

CELL RESPONSE ON SILK FIBROIN SCAFFOLDS WITH VARYING TOPOGRAPHIES

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INTRODUCTION: Micro- and nanopatterning through soft lithography is an established technology for the fabrication of topographies suitable for oriented tissue engineering purposes. As a technically and economically more attractive alternative we focus here on the preparation of silk fibroin (SF) scaffolds with aligned nanofiber orientation by the technique of electrospinning. Application of electrospun scaffolds for oriented tissue engineering was evaluated with the use of human mesenchymal stem cells (hMSC).

METHODS: An aqueous solution of SF was blended with polyethylene oxide to improve processability during electrospinning¹. Random electrospun scaffolds were collected on a cylindrical target ($d = 4$ cm) rotating at 200 min^{-1} . To produce aligned scaffolds, the rotating speed of the cylindrical target ($d = 14$ cm) was increased from 1000 to 4000 min^{-1} . Low relative humidity (RH) was maintained during electrospinning to ensure the deposition of uniform, bead-free fibers. SF nanofibers were examined for their architecture using scanning electron microscopy (SEM). Cell response to the different scaffolds was monitored by SEM and confocal laser scanning microscopy (CLSM) after fluorescent labeling of cell membranes.

RESULTS: As determined by SEM, maintaining a low relative humidity ($< 30\%$ RH) during electrospinning was a critical process factor to obtain uniform, bead free fibers. Control of fiber network anisotropy was by the rotational speed of the cylindrical target, with no alignment at the lowest and nearly complete SF fiber alignment achieved at the highest speed examined.

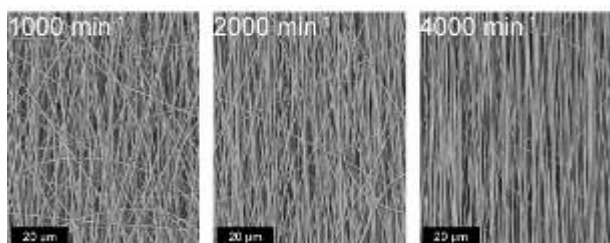


Fig. 1: Effect of rotational speed of cylindrical target during electrospinning on SF nanofiber deposition: increasing fiber orientation with increasing rotational speed (from left to right).

Upon cell seeding, different cell morphologies were observed depending on SF fiber orientation. hMSC adopted an elongated and spindle shaped morphology when cultured on aligned fibers, with the alignment of the cells being parallel to the prevailing fiber direction. This finding was in contrast to random electrospun fibers with no preferential cell orientation.

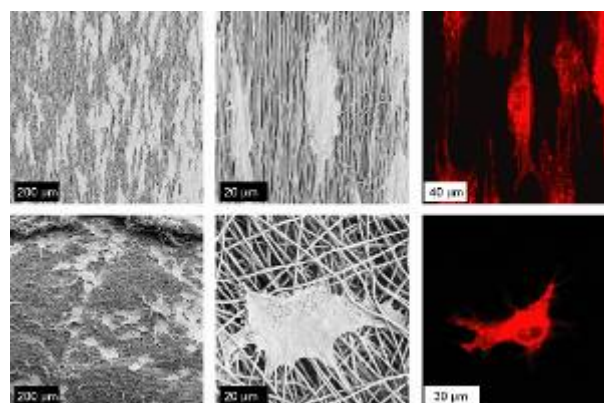


Fig. 2: SEM and CLSM micrographs showing hMSC on aligned (top row) and random SF nanofibers (bottom row).

DISCUSSION & CONCLUSIONS: Highly aligned and uniform SF scaffolds were manufactured by electrospinning under controlled conditions (relative humidity, rotational speed of cylindrical target). Anisotropic topography by fiber alignment allowed to control hMSC morphology and cell/tissue orientation mimicking the oriented architecture of the extracellular matrix in native tissue such as tendon or ligament, with implications for tissue engineering. Further studies will be dedicated to detail the impact of surface structure on the genomic profile of hMSC during attachment and differentiation.

REFERENCES: ¹ H.J. Jin, S.V. Fridrikh, G.C. Rutledge, et al. (2002) *Biomacromolecules* 3(6):1233-9.

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