Cell Infiltration and Growth in a Low Density, Uncompressed Three-Dimensional Electrospun Nanofibrous Scaffold

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Statement of Purpose

A limiting factor of traditional electrospinning is that the electrospun scaffolds consist entirely of tightly packed nanofiber layers that only provide a superficial porous structure due to the sheet-like assembly process. This characteristic hinders cell infiltration and growth throughout the nanofibrous scaffolds. Numerous strategies have been tried to overcome this challenge; however, these methods still produce sheet-like nanofibrous scaffolds with limited success to create a porous three-dimensional scaffold with good structural integrity. We have developed a three-dimensional cotton ball-like electrospun scaffold that consists of an accumulation of nanofibers in a low density and uncompressed manner. Instead of a traditional flat-plate collector, a grounded spherical dish and an array of needle-like probes were used to create a Focused, Low density, Uncompressed nanoFiber (FLUF) mesh scaffold. This method assembles a nanofibrous scaffold that is more advantageous for highly porous interconnectivity and demonstrates great potential for tackling current challenges of electrospun scaffolds.

Materials and Methods

Electrospun polycaprolactone nanofibers (ePCL) were fabricated using two methods: 1) The traditional method used a flat plate of aluminum foil as the nanofiber collection surface placed 28 cm from the nozzle of a syringe pump. 2) The new method replaced the flat sheet collector with a spherical foam dish lined on the back with aluminum foil. In addition, an array of 1 inch stainless steel needles was embedded into the dish. This method created ePCL nanofibers with a cotton ball-like structure. The morphologies of nanofibers fabricated using both methods were characterized with scanning electron microscopy (SEM). INS-1 rat insulinoma cells were seeded on the scaffolds, and cell infiltration was evaluated using H&E staining. Cell proliferation was measured using cell counting kit-8 after 1, 3, and 7 days.

Results

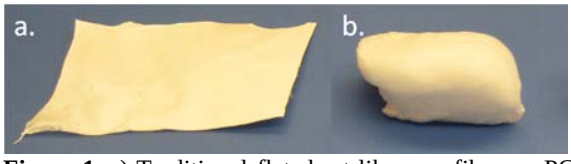

Figure 1. a) Traditional flat sheet-like nanofibrous ePCL scaffold. b) Cotton ball-like nanofibrous ePCL scaffold.

Fig. 1b shows the successful fabrication of a nanofibrous ePCL scaffold with a fully developed three-dimensional structure resembling a cotton ball. This structure is airy and porous in appearance in contrast to the traditional flat sheet-like ePCL scaffold in *Fig. 1a*. *Fig. 2* shows that the nanofiber diameters and morphologies of the three-dimensional cotton ball-like ePCL nanofibers (*Fig. 2b*) are similar (dia = 300-700 nm) to the traditional, flat sheet-like ePCL nanofibers (*Fig.* 2a). Cotton ball-like ePCL nanofibers also appear to have larger pore sizes as compared to traditional ePCL.

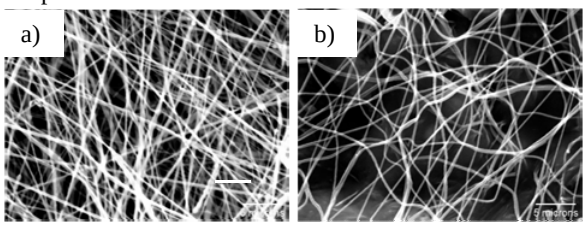

Figure 2. SEM images of ePCL nanofibers at 5000X. a) Flat sheet-like nanofibers. b) Cotton ball-like nanofibers. Scale bars = 5 μm.

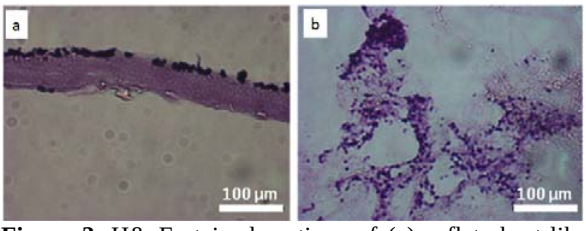

Figure 3. H& E stained sections of (a) a flat sheet-like scaffold and (b) a cotton ball-like scaffold after 7 days.

Fig. 3 shows very limited cell infiltration beyond the top surface in a flat sheet-like scaffold fabricated using a traditional method (*Fig 3a*). In contrast, there was a constant progression of cell infiltration over 200 microns through the cotton ball-like ePCL scaffold (*Fig 3b*).

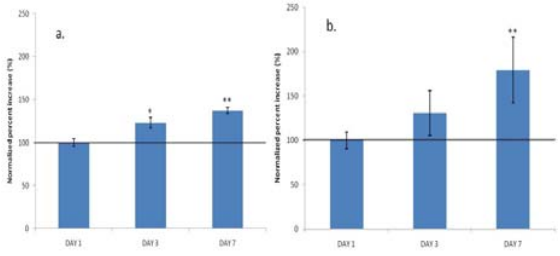

Figure 4. Normalized INS-1 cells growth on (a) the traditional ePCL scaffolds (b) cotton ball-like scaffolds. * ($p<0.05$). ** ($p<0.05$). Error bars: ± st.dev. n=4.

Over 7 days, the cell number increased 137.35 ± 3.14 % (as normalized to Day 1) on the traditional ePCL scaffolds, whereas the value for the cotton ball-like ePCL scaffolds jumped to 178.96 ± 37.09 %. Cells on the traditional ePCL scaffolds quickly proliferated to fill the available space on the top surface of the scaffold by day 3, after which the growth rate slowed due to poor cellular infiltration. Hence, there was only ~11% growth between days 3 and 7 on the traditional ePCL scaffold, compared to ~37% for the cotton ball-like ePCL.

Conclusion

This innovative strategy provides a new solution for overcoming the current challenges facing the electrospinning process and has great potential across a wide range of tissue engineering applications.

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