# Advances in the application of electrohydrodynamic fabrication for tissue engineering

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# Advances in the application of electrohydrodynamic fabrication for tissue engineering

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**Abstract**. Tissue engineering and cell-based therapy approaches require artificial scaffolds as extracellular matrix (ECM) and three-dimensional (3D) environment for clinically relevant cells to attach, be metabolically active and proliferate. Moreover, these constructs must possess mechanical and physical-chemical properties matched with certain implantation site. If all the required conditions are met, a tissue-engineered construct is considered as functional and will regenerate or replace the damaged tissue after implantation. In this work, we give a short overview of so-called electrohydrodynamic approach (EHD), e.g. with an application of electric field, to fabricate nano- and microstructured porous polymeric networks. This includes the application of electrospinning (networks) and electrospraying (micro- and macrospheres) to produce scaffolds and semipermeable hydrogel structures as a basis for tissue engineering and cell-based therapies.

## 1. Introduction

The field of tissue engineering is a subject of investigations by numerous researchers worldwide. It is involved in regenerative medicine as a promising approach for tissue regeneration, wound healing, treatment of acute and chronic diseases and for novel drug delivery strategies. This methodology includes the application of artificial tissue-engineered constructs (TECs). Scaffolds and 3D gel structures are widely applied types of TECs, which can be generated from a wide range of biomaterials. Selection of a suitable material for the generation of a scaffold is a highly crucial factor for tissue engineering; namely due to the specific response of stem cells to the surrounding environment in place of transplantation, stem cell niche [1]. In turn, the artificial tissue engineered stem cell niche should possess a range of parameters, including mimicking the ECM, cell-cell and cell-matrix interactions, mechanical and physical properties, cell recognition factors and cytokines [2]. However, these parameters may differ significantly depending on the site of transplantation and application of cell-based therapies; this has to be carefully taken into consideration and initially optimized for the application of novel 3D scaffold-based system [3].

### *1.1. Electrospinning*

There is a number of methods used to create scaffolds for tissue engineering. Among them, electrospinning has gained significant attention since its first patenting by Anton Formhals between 1931 and 1944 [4]. A significant contribution to electrospinning was made by Geoffrey Ingram

Taylor, who mathematically modeled a conical fluid droplet under the influence of electric field, so-called Taylor cone [5]. The main principle of the electrospinning is shown in figure 1(a).

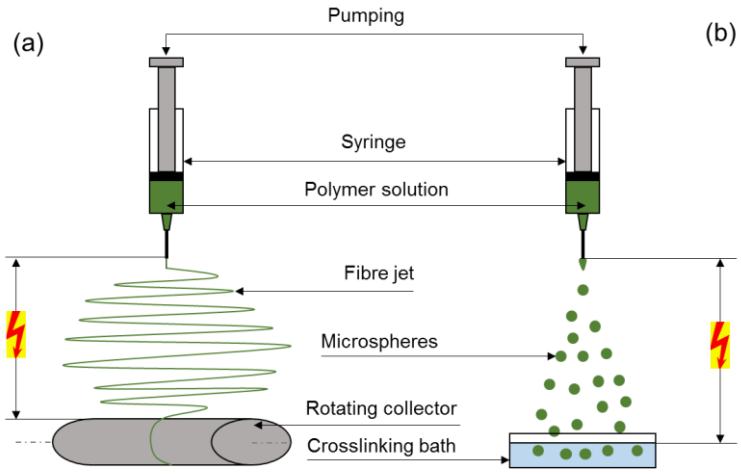


**Figure 1.** The general concept of the electrohydrodynamic process: electrospinning (a) and electrospraying (b). Drawings are not to scale.

A polymeric solution (either from natural or synthetic polymers) with relatively high viscosity is placed into a syringe and is pumped at defined flow rate through a connected needle. Then, the electric field (0.5-3 kV/cm) is applied between the needle (positive) and a collector (grounded), which also serves to collect the final material. With increasing voltage (strength of electric field), charge density within a droplet is formed resulting in its distortion. When the strength of the electric field overcomes the surface tension of the droplet solution, the Taylor cone releases thin fibres. First, it is developed into one fibre jet following the instability region (figure 1(a)). On the fibres' way to the collector, the solvent undergoes fast evaporation due to high surface-to-volume ratio of the fibre that in turn gets randomly deposited on the collector. Thus, a number of process parameters affect the electrospinning process. The main ones can be divided into solution, process, and environmental properties and are described in figure 2.

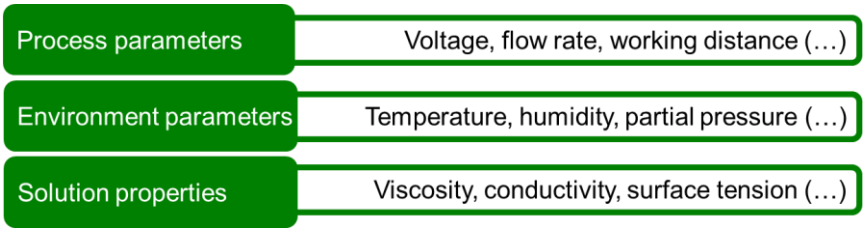


**Figure 2.** Key parameters influencing the electrohydrodynamic process

*1.2. Electrospraying*

With slight modifications to electrospinning set up and decrease in solution viscosity, this method becomes electrospraying allowing generation of polymeric microspheres [6]. The main principle of electrospraying will be explained based on the application of this method to generate alginate (naturally occurring block copolymer) microspheres for cell encapsulation and drug delivery.

The high-voltage electrospraying encapsulation applies the strength of electric field higher than 1 kV/cm for detaching alginate beads from the needle. Here, the alginate solution is kept in a syringe and pumped through the needle at defined flow rate, whereas high voltage is applied between the

positive and grounded electrode (in this case stainless steel ring immersed into crosslinking solution). The alginate droplet is getting positively charged. If the accumulated charge is enough to overcome the surface tension of alginate solution, the alginate droplet detaches from the tip of the needle and is transported to the bath containing stirred cross-linking solution with $Ca^{2+}$ ions (figure 1(b)). In the following, calcium ions replace sodium ions, causing shrinkage of alginate during the short period of time, thereby forming a gel-like structure. The flow rate of the alginate solution is controlled using a syringe pump. Upon gelling, the alginate beads are stirred at 200 rpm by a magnet in a Petri Dish using a stirrer in order to achieve homogeneous cross-linking.

## 2. Results and discussion

Electrospinning and electrospraying techniques have a common principle. The main parameters mainly affecting the size of fibres and microspheres include the strength of electric field and polymer concentration. Increase in polymer concentration results in an increase in fibre and microsphere size, whereas higher strength of electric field generates thinner fibres and smaller microspheres.

### *2.1. Electrospinning*

In the case of electrospinning, different collectors can be designed to produce various types of scaffolds including flat, tubular, and more complex 3D networks such as artificial heart valves. In the case of rotating collectors, the fibres get aligned with increasing rotation speed. This is very important for tendon regeneration, heart leaflets or other application, where fibre alignment is worth to consider. Variation in needle appearance, e.g. single, coaxial, multi-nozzle, allows relative process scaling up (this is, however, challenging due to electrostatic repulsion) and production of coaxial fibre networks for cell encapsulation and drug release applications with controlled release profile. The thickness of electrospun fibre mats depends on electrospinning time but is limited due to collector isolation upon spinning. Figure 3 exemplarily shows an appearance of electrospun tubular scaffolds from PCL-PLA (a blend of polycaprolactone and poly(l-lactide)) (figure 3(a)) and PCL-based heart valve (figure 3(d)). Figures 3(b-c) show the morphology of flat PCL-PLA fibre mats, as can be seen from images of scanning electron microscopy, the fibre diameter range is 0.5-1.5 µm, whereas thickness is 100 µm (15 kV, 25 cm, 100 mg/ml PCL and 50 mg/ml PLA blend, flow rate 3 ml/h).

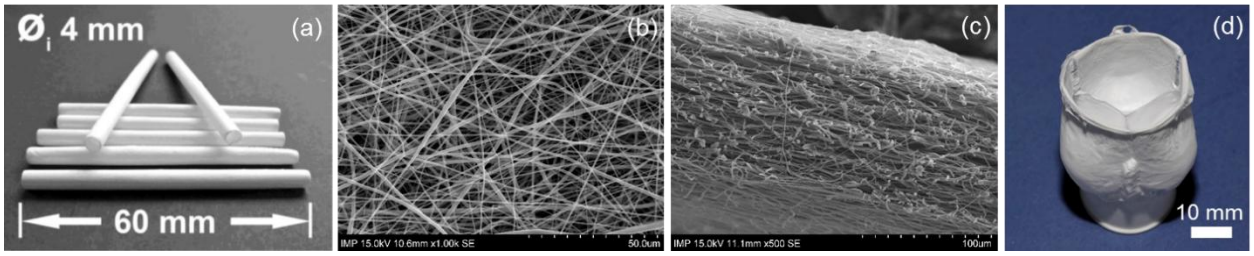


**Figure 3.** Electrospun materials: PCL-PLA tubular scaffolds (a), surface morphology (b) and thickness (c) of PCL-PLA flat scaffolds and artificial PCL heart valve (d).

Figure 4(a) shows the effect of fibre mat side (up – spinning side, down – collector side) on fibre diameter, whereas figure 4(b) depicts the effect of spinning time on the thickness of the final electrospun fibre mats. As can be seen, fibre diameter is not significantly affected by the collector, whereas the thickness of the mats can be adjusted by varying spinning time.

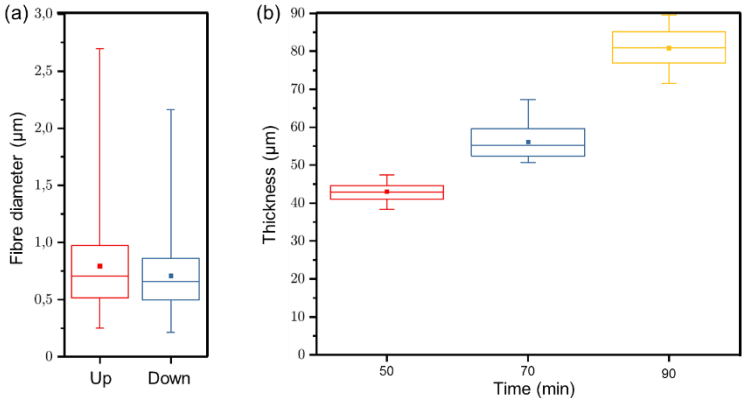


**Figure 4.** Effect of fibre mat position during electrospinning on the fibre diameter (a) as well as spinning time on the thickness of the final electrospun PCL/PLA (100:50) fibre mat. Electrospinning parameters: voltage 15 kV, spinning distance 25 cm, flow rate 3 ml/h, temperature 22°C, humidity 40%, rotation speed 250 rpm, collector diameter 15 cm.

Another important parameter of the electrospun materials is their biocompatibility. As it has been shown, electrospun PCL scaffolds possess low biocompatibility, since when seeded the cells become apoptotic even during first 3 days of culture [7]. However, its combination with PLA or gelatine increases noticeably the scaffold biocompatibility. As can be seen from fluorescence images (figure 5) of NIH 3T3 cell line cultured on electrospun flat PCL-PLA scaffolds (from figure 3(b)), only a few cells remain dead on the sixth day of culture thus showing high biocompatibility of PCL-PLA scaffolds. Quantification of cell viability showed that 95 % of cells were alive on day 6.

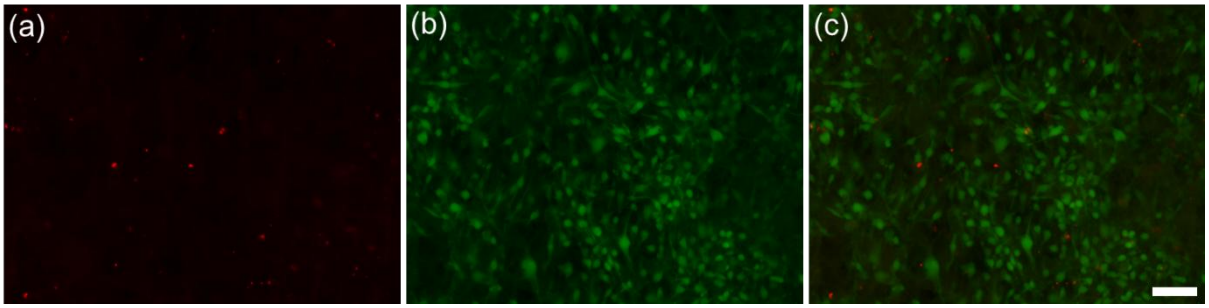


**Figure 5.** Fluorescence images of NIH 3T3 cells seeded on PCL-PLA (ratio 100:50) electrospun scaffolds on day 6: dead cells (a) stained with Ethidium Homodimer-1, live cells (b) stained with Calcein AM and their merge (c). Cell seeding density was $25\times10^3$ cell/cm$^2$. Scale bar is 100 μm.

*2.2. Electrospraying*

Encapsulation of cells, cellular component, and drugs in alginate microspheres has been advantageous for immunoisolation and drug delivery due to the semipermeable property of alginate structure after crosslinking. Alginate can be cross-linked with divalent metal ions, such as $Sr^{2+}$, $Ba^{2+}$, $Ca^{2+,}$ and others. $Ba^{2+}$-crosslinked spheres are more stable at physiological conditions; due to the toxicity of barium salts, the most commonly used gelling agent is $Ca^{2+}$. Type of gelling agent, alginate, its structural properties, and crosslinking density affect mechanical and biological properties of these microspheres. With electrospraying approach, it is possible to produce alginate microspheres with diameters ranging from 50-100 μm (the upper limit depends on the diameter of the needle used; for the single-needle approach, it is normally up to 3000 μm). For cell encapsulation, the required cell type with certain cell concentration is combined with alginate solution and gently mixed for homogeneous cell distribution.

With electrospraying, it is possible to produce solid alginate beads (figure 6(a)), multistructural alginate beads mimicking *Volvox* spheres (figure 6(b)) as well as coaxial alginate capsules (figure 6(c)).

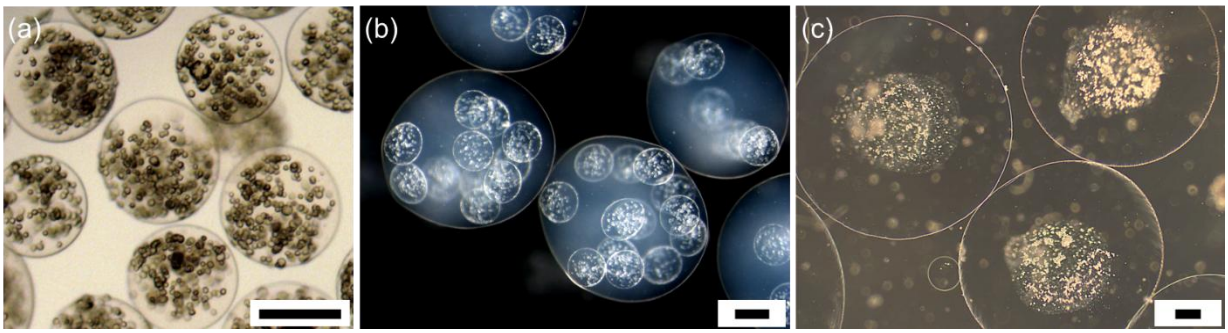


**Figure 6.** Different alginate structures produced using electrospraying: solid alginate beads with encapsulated amniotic mesenchymal stromal cells (a), multi-structural alginate spheres with solid beads inside (b), and coaxial alginate capsules with formed 3D cell aggregates (c). Scale bar is 250 µm.

All these systems have their advantages and drawbacks. Solid alginate beads are of high importance to the development of bioartificial liver support and immunoisolation, multi-structural beads can be utilized for separate co-culture strategies, whereas coaxial alginate capsules are of high importance to design models of 3D cell culture.

*2.3. Cryopreservation*

As soon as a functional tissue-engineered construct has been designed, it is of utmost interest to find optimal approaches for their storage upon demand. Among storage methods, cryopreservation is the most efficient process yielding high cell viability and functionality after long-term storage and thawing. Cryopreservation includes application of so-called cryoprotective agents (CPAs) in order to protect cells and tissues during cryopreservation (membrane stabilization, ice recrystallization inhibition etc.) to cells and tissue-engineered constructs, cooling down to glass transition temperature (Tg, can be measured using differential scanning calorimetry and is specific of certain CPAs), storage below this Tg, heating back to room temperature upon demand, CPA removal, cultivation or construct perfusion for 24 hours [8]. Cryopreservation includes mainly slow cooling and vitrification. Both approaches have their own advantages and drawbacks. In figure 7, a novel concept on cryopreservation of cell-seeded tissue-engineered constructs (figure 7(a)) within cryobags using slow cooling (figure 7(b)) and vitrification (figure 7(c)) is shown. Application of cryobags will improve heat transfer significantly as compared to common freezing in cryovials.

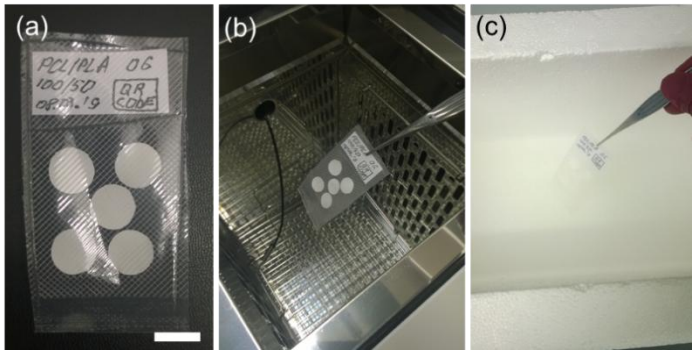


**Figure 7.** Cryopreservation strategies for cell-seeded electrospun flat scaffolds: application of cryobags (a) for slow cooling in a controlled-rate freezer (b) and vitrification using direct immersion into liquid nitrogen (c). The scaffolds in (a) were punched to a size of 10 mm. Scale bar in (a) is 10 mm.

Cryopreservation of cells within alginate structures produced using electrospraying could improve cell viability and functionality after thawing. This is mainly due to the viscoelastic properties of these structures, which can also serve as a reservoir for CPAs. Moreover, the alginate structure can resist ice formation during freezing and recrystallization upon thawing thus protecting the encapsulated cells

from damaging factors during cryopreservation [9]. Figure 8(a) shows the cell-free alginate formulations before and after cryopreservation. As can be seen, optimal cryopreservation parameters for big capsules have still to be optimized to yield intact structures after thawing. Figure 8(b) represents the effect of electric field and mixing ratio on number of inner microspheres for multistructural formulation before cryopreservation.

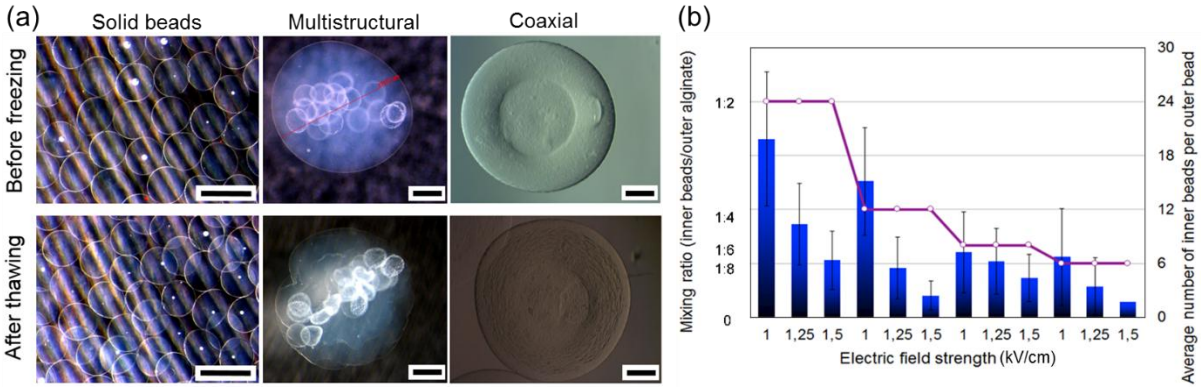


**Figure 8.** Cryopreservation of stem cells within cell-free alginate structures: (a) first raw – before freezing, last row – after cryopreservation with 10% (v/v) dimethyl sulfoxide, freezing at 1 K/min cooling rate and subsequent thawing in a 37 °C water bath; (b) effect of strength of electric field and mixing ratio on number of inner microspheres for multistructural formulation before cryopreservation. Scale bars in (a) represent 500 μm.

## 3. Concluding remarks

The methods of electrohydrodynamic fabrication including electrospinning and electrospraying are promising approaches to production of scaffolds and encapsulation strategies for tissue engineering, regenerative medicine, and cell-based therapies. In order to design functional tissue-engineered construct it is important to consider the properties and cell niche of transplantation site, and to use highly biocompatible components to avoid immune rejection after transplantation.

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