INTRODUCTION
Disruption of the annulus fibrosus (AF) due to degenerative disc disease (DDD) affects its ability to resist tensile forces. DDD is a very common and treatment options for persistent chronic pain are limited. As there is no optimal management for this disorder, repair of the damaged annulus fibrosus (AF) by AF cell-seeded scaffolds would be a novel approach. In a previous study, we developed a method for growing AF cells consisting of maintaining AF cell-seeded porous silk scaffolds (200um pore size) under continuous dynamic conditions using spinner flasks. AF tissue developed using this method and contained abundant collagen. As pore size affects surface area available for cell attachment as well as cell infiltration and mass transport into the scaffold the purpose of this study was to determine the optimal pore size to facilitate tissue formation and distribution within the scaffold.

MATERIALS AND METHODS
Preparation of porous silk scaffold: Silk fibroin was prepared from cocoons of Bombyx mori as described previously [1]. To generate porous scaffolds with different pore sizes the silk fibroin was dissolved in hexafluorosilicic acid containing NaCl granules of different sizes. This resulted in scaffolds (4mm diameter x 2mm height) with an average pore size of either 200um, 600um, or 1000um.

AF Tissue Formation: IVDs were harvested from bovine caudal spines, as described previously [1]. To generate porous scaffolds with different pore sizes the silk fibroin was dissolved in hexafluorosilicic acid containing NaCl granules of different sizes. This resulted in scaffolds (4mm diameter x 2mm height) with an average pore size of either 200um, 600um, or 1000um.

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RT-PCR: Total RNA was isolated by Trizol extraction, reverse-transcribed (Superscript II), and gene expression semi-quantified following PCR using sequence specific primers. The PCR products were run on a 1.5%-agarose gel and the intensity semi-quantified by densitometry following staining with ethidium bromide.

Biochemical analysis: Aliquots of papain digested tissues were assayed separately for proteoglycan and collagen contents. The proteoglycan content was estimated by quantifying the amount of sulfated glycosaminoglycans using the dimethylmethylene blue dye-binding assay and spectrophotometry. To determine collagen content the papain digests were hydrolyzed in 6 N HCl at 110°C and assayed for DNA content using the Hoechst 33258 dye binding assay and fluorometry. [2]

Scanning Electron Microscopy (SEM): In all the experiments, the scaffolds with different pore sizes were subjected to SEM. The cells on the different scaffolds were similar in appearance.

DISCUSSION
This study demonstrates that the pore size of the scaffold affects tissue formation by AF cells. More cells attached to silk scaffolds with an average pore size of 200 or 600um and these cells accumulated more collagen when compared to cells grown on scaffolds with pore sizes of 1000um. The mechanism whereby pore size influences tissue formation is not known but a similar response has been observed for chondrocytes [3]. It is likely that the amount of tissue that forms is a result of the interplay between total surface area and nutrient diffusion. In both the 200um and 1000um pore size scaffolds, there were many interior pores that were devoid of any tissue. This can not be purely related to the amount of scaffold available for attachment as the 200um pore sized scaffolds showed no significant difference in cellularity (DNA (mean±SEM): 200um: 4.5 ± 0.46ug; 600um: 6.1 ± 0.89ug and 1000um: 3.7 ± 0.37ug at 28 days).

Determination of Proteoglycan and Collagen Contents: There was no optimal management for this disorder, repair of the damaged annulus fibrosus (AF) by AF cell-seeded scaffolds would be a novel approach. In a previous study, we developed a method for growing AF cells consisting of maintaining AF cell-seeded porous silk scaffolds (200um pore size) under continuous dynamic conditions using spinner flasks. AF tissue developed using this method and contained abundant collagen. As pore size affects surface area available for cell attachment as well as cell infiltration and mass transport into the scaffold the purpose of this study was to determine the optimal pore size to facilitate tissue formation and distribution within the scaffold.

REFERENCES:

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