BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
13 (2017) 1563–1573

Original Article

nanomedjournal.com

# Three-dimensional functional human neuronal networks in uncompressed low-density electrospun fiber scaffolds

Albin Jakobsson, MSc[a,b,1], Maximilian Ottosson, MSc[a,b,1], Marina Castro Zalis, MSc[a], David O'Carroll, PhD[b], Ulrica Englund Johansson, PhD[a,2], Fredrik Johansson, PhD[b,*,2]

[a]*Inst. Clinical Sciences in Lund, Dept. Ophthalmology, Lund University Hospital, Lund, Sweden*
[b]*Dept. Biology, Unit of Functional Zoology, Bio-interface Group, Lund University, Lund, Sweden*

Received 13 April 2016; accepted 30 December 2016

**Abstract**

We demonstrate an artificial three-dimensional (3D) electrical active human neuronal network system, by the growth of brain neural progenitors in highly porous low density electrospun poly-ε-caprolactone (PCL) fiber scaffolds.

In neuroscience research cell-based assays are important experimental instruments for studying neuronal function in health and disease. Traditional cell culture at 2D-surfaces induces abnormal cell–cell contacts and network formation. Hence, there is a tremendous need to explore *in vivo*-resembling 3D neural cell culture approaches. We present an improved electrospinning method for fabrication of scaffolds that promote neuronal differentiation into highly 3D integrated networks, formation of inhibitory and excitatory synapses and extensive neurite growth. Notably, in 3D scaffolds *in vivo*-resembling intermixed neuronal and glial cell network were formed, whereas in parallel 2D cultures a neuronal cell layer grew separated from an underlying glial cell layer.

Hence, the use of the 3D cell assay presented will most likely provide more physiological relevant results.


*Key words:* Nanofibers; Electrospinning; Neuroscience; Stem cells; Electrophysiology

Three-dimensional (3D) modeling of neuronal networks holds great promise for new insights into central nervous system (CNS) development, function and disease pathology.

Proper development and function of the CNS in 3D rely on interactions both with other cells and with the extracellular matrix (ECM). ECM regulates key cellular functions during development such as migration, differentiation, and synapse formation, and is in adulthood important for homeostasis.[1,2] Neural cell cultures have been an invaluable tool for decades for studies to increase our understanding of brain development and normal function, as well as for exploring disease mechanisms and drug discovery. Conventional neural cell culture systems include the growth of adherent cell monolayers on flat and rigid 2D substrates; however, their physiological relevance is questioned.

Pioneering studies on the impact of 3D culture, using *e.g.* cell-aggregate cultures and hydrogels, show longer survival, different

*Abbreviations:* 3D, three dimensional; PCL, poly-ε-caprolactons; CNS, central nervous system; ECM, extracellular matrix; CAD, computer aided design; PLA, polylactid acid; PLL, poly-L-lysine; RT, room temperature; HRP, horseradish peroxidase; PFA, paraformaldehyde; PBS, phosphate buffered saline; SEM, standard error of mean; SEM, scanning electron microscope; DCX, doublecortin.

Disclosure: The following authors are to different degrees affiliated with the electrospinning technique company Cellevate AB: Albin Jakobsson (Chief technology officer), Maximilian Ottosson (Chief executive officer) and Fredrik Johansson (Technical advisor). No financial support was provided by the company.

For support we gratefully thank Med. Faculty Lund Univ., The Crafoord foundation, The Royal Physiographic Society of Lund, NanoLund and the O. Engqvist Foundation.

*Corresponding author at: Lund University, Dept. of Biology, Unit of Functional Zoology, Bio-interface Group, 223 62 Lund, Sweden.
*E-mail address:* fredrik.johansson@biol.lu.se (F. Johansson).

[1] Shared first authors.
[2] Shared last authors.

http://dx.doi.org/10.1016/j.nano.2016.12.023


Please cite this article as: Jakobsson A, et al, Three-dimensional functional human neuronal networks in uncompressed low-density electrospun fiber scaffolds. *Nanomedicine: NBM* 2017;13:1563-1573, http://dx.doi.org/10.1016/j.nano.2016.12.023

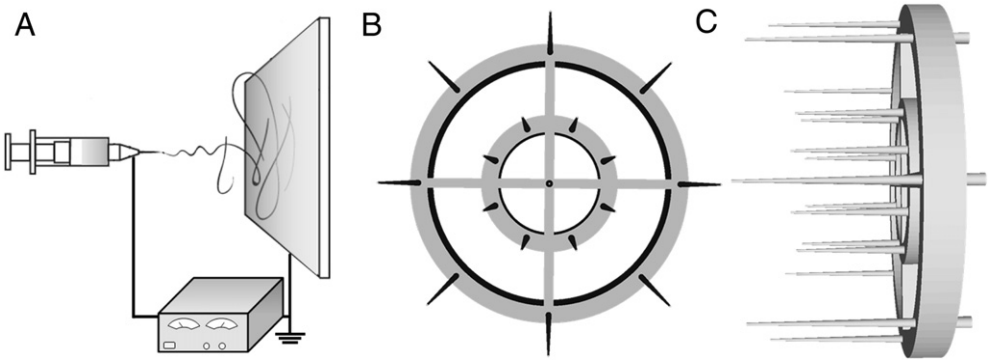


Figure 1. (**A**) Schematic representation of traditional electrospinning setup. The polymer solution in the syringe is jetted towards the grounded collector, following electrostatic forces generated by the applied voltage difference. The fibers will gather as compressed layers on the flat collector. (**B**) Front view and (**C**) side view of CAD model over 3D-printed spherical collector. The needles allow for accumulation of fibers without the compression against a flat surface.

differentiation pattern, longer neurite outgrowth and formation of higher density networks compared to 2D cell cultures.[3–6] It is thus of major interest to fully translate and explore the influence of the 3D environment on neural cell behavior utilizing biomimetic culture scaffolds. In addition, since the CNS has little or no capacity to spontaneously regenerate following injury or disease,[7,8] such biomimetic scaffolds may be able to coordinate formation of axonal growth cones, provide neurotrophic factors, inhibit formation of glial scars and support functional integration of cells in restorative approaches.[9–11]

Lately, different ECM-mimicking scaffolds have been fabricated, including sponges, fibers and hydrogels.[12,13] Electrospinning is an advantageous method for fabrication of scaffolds where the major physical (*e.g.* pore and fiber size), chemical (*e.g.* adhesion molecules) and mechanical (*e.g.* elasticity) properties of the ECM can be replicated and modified.[14] Previously, electrospun fiber scaffolds showed beneficial effects on neural cell proliferation, survival, differentiation,[15–18] and neurite formation.[11–14,19–21] Also, using electrospun fiber substrates, fiber diameter and pore size were reported to significantly affect crucial aspects of neural cell behavior, including migration, phenotypic differentiation and neurite extension.[22–26] In addition, the ability to functionalize electrospun scaffolds with active compounds such as the ECM protein laminin can control cell adhesion and neurite growth.[17,27–29] However, standard electrospinning methods, usually yield dense and tightly packed fiber meshes, not allowing full infiltration and integration of neural cells and neurites in 3D, but rather behave as a structured 2D surface. Furthermore, for *in vivo* trials, interconnecting pores are crucial for vascularization, nutrient flow and waste removal.[30]

A method for fabrication of stable and highly porous electrospun fibrous structures is warranted for further proper investigation of neural culture in 3D. Many techniques have been proposed, but unsuccessful in promoting satisfactory cell infiltration into the scaffolds.[31–33] In 2011, a method for producing 3D low density porous scaffolds without the loss of interconnecting pores was reported.[34]

Here, we firstly explored an improved electrospinning method yielding highly porous low-density fiber scaffold with maintained interconnecting pores. In order to remove the compact fiber layer-on-layer effect generated by a flat and static collector,[34] we designed and tested a novel collector comprising a concave semi-spherical array of metal needles mounted on a non-conductive base. The polymer poly-ε-caprolactone (PCL) was used since it is non-toxic, biodegradable, biocompatible, has been widely used in *in vitro* and *in vivo* experimental studies, and is approved for certain applications in the body by the US Food and Drug Administration.[11,29,35,36]

The resulting uncompressed low-density fiber scaffolds were then evaluated for the capacity of cultured human brain neural progenitor cells to survive, infiltrate, and phenotypically differentiate and form functional 3D neuronal networks, in comparison to culture at traditional 2D glass slides. We have previously reported the usefulness of such expandable and multipotent progenitors in exploration of neurorestoration *in vitro* and *in vivo*.[37,38]

Here human brain neural progenitors were seeded on the 3D fibrous scaffolds and on standard flat cell culture glass as control substrates, for the comparison of cell behavior. We describe, by using immunocytochemical, morphometric, confocal microscopy and electrophysiological analysis that uncompressed low-density nanofiber scaffolds can support the maturation of human brain progenitors into functional neuronal networks comprising intermixed neurons and glial cells expanding into the entire scaffold volume.

## Methods

### *Design and development of electrospinning collector*

The electrospinning collector baseplate (Figure 1) was designed in the free CAD software OpenSCAD and printed in biocompatible polylactic acid (PLA) on a Makerbot Replicator 2 (Makerbot Industries, USA). The collector was designed to hold two circular arrays of stainless steel needles (35 and 25 mm long respectively). The outer array had a diameter of 60 mm with needles placed at 23.5 mm intervals while the inner diameter was 30 mm and the needles placed in 11.8 mm intervals. All needles were connected to a common ground.

### *Electrospinning of scaffolds*

PCL pellets ($M_n$: 80,000, Sigma Aldrich, USA), 100 mg/mL were dissolved in a mixture of chloroform and methanol (1:1 v/v). The polymer solution was mixed for 24 h at room temperature (RT)