

Engineering niches for intervertebral disc cells using random and aligned silk nano fibres

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INTRODUCTION: Low back pain linked to intervertebral disc (IVD) degeneration is a highly abundant problem in the aging modern society. Until today there is no biological solution available based on the patient's autologous cells to restore or repair the IVD. We hypothesized that electrospun silk scaffolds can mimic the extracellular matrix of the IVD cells: i) a random orientation of the fibres would be ideal for nucleus pulposus cells (NPC) and ii) an alignment of the fibres would be favourable for annulus fibrosus cells (AFC).

METHODS: *Silk liquefaction:* Silk fibres from *Bombyx mori* (SwissSilk) were cut in small pieces and boiled in 0.2M Na₂CO₃ for 30 min to remove the sericine. Then, the silk fibres were rinsed three times in ultrapure water (UPW) and dried overnight. The dry silk was then dissolved in 9.3 M LiBr solution and dialysed against UPW for 48 h and purified by high speed centrifugation.¹ *Electrospinning:* Silk was mixed with 5% (wt/vol) 900kDa-PEO to generate a solution of 6.4% silk and 1 % (wt/vol) PEO. This solution was electrospun on a flat collector for randomly oriented fibres and on a rotating mandrel for aligned fibres. Of each electrospun mat N=40 samples of 6mm diameter were punched out. N=20 of the randomly aligned samples were ultra-sonicated for 1 min at 80 Watts to increase their porosity.² *Cyto-compatibility:* 40k human derived NPC and AFC (ethically approved) were seeded per carrier and grown for 7 days. On day 1 and 7 cell spreading (cLSM) and cell activity (Alamar Blue) and DNA content was monitored.

RESULTS: The electro-spinning process revealed two completely different scaffold and micro environments for cells as confirmed by SEM (Fig. 1, A,B) Live/dead stain of IVD cells confirmed their alignment in the direction of the parallel-oriented fibres (Fig. 1, C, D). On day 7 cell activity decreased for AFC to the contrary for NPC were it increased per cell (Fig. 2) Generally, it was noted that cells adhered and proliferated better on ultra-sonicated non-oriented scaffolds than on aligned scaffolds (data not shown).

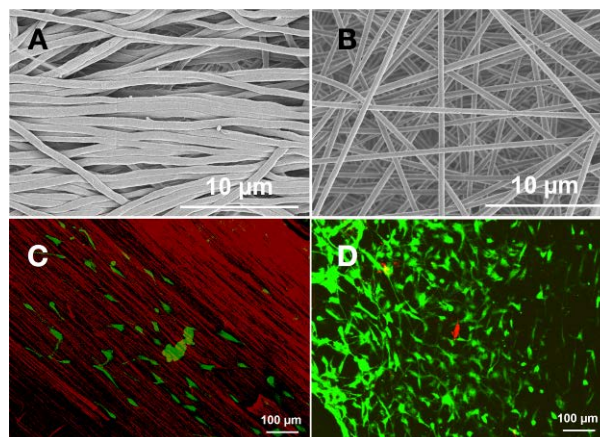


Fig 1: A-B) Scanning electron microscopy images of scaffolds A) Aligned silk fibres (mandrel speed=30Hz) B) Randomly distributed silk fibres C-D) Human IVD cells on scaffolds C) AFC aligned in the direction of silk fibres (red) D) NPC randomly oriented in randomly aligned silk nano fibres.

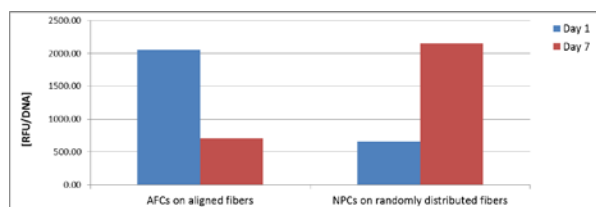


Fig. 2: Cell activity of human NPC and AFC measured with Alamar Blue Assay on day 1 and 7.

DISCUSSION & CONCLUSIONS: By modification of the silk composition and the electro-spinning parameters the 3D environment of a scaffold can be controlled. This is crucial for cell adhesion and proliferation of primary cells. The general direction of cell growth can be controlled by the arrangement of the silk nano fibres. Future research will focus on the control of porosity and integration of adhesion molecules and cytokines to tailor the IVD cell specific niche.

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