

Chondrocyte Growth in Porous Spider Silk 3D-Scaffolds

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INTRODUCTION: Silk is rediscovered the past decade as a possible biomaterial. As a strong and flexible natural material that biodegrades slowly, it's promising for tissue engineering applications [1, 2]. Porous 3D scaffolds have been made with silk fibroin for these purposes [3]. Spider silk exceeds the mechanical properties of silkworm silk. This explains the many attempts to produce synthetic spider silk. In this research spider cocoon silk is used to create porous 3D scaffolds for cartilage regeneration. Human chondrocytes were seeded on it and cultured for several weeks to establish the scaffolds are not cytotoxic and cells can migrate, attach, grow and express their ECM in the pores of the spider silk matrix.

METHODS: Spider is harder to dissolve than silkworm silk. But after washing a few times in Marseille-soap, the fibres do not only lose their yellow colour but also their protection against strong salts. The fibres can be dissolved in LiBr. After dialysis against water and freeze-drying, the spider silk fibroins can be re-dissolved in formic acid and mixed with NaCl particles. These particles are sieved for a size between 100-200µm and are ten times more abundant than the spider silk fibroin. The typical secondary structure can be regenerated with methanol before the NaCl particles are leached out of the material to leave the pores behind.

Human Chondrocytes were isolated from knee articular cartilage and seeded on the scaffolds. After 1, 3 and 6 weeks of culture cross-sections were made from top to bottom of the scaffold and coloured for cell-viability and expression.

RESULTS: The obtained spider silk scaffolds do not excel in tensile strength, but are quite flexible, very compressible and absorb fluid. The interconnectivity of the pores was proven by an ink-absorption-test and was clear when we saw the diffusion of the chondrocytes through the scaffold. The cells weren't found in every pore, but spread all over the scaffold from top to bottom and

attached to the spider silk. The immunohistochemical colouring of the ECM showed some dedifferentiation, but the cells growing in the pores were still round and shaped chondrocyte-like.



Fig.1: a) a porous, cylindrical scaffold, made with spider silk. B) Chondrocytes growing in the pores..

DISCUSSION & CONCLUSIONS: Even with small amounts of spider silk, it is possible to create a porous 3D scaffold that exists of spider silk fibroin only. As the pores are interconnected and porosity and pore-size and can be controlled, medium, cells and tissue can migrate through the matrix. As cells could be grown in the matrix for at least 6 weeks and their ECM-expression could be detected, it could definitely be used as a plug in cartilage regeneration.

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