Electrospinning Process Temperature Modulates 3D Architecture but Not Cellular Behavior

M. White[1], J. Henry[2], G. Voskerician[1,3].
[1.] Proxy Biomedical Ltd., Galway, Ireland.
[2.] Department of Mechanical & Biomedical Engineering, National University of Ireland Galway, Ireland
[3.] Department of Biomedical Engineering Case Western Reserve University, Cleveland, Ohio, USA

Introduction: The structural hierarchy of a scaffold for tissue engineering determines how well it balances the conflicting needs between mechanical strength, biological compatibility and mass-transport [1]. The fundamental importance of scaffold porosity and fiber diameter is illustrated by mass-transport requirements for cell nutrition, interconnected channels for cell migration, and surface features for cell attachment. Electrospinning has lately shown great potential as a novel scaffold fabrication technique for tissue engineering. Scaffolds are produced by spinning a polymeric solution in fibers onto a support using an electric field. Such structures have been found effective in terms of tissue regeneration, as cells can bridge the scaffold pores and fibers, resulting in a fast and homogeneous tissue growth. The critical processing parameters during electrospinning include solution viscosity, electric potential, distance between the needle and the target, the type of solvent used, and the processing temperature [2].

The aim of this study was to investigate the role of electrospinning process temperature in modulating the scaffold architecture (porosity and fiber diameter) and the effect of such differences on cell migration. These findings contribute to the field of cell-material interaction and lead to the fabrication of intelligent scaffolds which can manipulate cell morphology and properties to release the necessary biological signals leading to tissue regeneration.

Materials and Methods: *Electrospinning:* Poly(ε-caprolactone) (PCL), poly(p-dioxanone) (PDO) and poly lactide-co-poly(ε-caprolactone) (PLA-PCL) polymers were used in this study. Various solutions (w/v) (PCL-15%, PDO-11% and PLA-PCL-6%) were produced by dissolving the polymer in chloroform, hexafluorosiopropanol (HFIP) and acetone, respectively) [3-4]. All scaffolds were processed similarly at two separate processing temperatures, -70°C and 23°C. *Scaffold morphology:* Prior to cell seeding, scaffold morphologies (fiber diameter and pore size) were quantified using scanning electron microscopy (SEM – Hitachi S-4700 SEM). *Cell Seeding:* Scaffolds (n=4) were seeded with immortalized National Institute of Health (NIH) 3T3 mouse fibroblasts at a density of 20 x 10^3/scaffold in a sterile polystyrene 24 well plate. Blank wells were also seeded to act as a control. Three time points were investigated (1, 3, and 7 days) for all samples. *Cell migration:* At each time point, the scaffolds were removed and fixed in 10% formaldehyde. The number of cells at the base of the well plates was used in conjunction with the control data to estimate the cell population within each electrospun sample.

Results & Discussion: Assessment of scaffold morphology by SEM revealed a significant difference (p<0.05) between the pore sizes of the scaffolds processed at -70°C and those at 23°C, regardless of polymer variant (Table 1). The pore size (µm) was consistently higher in the -70°C compared to the 23°C processed scaffolds, as shown in Table 1. By estimating the number of cells within the scaffold, no statistical difference was found between cell migration into -70°C and 28°C processed scaffolds regardless of chemistry (Figure 1).

Table 1. Pore size data of electrospun scaffolds and their processing temperature, mean ± SD. * Indicates statistical difference (p<0.05)

Polymer	Fibre Diameter (µm)	Pore size (µm)	Target temperature (°C)
PCL	2.91±1.03	17.54±3.57*	23°C
PCL	4.45±1.83	23.85±6.61*	-70°C
PDO	0.85±0.31	7.13±1.31*	23°C
PDO	0.96±0.28	8.90±1.54*	-70°C
PLA-PCL	1.77±0.36	9.93±1.41*	23°C
PLA-PCL	1.57±0.29	13.14±1.81*	-70°C

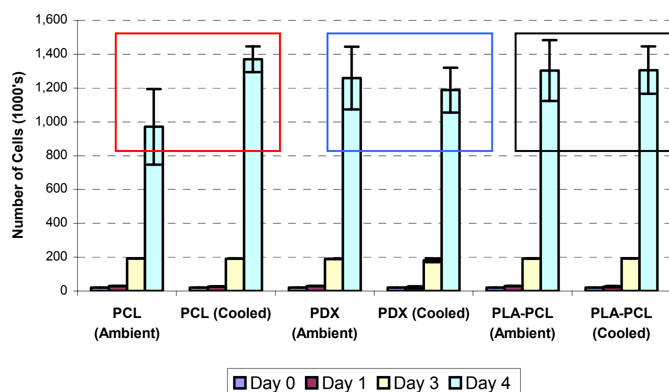

Figure 1. Estimated cell population within the individual scaffolds.

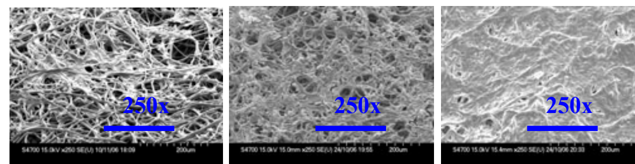

Figure 2. SEM images of cell seeded constructs (PCL cool processed left to right - 1 day, 3 days, and 7days).

SEM analysis confirmed the increase in cell density with increasing time periods. It also revealed that at Day 7 a very large number of cells have remained at the surface, unable to migrate possibly due to overpopulation (Figure 2).

Conclusions: The architectural properties and material chemistry of the scaffold play a secondary role to surface properties. Future work will assess the cell proliferation within the scaffolds and investigate the role of fiber and surface topography in modulating cellular behavior.

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References

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