The effect of substrate topography on the proliferation and morphology of chondrocytes

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Introduction: Fiber produced by electrospinning has morphological similarity to ECM, and this has been the main driver behind the rapid expansion in the use of this technique as it is assumed to promote cell proliferation and viability. However, the topographical effects of electrospun fiber on biology have not been compared to other topographies involving chemically identical materials. In this work, we compared proliferation, cell adhesion and cellular morphology of chondrocytes on 3 different topographically varied (electrospun fiber, semi-porous, and dense) polycaprolactone (PCL).

Methods: Different topographical features were prepared on 18 mm glass coverslips. The nanofiber sample was prepared using 12 wt% PCL in acetone electrospun at 100 µm of thickness. The glass coverslip was pre-coated with 0.5 wt% PCL in acetone by spin-coating to provide better adhesion of the subsequently deposited electrospun fibers. The voltage, the distance between the needle tip and the collecting plate, and the flow rate of solution were 24 kV, 20 cm and 24 ml/hr, respectively. Semi-porous structures with pore sizes ranging from 0.5 to 3 µm were produced by dip-coating coverslips in a solution of 5 wt% PCL in acetone. Dense surfaces were produced by dip-coating in a solution of 12 wt% PCL in acetone followed by gentle heating to 180°C for 15 min. To ensure that identical chemistries existed in all cases, previously electrospun PCL was used to produce the PCL-in-acetone solutions for both the semi-porous and dense surfaces. $6x10^5$ of primary rat chondrocytes were seeded onto each sample. Proliferation was assessed by colorimetry using crystal violet staining at d 4, 8 and 11. Bare glass coverslips were used as controls, and 5 samples of each condition were averaged. Cell attachment was observed by fluorescence microscopy with actin staining on d 11. The samples on day 11 were fixed with glutaraldehyde and OsO_4, followed by dehydration with ethanol series and HMDS. The dehydrated samples were coated with ~12 nm Os prior to SEM.

Results / Discussion: The proliferation of chondrocytes on nanofiber was superior to semi-porous and dense surface at d 4 but had apparently reached its plateau. Proliferation on glass and the semi-porous and dense PCL surfaces followed an upward trend (Fig. 1. (A)). The fluorescence microscopy images showed considerably greater stress fiber development in the cells on glass versus those on PCL fiber, semi-porous and dense surfaces (Fig. 1. (B)) with the greatest resemblance to glass being the dense PCL. In the SEM the cells on the glass were the most adherent in agreement with fluorescence microscopy results. The cells on fiber were well adhered to the underlying structures (Fig. 1. (C)). The morphology of the cells on semi-porous and dense surfaces was more rounded compared to that on fiber. The pseudopodia of the cells on the semi-porous surfaces adhered to the flat surfaces between the pores but displayed a close interaction with the pores themselves. The cells on dense PCL were the most rounded but appeared to follow spherulite boundaries.

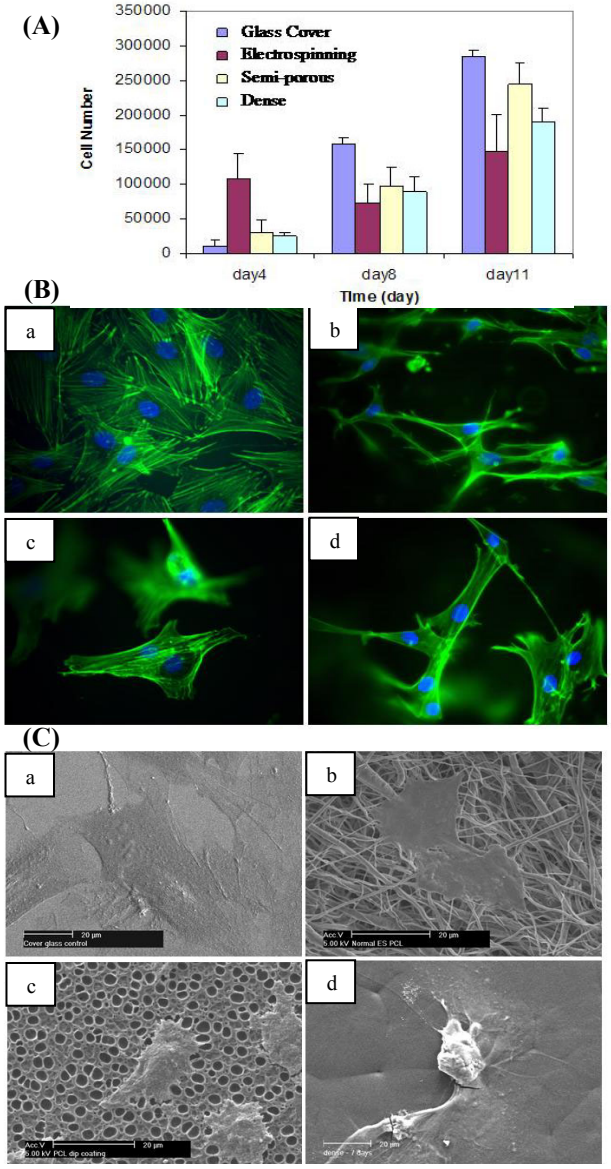

Fig. 1. (A) Proliferation of chondrocytes (B) Fluorescence images (C) SEM images (a; glass cover, b; nanofiber, c; semi-porous, d; dense)

Conclusions: Fibers produced by electrospinning appeared to promote initial adhesion and proliferation. In a subsequent time period the non-fibrous PCL substrates appeared to provide higher levels of proliferation. Both the semi-porous and the dense surfaces exhibited topographic control of the cytoskeleton. On-going work is expected to establish relative phenotypic stability under these conditions.