3 ligament fibroblasts and placed on an orbital shaker at 100rpm. Media was changed every 2 days and proliferation was measured by determined the DNA content after 7 days with the picogreen assay (Invitrogen).

For SEM scaffolds were freeze dried, and then freeze fractured and gold coated. Images were taken with a JEOL JSM6310. For FTIR discs were pressed using roughly 2mg of powdered scaffold and 30mg of KBr (Sigma). FTIR spectra were an average of 100 scans at a resolution of $4cm^{-1}$ recorded on a Bruker Equinox 55 spectrophotometer.

RESULTS: SEM images comparing adding salt to the mould before and after silk showed that adding silk before salt resulted in a more replicable structure. The images also confirmed that it was possible to make scaffolds with small well connected pores. The scaffolds have an unusual fibrous structure; reducing the concentration of silk in the solvent results in sparser fibres (Figure 1).

FTIR spectra show that silk in the scaffolds is in silk II form (β-sheets), adding methanol does not change the spectra.

There was no significant difference between the DNA produced after 7 days: 10% w/v silk 330 +/- 94ng and 7.5% w/v silk 454+/-293ng.

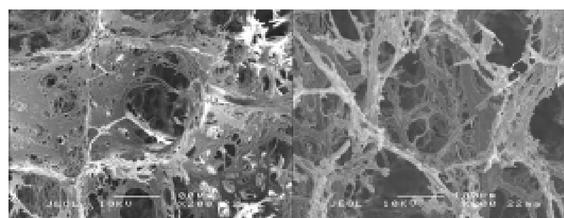

Fig. 1: SEM images of fibrous silk scaffold: 10% w/v silk (left) and 7.5%w/v silk (right)

DISCUSSION & CONCLUSIONS: A method to fabricate novel fibrous porous silk scaffolds has been developed. By changing the salt particle size it is possible to fabricate scaffolds with a large range of pore sizes. These pores are smaller than those of previous silk scaffolds with well connected, defined size pores. In addition the pore structure can be changed by varying the concentration of silk. Cell proliferation data suggests that these scaffolds are suitable for tissue engineering. Future work is to use these scaffolds to examine the effects of different pore sizes and pore structures on ligament fibroblast proliferation and matrix synthesis.

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