A Novel Three-Dimensional Cotton Ball-like Electrospun Nanofibrous Scaffold

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Statement of Purpose

A current major obstacle of electrospinning is that the scaffolds produced have tightly packed layers of nanofibers with only a superficial porous structure. This unavoidable characteristic limits cell infiltration and growth through the nanofibrous scaffolds. Numerous strategies have been tried to overcome these challenges, including incorporation of nanoparticles, hydrogels, relatively large microfibers, or the removal of salt or water-soluble fibers. However, these methods still produce flat sheet-like nanofibrous scaffolds, falling short of creating a porous three-dimensional scaffold with high structural integrity. Thus, we have developed a novel three-dimensional cotton ball-like electrospun nanofibrous scaffold by enabling a focused accumulation of low density, uncompressed nanofibers. Instead of the traditional flat-plate collector, a grounded spherical dish and an array of needle-like probes was used to generate this three-dimensional electrospun nanofiber scaffold. This system manipulates the electric field through which the nanofibers travel, thus causing them to accumulate in mid-air with a three-dimensional, cotton ball-like structure. This novel method creates a nanofibrous scaffold with a highly interconnected porous network and high structural integrity. This new method of fabricating a cotton ball-like three-dimensional nanofibrous scaffold clearly demonstrates potential to overcome the current major obstacle in electrospinning technology, and has various tissue engineering applications.

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. This method created ePCL nanofibers which deposited as layered flat sheets on the collector. 2) The new method replaced the flat sheet collector with a spherical (dia = 6 inch) 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). After sterilization with ethanol, human mesenchymal stem cells (hMSCs) were seeded on the scaffolds, and cell infiltration was evaluated at 1, 3, 7, and 10 days using H&E staining.

Results

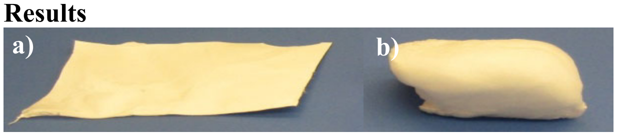

Fig. 1. a) Traditional flat sheet-like nanofibrous ePCL scaffold. b) Cotton ball-like nanofibrous ePCL scaffold.

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*.

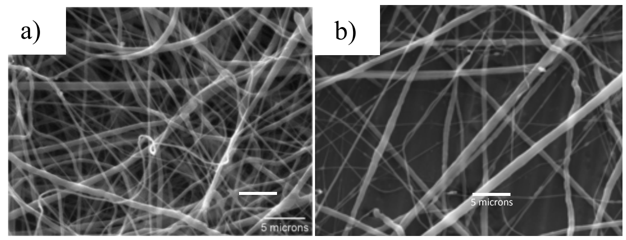

Fig. 2. SEM image of ePCL nanofibers at 5000X. a) Flat sheet-like nanofibers. b) Cotton ball-like nanofibers. Scale bars = 5 μm.

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). Moreover, the cotton ball-like ePCL nanofibers appear to have larger pore sizes as compared to the flat sheet-like ePCL nanofibers.

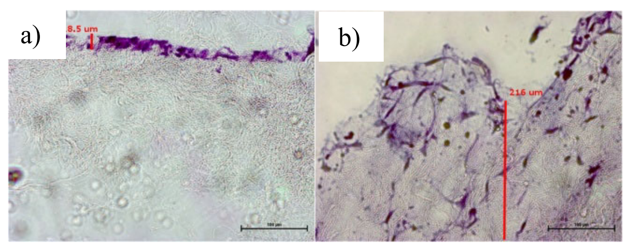

Fig. 3. Histology of cotton ball-like ePCL scaffold from a) day 1 (red bar 18.5 um). b) day 10 (red bar 216 um)

Fig. 3 shows a constant progression of cell infiltration from the surface (day 1, *Fig. 3a*) to over 200 microns (day 10, *Fig. 3b)* through the cotton ball-like ePCL scaffold. However, the traditional, flat sheet-like ePCL nanofibers showed little infiltration beyond the top surface.

Conclusion

We have successfully fabricated an electrospun cotton ball-like nanofibrous scaffold using a novel strategy. This scaffold demonstrated deep cell infiltration, which has been a challenge with the traditional method. This new approach to electrospinning presents a new strategy to overcome the challenge of current techniques, and has a potential to broadly impact many areas of tissue engineering. Currently, cell growth studies in the scaffolds are under investigation.

Acknowledgements

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