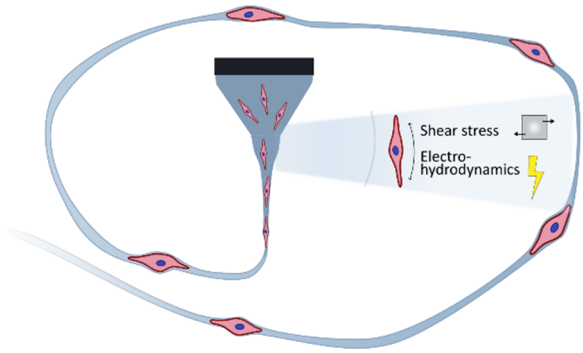
*Review*

# Cell Electrospinning: Electrohydrodynamic Effects on Cell Viability and Beyond

Anne-Kathrine Kure Larsen and Menglin Chen *

Department of Biological and Chemical Engineering, Aarhus University, 8000 Aarhus C, Denmark
* Correspondence: menglin@bce.au.dk
Received: 9 June 2025; Revised: 9 August 2025; Accepted: 13 August 2025; Published: 19 August 2025

**Abstract:** Electrospinning is a widely used technique for creating nano- to microscale fibers that resembles the fibrous structure of the extracellular matrix (ECM) environment, crucial for tissue engineering and disease modelling. Directly incorporating living cells into the electrospinning process, 'cell electrospinning', has evolved in the last two decades as a new biofabrication method combining homogenous cell loading with the potential of single cell resolution. However, keeping cells viable and functional during the electrohydrodynamic process is an ongoing challenge. In this review, key parameters in electrospinning affecting mammalian cell viability and functionality are assessed with the goal of identifying the most critical ones in the successful production of living cell-embedded fibers. The review further outlooks the potential mechanobiological and electrophysiological effects on the cells exposed under the electrohydrodynamic condition to layout a couple unexplored applications.




**Keywords:** cell electrospinning; biofabrication; tissue engineering; viability; mechanobiology; electrical stimulation

## 1. Introduction

Electrospinning is a versatile technique capable of generating continuous, ultrafine fibers with diameters at the nanometer to micrometer scale. The technique finds broad applications in diverse fields, including drug delivery [1], tissue engineering [2], air filtration [3], and food biosensors [4]. The underlying principle of electrospinning involves the formation of a "Taylor cone" from a charged jet of a liquid viscous solution subjected to a strong electric field, first described by Sir Geoffrey Taylor in 1964 [5]. However, it was until 1971 when Baumgarten obtained acrylic microfibers [6], electrospinning started to gain momentum as an efficient technique for the production of nano- and microfibers. In parallel with the development of electrospinning, electrospraying emerged as well. This technique, while utilizing similar principles as electrospinning, employs lower solution viscosity to generate micro- and nano-sized droplets instead of fibers [7].

In the biomedical research field, electrospinning has been widely applied to produce fibers that are capable of mimicking the fibrous morphology of the natural extracellular matrix (ECM) surrounding the cells in our bodies [2]. The ECM can differ in composition and mechanical properties, depending on the specific tissue requirement [8]. Being able to artificially mimic these fibrous ECM structures provides opportunities to study the effects of morphology, chemical composition and mechanical stiffness on cell response in a relatively controlled manner. One of the obstacles in the use of electrospun fibers for tissue engineering is the limited distribution of cells inside the volume of the densely packed fibrous matrix when seeding cells on top of an electrospun scaffold. To overcome this issue, an extension of electrospinning has emerged: Cell electrospinning. This technique involves the same principles, but instead of seeding on top of electrospun fibers, living cells are added directly into the electrospinning solution to ideally result in cell-embedded fibers [9], creating uniform and in-depth cell distribution within the fibrous matrix [10,11].

Other emerging biofabrication techniques such as 3D bioprinting, incorporating cells in the bioprinting materials, already offers a solution for in-depth cell distribution, but suffers from limited spatial resolutions, such as above 100 μm for extrusion-based bioprinting [12] and above 50 μm for light projection-based bioprinting, and subsequent suboptimal oxygen and nutrients diffusion [13,14]. Bioprinting techniques with higher resolution are highly desired for replicating physiological features at cellular scale [15]. Because of the inherent high resolution of electrospinning with fiber diameters of a few hundred nanometres to a few micrometres [16], electrospinning with living cells could potentially be a powerful tool to meet the needs of both higher resolution and better nutrient diffusion.

Cell viability is often compromised during the bioprinting process, especially in extrusion-based bioprinting where cells experience shear stresses during the extrusion [17]. Although cell viabilities above 90% have been achieved in bioprinting, a delicate balance between material stiffness, viscosity, processing parameters and resulting porosity is crucial for obtaining both high cell viability and printing fidelity [14]. Compared to bioprinting which is a much more established technology, obtaining viable cell-loaded, higher resolutions structures via cell electrospinning is still under development. While good viabilities above 90% have also been reported, some studies suffer from much lower viabilities down to 15–25% [18] and slight changes in parameters were found to influence them drastically [19,20].

Therefore, achieving high cell viability throughout the process of electrospinning is an ultimate prerequisite for the applications of cell electrospinning. There are several excellent and recent reviews on cell electrospinning, including materials (Maurmann et al. [9]), its critical processing parameters and biomedical applications (Elveren et al. [11]). Cell viability has been evaluated in some highlighted studies, emphasizing parameters important for cell fate; however, there is still ambiguity to which parameters play the most significant roles for cell survival and function during an electrospinning process. The aim of this review is to investigate the cell electrospinning process and solution parameters, and their possible interplay. This will be done through a thorough literature review with focus on discussing reported cell viabilities in relation to electrospinning parameters and resulting fiber morphologies. Cell electrospinning has been conducted on various cell types, including mammalian, bacteria and yeast cells; A few reviews have included the latter two [21–23], but in this review, we limit the scope to mammalian cell electrospinning for tissue regeneration application. The review will cover the majority of conducted studies on mammalian cell electrospinning and highlight the critical parameters focusing on their role in maintaining cell viability. We believe that a better understanding of the interplay between process parameters and cell fate will help improve the success rate for obtaining viable cell-embedded fibers, further leading to the broadening of the application of single cell-embedded fibers that may provide beyond morphological guidance.

## 2. Principles of Electrospinning and Cell Electrospinning

Theoretically pinned as electrohydrodynamics, which describes how an electric field influences a charged fluid, electrospinning is based on the extension of viscous liquid jet from a syringe by an applied electric field, forming sub-micron scale fibers. In the setup, the viscous liquid dispenser is placed above or next to a collector plate. While an electric field is applied across the needle and collector, and the liquid is pumped out at a controlled flow rate, a droplet emerges forming a Taylor cone [5] (Figure 1) to initiate a jet whipping into submicron fibers deposited on the collector. Various parameters affecting electrospinning performance and morphology of electrospun fibers can be modified such as the distance between needle and collector (typically 1–20 cm), the applied voltage (typically 1–20 kV), electric field strength, and the solution flow rate (Figure 1). Instead of a solid collector, the fibers can also be spun directly into a liquid coagulation bath [24].

Cell electrospinning shares the same principle and procedure, except incorporating living cells into the electrospinning solution. Cell electrospinning has been reported with a variety of components, cell types and process parameters. An overview of reported studies can be seen below (Table 1) with the most important parameters listed. In the next section, the challenges associated with cell electrospinning and the influence of the most dominant parameters on cell viability will be presented and discussed.

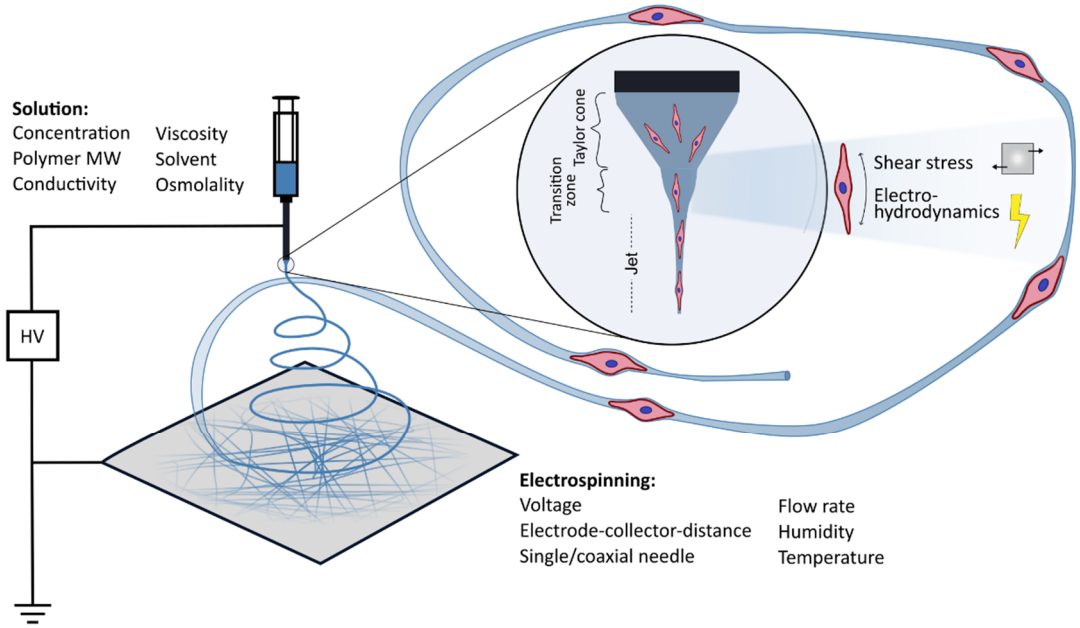



**Figure 1.** Illustration of Cell electrospinning, the typical electrospinning setup, crucial solution properties and operational parameters.

**Table 1.** Overview of publications using cell electrospinning and cell electrospraying and their parameters and highest obtained cell viabilities. Abbreviations: PDMS: Polydimethylsiloxane, PVA: Polyvinyl alcohol, PLLA: Poly(l-lactic acid), DMF: N,N-dimethyl formamide, DCM: dichloromethane, PEG: Polyethylene glycol, PEO: Poly(ethylene oxide), PCL: Polycaprolactone, FBS: Fetal Bovine Serum.

| Technique | Materials | Solvent | Voltage/Collector Distance | Electric Field Strength (kV/mm) | Flow Rate (mL/h) | Cell Type | Cell Viability | Year | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Electrospinning (coaxial) | Shell: PDMS, core: Cell suspension | Core: Cell medium | 9 kV/105 mm | 0.09 | 0.36/36 (core/shell) | EHDJ/1321N1 cells | 67.6 ± 1.9% (after fiber dissolution) | 2006 | [25] |
| Electrospinning | 8% PVA (MW = 205 kDa) | DI water/cell medium | 6 kV/150 mm | 0.04 | 0.5 | 3T3-L1 mouse fibroblasts | 15–25%/>90% in culture medium (electrospray) | 2011 | [18] |
| Electrospinning (coaxial) | Shell: 12% PLLA (MW = 140 kDa), core: 10% PEG (MW = 35 kDa)/PEO (MW = 900 kDa) + cells | Shell: DMF/DCM, Core: Aqueous solution | 20 kV/150 mm | 0.133 | 1.2/1.5 (core/shell) | PC12 cells | NA | 2012 | [26] |
| Electrospinning | 10% PVA | Milli-Q water | 21 kV/150 mm | 0.14 | 0.34 | Mononuclear cells (MNCs), mesenchymal stem cells (MSCs) | 8.38–19.6% | 2012 | [27] |
| Electrospinning (coaxial) | Shell: 12% PCL (MW = 70–90 kDa), Core: 2% PEO (MW = 600 kDa) + 2% FBS | Shell: Chloroform/DMF 60:40, Core: 50% FBS | NA | 0.15 | NA (ratio of 1:6 (core/shell)) | Human umbilical vein endothelial cells (HUVEC) | NA (but 97% in PEO/FBS solution compared to 3% in PEO alone) | 2014 | [28] |
| Electrospinning (coaxial) | Matrigel rich collagen biopolymer | NA | 8 kV/20 mm | 0.4 | NA | Primary cardiac myocytes | ~80% | 2014 | [29] |
| Electrospinning | 2% alginate, 2% PEO (MW = 900 kDa), 0.7% lecithin | PBS in water | 11.2 kV/70 mm | 0.16 | 0.5 | MG63 cells | >80% | 2015 | [30] |
| Electrospraying | Cell suspension | Cell culture medium | 15 kV/40 mm | 0.375 | 2.60 | Mesenchymal stem cells | 89 ± 4.6% | 2015 | [31] |
| Electrospinning | 2% alginate, 3% PEO (MW = 900 kDa) | Tri-distilled water | 0.7–1.75 kV/14 mm | 0.05–0.125 | 0.25 | C2C12 myoblasts | 90% | 2018 | [32] |
| Electrospinning (coaxial) | Shell: 20% PCL (MW = 70–90 kDa), core: 10% PVA (MW = 85–124 kDa) | Shell: Chloroform, Core: Cell culture medium | 6 kV/60 mm | 0.1 | 1/1 (core/shell) | PC12 | >95% | 2018 | [33] |
| Electrospinning | 1% Fibrinogen, 0.2% PEO | DI water w. 5% glucose | 4.5 kV/80 mm | 0.056 | 7.5 | C2C12 cells as cellular aggregates | NA (estimate: ~50–60%) | 2019 | [34] |
| Electrospinning | 0.5% Gelatin, 0.5% pullulan | Serum-free culture medium | 8 kV/75 mm | 0.107 | 1.8 | Adipose tissue-derived stem cells (hASCs) | 90% | 2020 | [20] |

**Table 1.** *Cont.*

| Technique | Materials | Solvent | Voltage/Collector Distance | Electric Field Strength (kV/mm) | Flow Rate (mL/h) | Cell Type | Cell Viability | Year | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Electrospinning (coaxial) | Shell: 15% poly(lactic-co-glycolic acid) (82/18 molar ratio), 5% PEO (MW = 10.065 kDa), core: 6% PVA (MW = 85–146 kDa) | Shell: Chloroform, core: DI water/cell medium | 10 kV/50 mm | 0.2 | 10/50 (core/shell) | Pheochromocytoma 12 (PC12) cells | NA | 2020 | [35] |
| Electrospinning | 2% Sodium alginate, 3% PEO (MW = 900 kDa) | Triple-distilled water | 10.5 kV/140 mm | 0.075 | 0.25 | Human umbilical vein endothelial cells (HUVECs) | 90% | 2020 | [36] |
| Electrospinning | 12% Poly(vinyl) alcohol (PVA, MW = 89.000–98.000) | 10:1 Deionized water/cell culture medium | 1–2 kV/70–100 mm | 0.029 | 1 | MC3T3 osteoblast precursor cells | 81% | 2021 | [37] |
| Electrospraying | Laminin modified Alginate | 20 mM HEPES | 18 kV/15 mm | 1.2 | 0.04 | Adipose derived stem cells (ADSCs) | 99% | 2021 | [38] |
| Electrospraying | 0.125% Gelatin, 0.125% pullulan | Serum-free media | 10 + 15 kV/70 mm | 0.143–0.214 | 12 | Human adipose-derived stem cells (hASCs) | 90 + 70% | 2021 | [39] |
| Electrospraying | Cell suspension | Cell culture medium | 9 kV/50 mm | 0.18 | 2 | C28/12 chondrocytes, primary chondrocytes | <91% | 2022 | [40] |
| Electrospinning | 2% Alginate/3% PEO (MW = 900 kDa) | Triple-distilled water | 10.5 kV/140 mm | 0.075 | 0.25 | Smooth muscle cells (SMCs) and Mesenchymal stem cells (MSCs) | >90% | 2023 | [19] |
| Electrospinning | 50% Gelatin (fish) (MW = 50–100 kDa) | PBS | 20 kV/50 mm | 0.40 | 2.4 | Mesenchymal stem cell (MSC) | >90% | 2023 | [41] |
| Electrospinning | 7.5% hydrazide functionalized POEGMA (POH), 2.5% PEO (MW = 600 kDa) | DMEM medium/PBS | 10 kV/10 mm | 1 | 0.9 | 3T3 fibroblasts and Psi2 12S6 epithelial cells | NA but ≈4-fold increase in cell density at day 14. | 2023 | [42] |
| Electrospinning | 7.5% hydrazide functionalized POEGMA (POH), 2.5% PEO (MW = 600 kDa) | PBS:DMEM 3:1 | 10 kV/100 mm | 0.1 | 0.9 | C2C12 mouse myoblast | >85% | 2023 | [43] |
| Electrospinning | 10% polyvinylpyrrolidone (PVP) (MW = 360 kDa) | PBS | 8 kV/100 mm | 0.08 | NA | Human umbilical cord mesenchymal stem cells (HUCMSCs) | 88 ± 4.3% | 2024 | [44] |

## 3. Challenges in Cell Electrospinning

Cell electrospinning is demanding in terms of the choice of materials and solvents. The options are limited compared to conventional electrospinning where organic solvents often are used to dissolve polymers, which should naturally be avoided when incorporating living cells. In addition, the materials should be biocompatible, non-toxic, and the degradability should be considered depending on the application. Furthermore, the electrospinning parameters should be fine-tuned to maintain both viable cells and spinnability. Collectively, the spinnability of the solution and inclusion of living cells in the extruded fibers is a delicate balance [11]. The limited number of publications using cell electrospinning underlines the necessity to better understand the parameters that affect both spinnability and viability, thus obtaining fully viable and functional cells inside thin single cell-sized fibers is still an essential challenge [32,34]. In the next sections, the different parameters in cell electrospinning will be discussed and assessed for their influence on cell viability and function with examples from literature.

### *3.1. Solution Components*

Solution composition is critical in cell electrospinning since living cells are incorporated. The main criteria to consider are the use of non-cytotoxic and biocompatible materials and water as solvent, in contrast to the typical organic solvents such as hexafluoro-isopropanol, chloroform, dichloromethane or dimethylformamide in conventional electrospinning [45]. The choice of solvent will affect spinnability of the polymer together with the resulting fiber diameter and morphology [46]. When a polymer solution is extruded as a jet into the air, fibers are formed from electrostatic tensile forces and solidification of the jet as the solvent evaporates. The behaviour of the jet is furthermore influenced by the viscosity and the viscoelasticity of the polymer solution. At high viscosities, larger jets and fibers are formed, but below a certain threshold of viscosity, the jet can start breaking into droplets resulting in electrospraying instead of spinning [46,47].

The use of aqueous solution for preserving cell viability requires hydrophilic polymers. These are often natural, biobased polymers e.g., proteins such as collagen, silk fibroin, fibrinogen or collagen-derived gelatine [48], polysaccharides like sodium alginate [49], pullulan [50] and glycosaminoglycans such as hyaluronic acid [51], or synthetic but biocompatible polymers such as poly(vinyl alcohol) (PVA). Polyelectrolytes alone have inherently low spinnability due to limited chain entanglement. This is the case for one of the most common polysaccharides in cell electrospinning, sodium alginate (SA), in which limited ability for the negatively charged alginate chains to entangle sufficiently hinders fiber formation [32]. SA is therefore usually co-electrospun together with other polymers such as poly(ethylene oxide) (PEO) as a carrier polymer to increase molecular entanglement and viscosity, and with processing agents such as lecithin [30,32,52–54]. Importantly, since the polymers are water soluble but would have to be stable in cell culture medium after cell electrospinning, rapid in situ crosslinking of the materials is needed for the fibers to maintain their structure. Various strategies have been applied varying depending on the material e.g., physical crosslinking, enzymatic crosslinking or click chemistry photocrosslinking. SA as an example, rapid ionic physical crosslinking is achieved by immersing fibers into a $CaCl_2$ solution in which $Ca^{2+}$ ions substitute $Na^{2+}$ ions and cause ionic bonding between $Ca^{2+}$ and the carboxyl groups on sodium alginate [55]. Enzymatic crosslinking has, on the other hand, been performed using fibrinogen by the addition of thrombin that converts fibrinogen molecule to insoluble fibrin [34].

A way to solve the limited choices of hydrophilic polymers with rapid crosslinkability for preparing aqueous cell-loading solutions, is the use of coaxial cell electrospinning. A coaxial needle is used to separate the core and shell flows, enabling the use of polymers requiring organic solvents in the shell compartment. By retaining the cells suspended in culture medium or aqueously dissolved polymers in the core, the two solvents are immiscible during electrospinning and thus the cells are not in contact with the organic solvent which is further evaporating quickly. As a benefit, crosslinking is optional as the shell is often a hydrophobic polymer, only needed if the core material has to be crosslinked as well [26,33].

Osmolarity is also an important parameter to consider for cell viability. Aqueous cell electrospinning solutions should thus also contain surfactants, cell culture additives and salts for creating a physiological relevant environment with the correct osmolarity. As an example, in a study of Ang. et al. they observed an increased viability from 2% to 97% for HUVECs in a 2% PEO solution by addition of 50% FBS, with significant increase in viability already seen at 5% FBS. The low viability in PEO alone was suspected to be caused by the hypotonic nature of the aqueous solution in relation to the cell interior, with a low osmolality of 0.41 mOsm/kg compared to physiological osmolality of 270–290 mOsm/kg, causing water influx across the cell membrane. Besides its nutritious effect, the addition of FBS to the cell-containing electrospinning solution was anticipated to increase osmolality closer to physiological conditions and also demonstrated enhanced cell survival [28]. 5% glucose has been used as an alternative to an ionic buffer such as PBS in order to match cellular osmolarity while keeping the

solution at low ionic concentrations to maintain conductivity suited for electrospinning and decrease the current on the cells [34]. These examples highlight the importance of considering physiological osmolarity for obtaining viable cells in electrospinning and other biofabrication processes.

The ability of a polymer to facilitate cell adhesion is also important for cell survival. For example, PVA has limited cell adhesion owing to the low protein adsorption, so various biopolymers such as gelatin and chitosan have often been co-electrospun with PVA as electrospun scaffolds, resulting in increased cell adhesion and survival upon seeding [56,57]. Porcine and fish gelatin have been popular choices in both conventional electrospinning and cell electrospinning, owing to its natural collagen origin, biocompatibility, low cost and cell adhesion properties. In both studies listed here, a very good viability has been obtained for electrospun fibers including gelatin of either fish or porcine origin [20,41]. Gelatins from different animal sources are different in their chemical structure, resulting in different sol-gel transition temperature. Fish gelatin has been proposed as an alternative to the commonly used porcine gelatin as cold-water fish gelatin has a lower sol-gel transition temperature, below ambient temperature opposed to porcine gelatin that gels at room temperature, requiring extra control over ambient temperature during electrospinning [58]. On the other hand, crosslinking leads to very different storage moduli, 723–7340 Pa for porcine and 294–464 Pa for fish gelatin, which could ultimately influence the behaviour of cells embedded in electrospun fibers [59].

### *3.2. Electric Field Strength*

The electric field strength, determined by the ratio of applied voltage to the distance between the electrode and the collector, is a critical parameter in electrospinning. However, while the main limit to the strength of the electric fields is directed by the range yielding good spinnability and suitable fiber diameters and morphologies in conventional electrospinning, the parameter seem to be more crucial in cell electrospinning. Excessive electric field strength has been stated to negatively affect cell viability as a too strong electric field can disrupt the cellular membrane [32,60,61], however no definite quantitative relationship between electric field strength or time and cell viability has been established yet.

It is well-known from the use of electroporation in DNA delivery methods, that cells can sustain a certain electric field, with electroporation typically using electric pulses at 0.5–1 kV/cm [62]. It is noteworthy that there is usually a fraction of the electroporated cells that do not survive it [63]. There may be similar effect in cell electrospinning, even though considerable differences in electric field strengths have been reported throughout the listed studies (Table 1), varying from 0.029 to 0.4 kV/mm (only taking into account the studies that reported viability results). For electrospraying, the electric fields are often much stronger and better tolerated by cells, however it fails to facilitate continuous fiber production. Yang et al. successfully produced laminin-alginate microspheres using cell electrospraying with a strong field at 1.2 kV/mm and resulting 99% viability of adipose derived stem cells [64]. It was already reported by Jayasinghe et al. as one of the first instances of cells processed in an electric field in 2006 that Jurkat cells could survive jetting in a 2 kV/mm electric field with no observable cell damage [65].

This difference in the range of electric fields that cells can sustain during electrospinning may vary among cell types. From electroporation studies with 15 different cell lines, O'Hare et al. reported that the different cell types showed markedly different levels of electrosensitivity, which could not be ascribed to cell size or culture conditions. From this study, they found lymphoid cells to be most sensitive, followed by epithelial and fibroblast cells [66]. However, in another study investigating electropermeabilization and electrosensitivity, no trend in electrosensitivities of specific tissue types was found, instead significant differences in electrosensitivities appeared among different tumor cell lines, even from the same tumor type [67]. However, the electric field strengths reported in the studies listed in this review vary greatly; it is reasonable to question if the difference could lie in other process parameters or composition of electrospinning material as well. There are examples where various field strengths have been employed on the same cell type, such as C2C12 in the studies of Yeo et al. [32] and Dawson et al. [43]. Yeo et al. reported cell viability at >80% when electrospinning a solution of 2% alginate and 3% PEO at up to 0.1 kV/mm while it decreased drastically to around 10% at 0.125 kV/mm (Figure 2a) [32]. Dawson et al. reported on similar viability at 0.1 kV/mm with same cell type in a solution of 7.5% hydrazide functionalized POEGMA (POH) and 2.5% PEO (Figure 2b,c). However, with electrospraying cell suspension in culture medium, even higher electric fields of up to 0.15 kV/mm could be applied without affecting cell viability noticeably (Figure 2d) [43]. This example highlights the importance of evaluating electric field strength effects in combination with other parameters such as solution composition and physical properties. For example, material conductivity and dielectric properties of both the solutes and the solvents are important to consider in relation to electric field strength. At the onset of electrospinning, the taylor cone formation is highly affected by the

availability of free charges in the solution that is further influenced by the dielectric property of the solution. As cell electrospinning is performed in aqueous solution such as medium or PBS buffer which include ionic salts for retaining the correct conditions for living cells, the conductivity is markedly increased compared to water. Cell culture medium and PBS buffer typically have conductivities around 1.5–2 S/m [68,69] and 1.4 S/m [70], respectively, compared to a conductivity of 5.5 µS/m for pure water, and when exposed to air, 0.1–0.2 mS/m [71]. While not directly shown for electrospinning, medium conductivity has shown to negatively influence the survival of Chinese hamster fibroblasts (DC3F) cells in electropermeabilization [69]. The electric field strength is therefore not the only parameter to consider when evaluating the effect of the electric field on living cells; besides affecting the electrospun cells, it also affects the electrospinning process itself, including electrospinnability and fiber morphology [72–74].

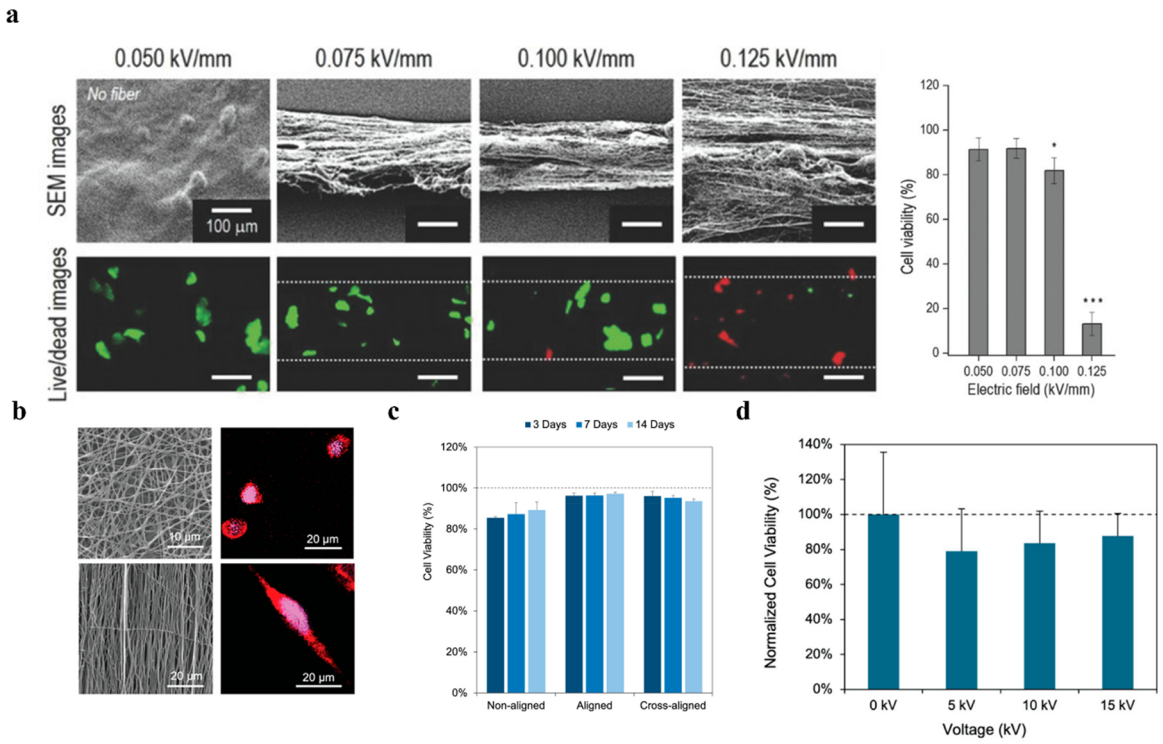



**Figure 2.** Electric field effects on cell viability. (**a**) C2C12 myoblast viability in increasing electric fields during cell electrospinning. * $p < 0.05$, and *** $p < 0.001$, scale bars = 100 µm [32], (**b**) C2C12 myoblasts electrospun in random and aligned fibers, red: CellTrace CFSE, blue: DAPI, their viabilities (**c**) at day 3, 7 and 14 at 0.1 kV/mm and (**d**) electrospray in cell suspension [43]. (**a**) Reproduced with permission from ref. [32]. Copyright 2018 John Wiley and Sons. (**b**) Reprinted (adapted) with permission from [43]. Copyright 2023 American Chemical Society.

### *3.3. Viscosity and Shear Force*

Viscosity is a critical parameter in electrospinning and fiber formation, however, in the context of cell electrospinning it is even more critical with a smaller process window. A certain viscosity is needed to reach good spinnability, but if the viscosity becomes too high, the charges initiating the spinning may be insufficient for stretching the viscous material into a fiber. At this point, spinning would require higher applied voltage, leading to heterogeneous fibers and would eventually result in a higher shear force which can be damaging to living cells. On the contrary, too low viscosity results in electrosprayed droplets instead of fibers [47]. The viscosity is therefore important to control and monitor, however, only two studies included in this review reports on actual viscosity [25,26]. Instead, polymer concentrations are typically given, but reproducing experiments with electrospinning solely based on published polymer concentrations is challenging. Variations in molecular weight, environmental factors like temperature, which significantly influences viscosity, can lead to unpredictable changes in fiber morphology or even render the results irreproducible [75].

The influence of shear force on cell viability during electrospinning is rather underrepresented. The shear force acting on an object in a viscous solution is partly determined by viscosity of the solution and is proportional to the viscosity at constant flow rate [28]. The higher the viscosity, the greater force is required to extrude the

solution, resulting in higher shear stress. This is also a well-known challenge in extrusion-based bioprinting [17] (Figure 3a). For example, it was observed by Kim et al. that cell viability decreased significantly to below 80% from 93% when the collagen content in their bioink was increased from <5% to >7% [76]. Shear stress effects were also observed biologically by Ang. et al., with upregulation of IL-8 expression in HUVECs endothelial cells post electrospinning (Figure 3b) [28]. Looking across studies using the same polymer such as PVA but achieving very different cell viabilities could also relate to viscosity. In these studies, 8–12% PVA is used either for single needle electrospinning or as the core with cells in coaxial electrospinning. The very low viabilities of 15–25% and 8.38–19.6% obtained in the studies by Canbolat et al. [18] and Zanatta et al. [27], respectively, share the use of higher molecular weight polymers compared to the other two studies by Wu et al. and Das et al. [33,37]. The use of high MW polymers must have resulted in higher viscosities and higher shear stresses on the cells in the electrospinning process. This is further supported by the fact that electrospraying cells in culture medium with low viscosity yielded much better viability (>90%), reported by Canbolat et al. [18]. In addition, these studies [18,27] yielding low cell viabilities used DI water/cell medium and MilliQ-water in the electrospinning solution, respectively, further indicating that an osmotic pressure mismatch could also have negatively influenced the cell viability.

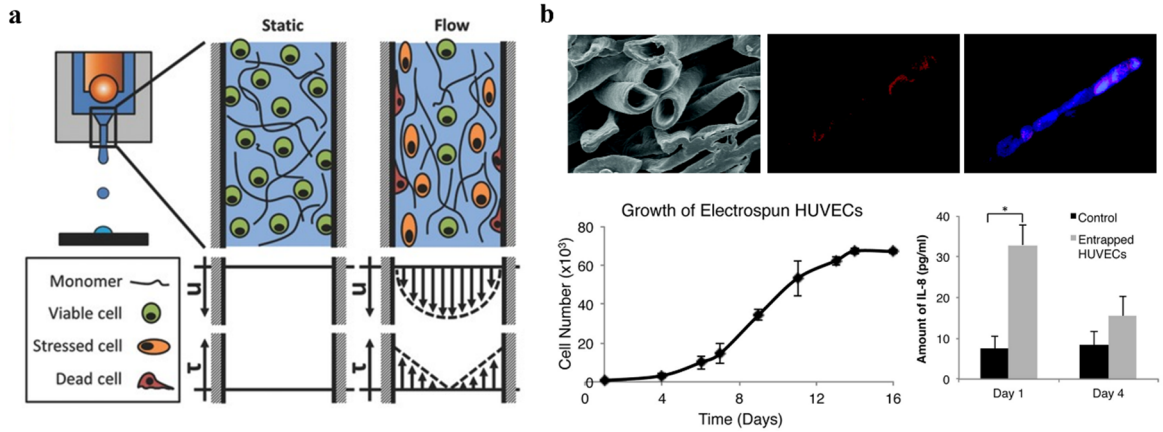



**Figure 3.** Viscosity and shear stress effects on cells in (**a**) standard extrusion-based bioprinting where flow-induced shear stress influences cell function and viability [77], (**b**) HUVEC cells in coaxial electrospun fibers showing increased shear stress marker, IL-8, expression 1 day post electrospinning, while decreased expression after 4 days of culture indicating cell recovery. Red: DiI (membrane dye), blue: DAPI [28]. (**a**) Reproduced with permission from ref. [77]. Copyright 2018 John Wiley and Sons. Adapted from ref. [28] (Creative Commons CC-BY-NC 3.0).

Coaxial electrospinning could be a solution to isolate cells from the shear stress induced by the polymer solution while extruded, by loading cells into the core in a low viscosity solution or cell medium. The core solution does not need to be electrospinnable, as long as the sheath solution is so and can drag the core solution with it [78]. The compartmentalization further expands the material choice to biocompatible, hydrophobic polymers. For example, PDMS or PCL shell solutions have been shown to successfully assist the electrospinning of cells only cell suspension in medium or a low concentrated polymer solution with cells in the core in only cell suspension. Townsend-Nicholson et al. found that the cell viability remained similar to the control group at 67.6% when locating the cell solution in the core of a coaxial needle with highly viscous PDMS in the shell [25]. Wu et al. reported a 20% PCL shell with a cell-loaded 10% PVA core to be electrospun at 0.1 kV/mm with a cell viability >95% and no reported viability problems even when increasing voltages [33]. It should be noted that they used different cell types and are not fully comparable, however, it still gives a good indication of the protective role of shielding cells in the inner core. At the opposite, a certain viscosity might also be beneficial for protecting cells from the electric field as Wen et al. demonstrated. They observed that better cell viability was obtained when cell electrospinning was carried out with fish gelatin/PBS compared to electrospraying cells in PBS alone [41]. Another benefit of using the coaxial needle system relates back to the challenge of the limited availability of solvents and the need for crosslinking fibers before cell culture (Section 3.1). Studies demonstrated that the use of organic solvents for dissolving e.g., PLLA or PCL in the shell solution did not affect the electrospun cells when they were suspended in aqueous solution in the core [26,33].

### *3.4. Other Parameters*

Flow rate and dehydration time has also been suspected to affect the viability of electrospun cells. Typical flow rates used are in the range of 0.25 to 7.5 mL/h in single needle cell electrospinning according to our review. High flow rates may increase the shear force on the cells, but it was in contrast reported by Semitela et al. that a flow rate of 1 mL/h resulted in much higher cell death compared to flow rates at 2–7 mL/h [40]. It was further found in a study by Guo et al. that increased flow rate for stabler jet formation had a lower negative influence on cell viability than decreasing the distance between the electrode and collector which would directly enhance the electric field strength [34]. In coaxial electrospinning, the flow rates can be controlled separately for core and shell solution leading to increased complexity and a more challenging process. Typically, shell flow rates needs to be higher than core flow rates to draw along the core solution and by varying this ratio, the sizes of core/shell structures can also be manipulated in connection with solution viscosities [78–80]. In practice, the ratio of shell:core flow rates for previous cell electrospinning experiments in literature have varied from 1.2 to 100 (Table 1). Placing living cells in the core could then be a tool to protect cells from the higher flow rate, i.e., larger shear stresses from the sheath fluid.

Dehydration has been suspected to influence viability in electrospinning processes since fibers are often deposited on a dry collector after solvent evaporation [18]. However, Fei Xu et al. tested this with 3T3 fibroblasts and Psi2 12S6 epithelial cells where cell viability was above 80% after 2 h of dehydration, and Dawson et al. tested the same on C2C12 myoblasts with acceptable viability after 1 h of dehydration (Figure 2b) [42,43].

### *3.5. Summary*

As clearly seen from previous sections, there are many variables both in the experimental settings and properties of the cell-containing electrospinning solutions that influence electrospinning performance, cell viability and functionality of post-processed cells. There is of course an operational window of the electric fields strengths and other physical parameters like needle-to-collector distance, flow rate and dehydration time that a cell can tolerate. However, putting emphasis on the physical and chemical properties of the bioinks seems to be even more important. Specifically, one of the main causes of cell death is the high shear stress that cells are experiencing during the process, which is determined by the combination of both the physical process parameters such as electric field strength and the composition and mechanical properties of the bioink. As viscosity and viscoelasticity are important parameters in determining effective shear stress, we believe reporting this parameter is of underestimated value when publishing papers on cell electrospinning.

## 4. Tissue Engineering Applications

Current applications of cell electrospinning of mammalian cells are mostly in tissue engineering, spanning from wound healing, neuron tissue engineering, to skeletal muscle tissue engineering, where the extracellular matrix and tissue morphology and characteristics are mimicked to regenerate the lost tissue. Here, cell electrospinning benefits from having cells homogeneously localized all over a scaffold instead of only on the top layer.

Wen et al. included mesenchymal stem cells (MSCs) into fish gelatin electrospun fibers for wound healing. The cell-loaded fibers were deposited onto a wound on mouse in vivo and was shown to promote healing by enhancing ECM remodelling with increased collagen deposition and decreased expression of the inflammation marker IL-6. It also promoted angiogenesis by increasing the expression of vascular endothelial growth factor (VEGF) and demonstrated to be an efficient approach for stem cell delivery [41].

Another application is in nerve regeneration as also envisioned with conventional electrospinning [81–83]. Wu et al. successfully incorporated PC12 cells and differentiated them inside hollow coaxial electrospun PVA/PCL fibers without the addition of nerve growth factors NGF) (Figure 4a,b) [33].

Skeletal muscle tissue has also been engineered using cell electrospinning, specifically with a drum collector to facilitate alignment of fibers. Anisotropic cues are often used to mimic muscle tissue [84] and guide the formation of myotubes. Yeo et al. demonstrated cell electrospinning for the same purpose, incorporating C2C12 myoblast cells in electrospun alginate fibers, yielding highly aligned and multinucleated cell morphology that could facilitate myogenic differentiation (Figure 4c) [32].

Embedding cells within single cell-sized electrospun fibers may enable other unexplored functionality. First, fibers with diameters at the cellular scale can provide a more direct 3D localized morphological and mechanical cues for the encapsulated single cells [85–88]. 2D Surface topography is known to influence not only the shape of cells but also intracellular processes such as gene expression. This can happen through focal adhesion assembly change and cytoskeletal reorganization, which can further activate transcription coregulators, e.g., the well-known mechanosensing Yes-Associated Protein (YAP) [89]. 2D surface structures and guidance have been further proven

to direct differentiation of stem cells into specified cell types through mechanobiological pathways [90,91]. Encasing single cells inside a fiber structure is hypothesized to not only induce guided cell alignment but also provide 3D, subcellular mechanical cues around the single cell, potentially providing a new tool of utilizing mechanobiology for the production of novel high resolution 3D tissue models.

On the other hand, electrostatic ejection of a cell-embedded liquid jet in the electrospinning process may affect biophysical functions of the cell and tissue constructs [92]. Apart from mechanical cues, adverse or beneficial effects of electric field could be anticipated. Electrical stimulation of cells has been demonstrated using a variety of techniques, such as electromagnetic stimulation [93,94], piezoelectric scaffolds [95] and hybrid conductive microfibers [96] to affect cell behaviours like differentiation [95,96], proliferation and migration [97], nerve regeneration [98,99] and gene expression [100] etc. Seeing cell electro-spinning and -spraying as another tool for single-pulse electrical stimulation could open new perspectives in the utilization of the technology in tissue engineering.

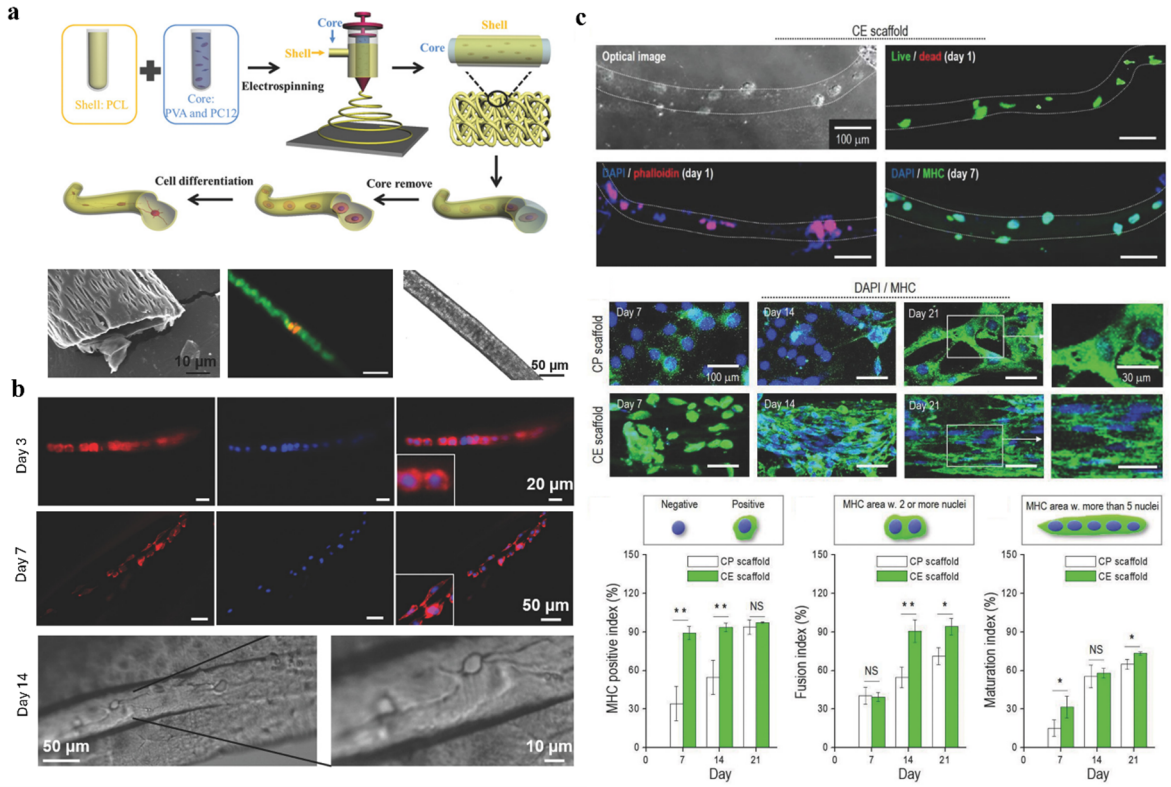



**Figure 4.** Examples of applications of cell electrospinning. (**a**) Coaxial electrospinning with living PC12 cells suspended in the core with (**b**) most cells surviving (green) and successful differentiation into neuron-like cells obtained after 7 days in culture in the fibers. Red: MAP2; blue: DAPI [33]. (**c**) C2C12 myoblasts encapsulated inside electrospun fibers via cell electrospinning. Myosin heavy chain (MHC) positive staining indicated successful myogenic differentiation, with cell electrospun fibers being superior in performance to a traditional cell printed scaffold. * $p < 0.05$, ** $p < 0.01$ [32]. (**a**,**b**) Reproduced with permission from ref. [33]. Copyright 2018 John Wiley and Sons Copyright. (**c**) Reproduced with permission from ref. [32]. Copyright 2018 John Wiley and Sons.

## 5. Conclusions and Outlook

While significant progress in cell electrospinning has been achieved, several key areas require further investigation to optimize the process and broaden its applicability. The fact that viscosity was reported in only two of the reviewed studies highlights a significant knowledge gap in technology transfer. Future studies should prioritize characterizing and controlling these key parameters to better understand its impact on cell viability and fiber formation. Furthermore, computational modelling of shear stress effect on cells during the extrusion in electrospinning initiation could help the prediction and optimization of experimental outcomes, leading to more efficient and reproducible results. Achieving a balance between electrospinnability, cell embedding efficiency, and cell viability remains a central challenge, demanding careful consideration of combined material properties and process parameters.

Beyond cell viability, it would also be crucial to study mechanobiological and electrophysiological effects on the cells when being processed through this electrohydrodynamic process. The former addresses the mechanical

influence of the polymer solution with its molecular entanglement, viscosity and resulting shear forces on a cell during syringe flow, Taylor cone formation and jetting. The latter exerts electrical interference on cell membrane. Generally, presenting cells to extracellular physical force such as shear stress, tension or compression has been proved to influence cell behaviour through mechanosensing pathways [101]. For example, cell stretching on a stiff substrate or simply compression of the nucleus using an AFM cantilever can result in translocation of the mechanotransduction regulated transcription factor YAP from cytosol to the nucleus [102]. One could therefore hypothesize that YAP translocation would also be affected by both the shear forces during the electrospinning process and the possible compression force that cells experience inside thin fibers. YAP translocation is known to influence gene transcription and thereby gene expression, potentially leading to interesting biological effects of cell electrospinning. This might open up new applications in the biomedical field.

Besides mechanobiological effect, electrophysiological effects might also lead changes in both cell phenotypes and genetics, for example as reported by Das et al. where global DNA hypomethylation was enhanced in cells subjected to electrical fields during electrospinning [37]. Other desirable electrophysiological effects have been indicated such as differentiation of PC12 cells inside electrospun fibers [33]. Furthermore, it could also be hypothesized that electroporation through cell electrospinning should in principle be possible.

In conclusion, if the ongoing challenges of cell electrospinning in terms of electrospinnability, and cell viability are well addressed, true applications of this technique will be unleashed, providing single cell models for investigating mechanobiological and electrohydrodynamic effects on diverse cell types, as well as contributing to the construction of high-resolution tissue engineering constructs with in-depth cell distribution and mechanobiological/electrohydrodynamic guidance.

**Author Contributions:** A.-K.K.L.: conceptualization, writing—original draft preparation; M.C.: conceptualization, supervision; writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Lundbeck Foundation, grant number R400-2022-1232, Marie Skłodowska-Curie Actions programme ENSIGN, grant number 101086226 and L4DNANO, grant number 101086227.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Luraghi, A.; Peri, F.; Moroni, L. Electrospinning for drug delivery applications: A review. *J. Control. Release* **2021**, *334*, 463–484. https://doi.org/10.1016/j.jconrel.2021.03.033.
2. Kishan, A.P.; Cosgriff-Hernandez, E.M. Recent advancements in electrospinning design for tissue engineering applications: A review. *J. Biomed. Mater. Res. Part A* **2017**, *105*, 2892–2905. https://doi.org/10.1002/jbm.a.36124.
3. Lu, T.; Cui, J.; Qu, Q.; Wang, Y.; Zhang, J.; Xiong, R.; Ma, W.; Huang, C. Multistructured Electrospun Nanofibers for Air Filtration: A Review. *ACS Appl. Mater. Interfaces* **2021**, *13*, 23293–23313. https://doi.org/10.1021/acsami.1c06520.
4. Mercante, L.A.; Scagion, V.P.; Migliorini, F.L.; Mattoso, L.H.C.; Correa, D.S. Electrospinning-based (bio)sensors for food and agricultural applications: A review. *TrAC Trends Anal. Chem.* **2017**, *91*, 91–103. https://doi.org/10.1016/j.trac.2017.04.004.
5. Taylor, S.G. Disintegration of water drops in an electric field. *Proc. R. Soc. London. Ser. A Math. Phys. Sci.* **1964**, *280*, 383–397. https://doi.org/10.1098/rspa.1964.0151.
6. Baumgarten, P.K. Electrostatic spinning of acrylic microfibers. *J. Colloid Interface Sci.* **1971**, *36*, 71–79. https://doi.org/10.1016/0021-9797(71)90241-4.
7. Boda, S.K.; Li, X.; Xie, J. Electrospraying an enabling technology for pharmaceutical and biomedical applications: A review. *J. Aerosol. Sci.* **2018**, *125*, 164–181. https://doi.org/10.1016/j.jaerosci.2018.04.002.
8. Jansen, K.A.; Donato, D.M.; Balcioglu, H.E.; Schmidt, T.; Danen, E.H.; Koenderink, G.H. A guide to mechanobiology: Where biology and physics meet. *Biochim. Biophys. Acta* **2015**, *1853*, 3043–3052. https://doi.org/10.1016/j.bbamcr.2015.05.007.
9. Maurmann, N.; França, F.S.; Girón, J.; Pranke, P. Cell Electrospinning: A Review of Materials and Methodologies for Biofabrication. *Adv. Biol.* **2023**, *7*, 2300058. https://doi.org/10.1002/adbi.202300058.
10. Nosoudi, N.; Hasanzadeh, A.; Hart, M.; Weaver, B. Advancements and Future Perspectives in Cell Electrospinning and Bio-Electrospraying. *Adv. Biol.* **2023**, *7*, 2300213. https://doi.org/10.1002/adbi.202300213.
11. Elveren, B.; Kurečič, M.; Maver, T.; Maver, U. Cell Electrospinning: A Mini-Review of the Critical Processing Parameters and Its Use in Biomedical Applications. *Adv. Biol.* **2023**, *7*, 2300057. https://doi.org/10.1002/adbi.202300057.
12. Karvinen, J.; Kellomäki, M. Design aspects and characterization of hydrogel-based bioinks for extrusion-based bioprinting. *Bioprinting* **2023**, *32*, e00274. https://doi.org/10.1016/j.bprint.2023.e00274.

13. He, C.-F.; Qiao, T.-H.; Wang, G.-H.; Sun, Y.; He, Y. High-resolution projection-based 3D bioprinting. *Nat. Rev. Bioeng.* **2025**, *3*, 143–158. https://doi.org/10.1038/s44222-024-00218-w.
14. Lee, M.; Rizzo, R.; Surman, F.; Zenobi-Wong, M. Guiding Lights: Tissue Bioprinting Using Photoactivated Materials. *Chem. Rev.* **2020**, *120*, 10950–11027. https://doi.org/10.1021/acs.chemrev.0c00077.
15. Zandrini, T.; Florczak, S.; Levato, R.; Ovsianikov, A. Breaking the resolution limits of 3D bioprinting: Future opportunities and present challenges. *Trends Biotechnol.* **2023**, *41*, 604–614. https://doi.org/10.1016/j.tibtech.2022.10.009.
16. Greiner, A.; Wendorff, J.H. Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibers. *Angew. Chem. Int. Ed.* **2007**, *46*, 5670–5703. https://doi.org/10.1002/anie.200604646.
17. Boularaoui, S.; Al Hussein, G.; Khan, K.A.; Christoforou, N.; Stefanini, C. An overview of extrusion-based bioprinting with a focus on induced shear stress and its effect on cell viability. *Bioprinting* **2020**, *20*, e00093. https://doi.org/10.1016/j.bprint.2020.e00093.
18. Canbolat, M.F.; Tang, C.; Bernacki, S.H.; Pourdeyhimi, B.; Khan, S. Mammalian Cell Viability in Electrospun Composite Nanofiber Structures. *Macromol. Biosci.* **2011**, *11*, 1346–1356. https://doi.org/10.1002/mabi.201100108.
19. Yeo, M.; Yoon, J.W.; Park, G.T.; Shin, S.-C.; Song, Y.-C.; Cheon, Y.-I.; Lee, B.-J.; Kim, G.H.; Kim, J.H. Esophageal wound healing by aligned smooth muscle cell-laden nanofibrous patch. *Mater. Today Bio* **2023**, *19*, 100564. https://doi.org/10.1016/j.mtbio.2023.100564.
20. Nosoudi, N.; Oommen, A.J.; Stultz, S.; Jordan, M.; Aldabel, S.; Hohne, C.; Mosser, J.; Archacki, B.; Turner, A.; Turner, P. Electrospinning Live Cells Using Gelatin and Pullulan. *Bioengineering* **2020**, *7*, 21. https://doi.org/10.3390/bioengineering7010021.
21. Diep, E.; Schiffman, J.D. Electrospinning Living Bacteria: A Review of Applications from Agriculture to Health Care. *ACS Appl. Bio Mater.* **2023**, *6*, 951–964. https://doi.org/10.1021/acsabm.2c01055.
22. Schulte-Hermann, J.; Riessland, H.; MacKinnon, N.; Korvink, J.G.; Islam, M. Biomineralization of Electrospun Bacteria-Encapsulated Fibers: A Relevant Step toward Living Ceramic Fibers. *ACS Appl. Bio Mater.* **2024**, *7*, 7936–7943. https://doi.org/10.1021/acsabm.4c00715.
23. Zussman, E. Encapsulation of cells within electrospun fibers. *Polym. Adv. Technol.* **2010**, *22*, 366–371. https://doi.org/10.1002/pat.1812.
24. Zhang, Y.-Q.; Wang, P.; Shi, Q.-F.; Ning, X.; Chen, Z.; Ramakrishna, S.; Zheng, J.; Long, Y.-Z. Advances in Wet Electrospinning: Rich Morphology and Promising Applications. *Adv. Fiber Mater.* **2025**, *7*, 374–413. https://doi.org/10.1007/s42765-024-00493-7.
25. Townsend-Nicholson, A.; Jayasinghe, S.N. Cell Electrospinning: a Unique Biotechnique for Encapsulating Living Organisms for Generating Active Biological Microthreads/Scaffolds. *Biomacromolecules* **2006**, *7*, 3364–3369.
26. Shih, Y.-H.; Yang, J.-C.; Li, S.-H.; Yang, W.-C.; Chen, C.-C. Bio-electrospinning of poly(l-lactic acid) hollow fibrous membrane. *Text. Res. J.* **2012**, *82*, 602–612. https://doi.org/10.1177/0040517511420756.
27. Zanatta, G.; Steffens, D.; Braghirolli, D.I.; Fernandes, R.A.; Netto, C.A.; Pranke, P. Viability of mesenchymal stem cells during electrospinning. *Braz. J. Med. Biol. Res.* **2012**, *45*, 125–130. https://doi.org/10.1590/s0100-879x2011007500163.
28. Ang, H.Y.; Irvine, S.A.; Avrahami, R.; Sarig, U.; Bronshtein, T.; Zussman, E.; Boey, F.Y.C.; Machluf, M.; Venkatraman, S.S. Characterization of a bioactive fiber scaffold with entrapped HUVECs in coaxial electrospun core-shell fiber. *Biomatter* **2014**, *4*, e28238. https://doi.org/10.4161/biom.28238.
29. Ehler, E.; Jayasinghe, S.N. Cell electrospinning cardiac patches for tissue engineering the heart. *Analyst* **2014**, *139*, 4449–4452. https://doi.org/10.1039/c4an00766b.
30. Yeo, M.; Kim, G. Fabrication of cell-laden electrospun hybrid scaffolds of alginate-based bioink and PCL microstructures for tissue regeneration. *Chem. Eng. J.* **2015**, *275*, 27–35. https://doi.org/10.1016/j.cej.2015.04.038.
31. Braghirolli, D.; Pranke, P.; Zamboni, F.; Acasigua, G.A.X. Association of electrospinning with electrospraying: A strategy to produce 3D scaffolds with incorporated stem cells for use in tissue engineering. *Int. J. Nanomed.* **2015**, 5159-5170. https://doi.org/10.2147/ijn.s84312.
32. Yeo, M.; Kim, G.H. Anisotropically Aligned Cell-Laden Nanofibrous Bundle Fabricated via Cell Electrospinning to Regenerate Skeletal Muscle Tissue. *Small* **2018**, *14*, 1803491. https://doi.org/10.1002/smll.201803491.
33. Wu, Y.; Ranjan, V.D.; Zhang, Y. A Living 3D In Vitro Neuronal Network Cultured inside Hollow Electrospun Microfibers. *Adv. Biosyst.* **2018**, *2*, 1700218. https://doi.org/10.1002/adbi.201700218.
34. Guo, Y.; Gilbert-Honick, J.; Somers, S.M.; Mao, H.Q.; Grayson, W.L. Modified cell-electrospinning for 3D myogenesis of C2C12s in aligned fibrin microfiber bundles. *Biochem. Biophys. Res. Commun.* **2019**, *516*, 558–564. https://doi.org/10.1016/j.bbrc.2019.06.082.
35. Ranjan, V.D.; Zeng, P.; Li, B.; Zhang, Y. In vitro cell culture in hollow microfibers with porous structures. *Biomater. Sci.* **2020**, *8*, 2175–2188. https://doi.org/10.1039/c9bm01986c.

36. Yeo, M.; Kim, G. Micro/nano-hierarchical scaffold fabricated using a cell electrospinning/3D printing process for co-culturing myoblasts and HUVECs to induce myoblast alignment and differentiation. *Acta Biomater.* **2020**, *107*, 102–114. https://doi.org/10.1016/j.actbio.2020.02.042.
37. Das, P.; Hore, A.; Ghosh, A.; Datta, P. Bone tissue engineering construct fabricated using a cell electrospinning technique with polyglutamic acid biopolymer. *J. Polym. Res.* **2021**, *28*, 255. https://doi.org/10.1007/s10965-021-02612-z.
38. Yang, I.-H.; Chen, Y.-S.; Li, J.-J.; Liang, Y.-J.; Lin, T.-C.; Jakfar, S.; Thacker, M.; Wu, S.-C.; Lin, F.-H. The development of laminin-alginate microspheres encapsulated with Ginsenoside Rg1 and ADSCs for breast reconstruction after lumpectomy. *Bioact. Mater.* **2021**, *6*, 1699–1710. https://doi.org/10.1016/j.bioactmat.2020.11.029.
39. Nosoudi, N.; Hart, C.; Mcknight, I.; Esmaeilpour, M.; Ghomian, T.; Zadeh, A.; Raines, R.; Ramirez Vick, J.E. Differentiation of adipose-derived stem cells to chondrocytes using electrospraying. *Sci. Rep.* **2021**, *11*, 24301. https://doi.org/10.1038/s41598-021-03824-5.
40. Semitela, Â.; Ramalho, G.; Capitão, A.; Sousa, C.; Mendes, A.F.; Aap Marques, P.; Completo, A. Bio-electrospraying assessment toward in situ chondrocyte-laden electrospun scaffold fabrication. *J. Tissue Eng.* **2022**, *13*, 204173142110693. https://doi.org/10.1177/20417314211069342.
41. Wen, Z.; Chen, Y.; Liao, P.; Wang, F.; Zeng, W.; Liu, S.; Wu, H.; Wang, N.; Moroni, L.; Zhang, M.; et al. In Situ Precision Cell Electrospinning as an Efficient Stem Cell Delivery Approach for Cutaneous Wound Healing. *Adv. Healthc. Mater.* **2023**, *12*, 2300970. https://doi.org/10.1002/adhm.202300970.
42. Xu, F.; Dawson, C.; Hoare, T. Multicellular Layered Nanofibrous Poly(Oligo Ethylene Glycol Methacrylate) (POEGMA)-Based Hydrogel Scaffolds via Reactive Cell Electrospinning. *Adv. Biol.* **2023**, *7*, 2300052. https://doi.org/10.1002/adbi.202300052.
43. Dawson, C.; Xu, F.; Hoare, T. Reactive Cell Electrospinning of Anisotropically Aligned and Bilayer Hydrogel Nanofiber Networks. *ACS Biomater. Sci. Eng.* **2023**, *9*, 6490–6503. https://doi.org/10.1021/acsbiomaterials.3c01013.
44. Lu, T.; Yang, L.; Li, Z.; Liu, Y.; Xu, S.E.; Ye, C. Immediate implantation of ultrafine fiber slow-release system based on cell electrospinning to induce osteogenesis of mesenchymal stem cells. *Regen. Biomater.* **2024**, *11*, rbad113. https://doi.org/10.1093/rb/rbad113.
45. Al-Abduljabbar, A.; Farooq, I. Electrospun Polymer Nanofibers: Processing, Properties, and Applications. *Polymers* **2022**, *15*, 65. https://doi.org/10.3390/polym15010065.
46. Lasprilla-Botero, J.; Álvarez-Láinez, M.; Lagaron, J.M. The influence of electrospinning parameters and solvent selection on the morphology and diameter of polyimide nanofibers. *Mater. Today Commun.* **2018**, *14*, 1–9. https://doi.org/10.1016/j.mtcommm.2017.12.003.
47. Xue, J.; Wu, T.; Dai, Y.; Xia, Y. Electrospinning and Electrospun Nanofibers: Methods, Materials, and Applications. *Chem. Rev.* **2019**, *119*, 5298–5415. https://doi.org/10.1021/acs.chemrev.8b00593.
48. Sell, S.A.; Wolfe, P.S.; Garg, K.; Mccool, J.M.; Rodriguez, I.A.; Bowlin, G.L. The Use of Natural Polymers in Tissue Engineering: A Focus on Electrospun Extracellular Matrix Analogues. *Polymers* **2010**, *2*, 522–553. https://doi.org/10.3390/polym2040522.
49. Yang, J.M.; Yang, J.H.; Tsou, S.C.; Ding, C.H.; Hsu, C.C.; Yang, K.C.; Yang, C.C.; Chen, K.S.; Chen, S.W.; Wang, J.S. Cell proliferation on PVA/sodium alginate and PVA/poly(gamma-glutamic acid) electrospun fiber. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2016**, *66*, 170–177. https://doi.org/10.1016/j.msec.2016.04.068.
50. Wang, Y.; Guo, Z.; Qian, Y.; Zhang, Z.; Lyu, L.; Wang, Y.; Ye, F. Study on the Electrospinning of Gelatin/Pullulan Composite Nanofibers. *Polymers* **2019**, *11*, 1424. https://doi.org/10.3390/polym11091424.
51. Seon-Lutz, M.; Couffin, A.C.; Vignoud, S.; Schlatter, G.; Hebraud, A. Electrospinning in water and in situ crosslinking of hyaluronic acid/cyclodextrin nanofibers: Towards wound dressing with controlled drug release. *Carbohydr. Polym.* **2019**, *207*, 276–287. https://doi.org/10.1016/j.carbpol.2018.11.085.
52. Nie, H.; He, A.; Wu, W.; Zheng, J.; Xu, S.; Li, J.; Han, C.C. Effect of poly(ethylene oxide) with different molecular weights on the electrospinnability of sodium alginate. *Polymer* **2009**, *50*, 4926–4934. https://doi.org/10.1016/j.polymer.2009.07.043.
53. Saquing, C.D.; Tang, C.; Monian, B.; Bonino, C.A.; Manasco, J.L.; Alsberg, E.; Khan, S.A. Alginate–Polyethylene Oxide Blend Nanofibers and the Role of the Carrier Polymer in Electrospinning. *Ind. Eng. Chem. Res.* **2013**, *52*, 8692–8704. https://doi.org/10.1021/ie302385b.
54. Kim, M.W. Surface activity and property of polyethyleneoxide (PEO) in water. *Colloids Surf. A Physicochem. Eng. Asp.* **1997**, *128*, 145 154.
55. Bt Ibrahim, S.F.; Mohd Azam, N.A.N.; Mat Amin, K.A. Sodium alginate film: The effect of crosslinker on physical and mechanical properties. *IOP Conf. Ser. Mater. Sci. Eng.* **2019**, *509*, 012063. https://doi.org/10.1088/1757-899x/509/1/012063.
56. Yang, Q.; Guo, J.; Zhang, S.; Guan, F.; Yu, Y.; Feng, S.; Song, X.; Bao, D.; Zhang, X. Development of cell adhesive and inherently antibacterial polyvinyl alcohol/polyethylene oxide nanofiber scaffolds via incorporating chitosan for tissue engineering. *Int. J. Biol. Macromol.* **2023**, *236*, 124004. https://doi.org/10.1016/j.ijbiomac.2023.124004.
57. Huang, C.-Y.; Hu, K.-H.; Wei, Z.-H. Comparison of cell behavior on pva/pva-gelatin electrospun nanofibers with random and aligned configuration. *Sci. Rep.* **2016**, *6*, 37960. https://doi.org/10.1038/srep37960.

58. Kwak, H.W.; Shin, M.; Lee, J.Y.; Yun, H.; Song, D.W.; Yang, Y.; Shin, B.S.; Park, Y.H.; Lee, K.H. Fabrication of an ultrafine fish gelatin nanofibrous web from an aqueous solution by electrospinning. *Int. J. Biol. Macromol.* **2017**, *102*, 1092–1103. https://doi.org/10.1016/j.ijbiomac.2017.04.087.
59. Young, A.T.; White, O.C.; Daniele, M.A. Rheological Properties of Coordinated Physical Gelation and Chemical Crosslinking in Gelatin Methacryloyl (GelMA) Hydrogels. *Macromol. Biosci.* **2020**, *20*, 2000183. https://doi.org/10.1002/mabi.202000183.
60. Xiao, S.; Guo, S.; Nesin, V.; Heller, R.; Schoenbach, K.H. Subnanosecond electric pulses cause membrane permeabilization and cell death. *IEEE Trans. Biomed. Eng.* **2011**, *58*, 1239–1245. https://doi.org/10.1109/TBME.2011.2112360.
61. Kotnik, T.; Rems, L.; Tarek, M.; Miklavcic, D. Membrane Electroporation and Electropermeabilization: Mechanisms and Models. *Annu. Rev. Biophys.* **2019**, *48*, 63–91. https://doi.org/10.1146/annurev-biophys-052118-115451.
62. Zu, Y.; Huang, S.; Lu, Y.; Liu, X.; Wang, S. Size Specific Transfection to Mammalian Cells by Micropillar Array Electroporation. *Sci. Rep.* **2016**, *6*, 38661. https://doi.org/10.1038/srep38661.
63. Harris, E.; Elmer, J.J. Optimization of electroporation and other non-viral gene delivery strategies for T cells. *Biotechnol. Prog.* **2021**, *37*, e3066. https://doi.org/10.1002/btpr.3066.
64. Yang, I.-H.; Chen, Y.-S.; Li, J.-J.; Liang, Y.-J.; Lin, T.-C.; Jakfar, S.; Thacker, M.; Wu, S.-C.; Lin, F.-H. The development of laminin-alginate microspheres encapsulated with Ginsenoside Rg1 and ADSCs for breast reconstruction after lumpectomy. *Bioact. Mater.* **2021**, *6*, 1699–1710. https://doi.org/10.1016/j.bioactmat.2020.11.029.
65. Jayasinghe, S.N.; Eagles, P.A.M.; Qureshi, A.N. Electric field driven jetting: An emerging approach for processing living cells. *Biotechnol. J.* **2006**, *1*, 86–94. https://doi.org/10.1002/biot.200500025.
66. O'Hare, M.J.; Ormerod, M.G.; Imrie, P.R.; Peacock, J.H.; Asche, W. Electropermeabilization and Electrosensitivity of Different Types of Mammalian Cells. In *Electroporation and Electrofusion in Cell Biology*; Springer: Berlin/Heidelberg, Germany, 1989; pp. 319–330.
67. ĈemazˆR, M.; Jarm, T.; Miklavĉiĉ, D.; Lebar, A.M.; Ihan, A.; Kopitar, N.A.; Serŝa, G. Effect of Electric-Field Intensity on Electropermeabilization and Electrosensitmty of Various Tumor-Cell Lines In Vitro. *Electro Magnetobiol.* **1998**, *17*, 263–272. https://doi.org/10.3109/15368379809022571.
68. Lang, Q.; Wu, Y.; Ren, Y.; Tao, Y.; Lei, L.; Jiang, H. AC Electrothermal Circulatory Pumping Chip for Cell Culture. *ACS Appl. Mater. Interfaces* **2015**, *7*, 26792–26801. https://doi.org/10.1021/acsami.5b08863.
69. Pucihar, G.; Kotnik, T.; Kanduser, M.; Miklavcic, D. The influence of medium conductivity on electropermeabilization and survival of cells in vitro. *Bioelectrochemistry* **2001**, *54*, 107–115.
70. Kucernak, A.R.; Wang, H.; Lin, X. Avoid Using Phosphate Buffered Saline (PBS) as an Electrolyte for Accurate OER Studies. *ACS Energy Lett.* **2024**, *9*, 3939–3946. https://doi.org/10.1021/acsenergylett.4c01589.
71. Ageev, I.M.; Rybin, Y.M. Features of Measuring the Electrical Conductivity of Distilled Water in Contact with Air. *Meas. Tech.* **2020**, *62*, 923–927. https://doi.org/10.1007/s11018-020-01714-2.
72. Sun, Z.; Deitzel, J.M.; Knopf, J.; Chen, X.; Gillespie, J.W. The effect of solvent dielectric properties on the collection of oriented electrospun fibers. *J. Appl. Polym. Sci.* **2012**, *125*, 2585–2594. https://doi.org/10.1002/app.35454.
73. Wendorff, J.H.; Agarwal, S.; Greiner, A. *Electrospinning: Materials, Processing and Applications*; Wiley-VCH: Weinheim, Germany, 2012.
74. Angammana, C.J.; Jayaram, S.H. Analysis of the Effects of Solution Conductivity on Electrospinning Process and Fiber Morphology. *IEEE Trans. Ind. Appl.* **2011**, *47*, 1109–1117. https://doi.org/10.1109/tia.2011.2127431.
75. Nezarati, R.M.; Eifert, M.B.; Cosgriff-Hernandez, E. Effects of humidity and solution viscosity on electrospun fiber morphology. *Tissue Eng. Part C Methods* **2013**, *19*, 810–819. https://doi.org/10.1089/ten.TEC.2012.0671.
76. Kim, Y.B.; Lee, H.; Kim, G.H. Strategy to Achieve Highly Porous/Biocompatible Macroscale Cell Blocks, Using a Collagen/Genipin-bioink and an Optimal 3D Printing Process. *ACS Appl. Mater. Interfaces* **2016**, *8*, 32230–32240. https://doi.org/10.1021/acsami.6b11669.
77. Blaeser, A.; Duarte Campos, D.F.; Puster, U.; Richtering, W.; Stevens, M.M.; Fischer, H. Controlling Shear Stress in 3D Bioprinting is a Key Factor to Balance Printing Resolution and Stem Cell Integrity. *Adv. Healthc. Mater.* **2016**, *5*, 326–333. https://doi.org/10.1002/adhm.201500677.
78. Yoon, J.; Yang, H.S.; Lee, B.S.; Yu, W.R. Recent Progress in Coaxial Electrospinning: New Parameters, Various Structures, and Wide Applications. *Adv. Mater.* **2018**, *30*, e1704765. https://doi.org/10.1002/adma.201704765.
79. Han, D.; Steckl, A.J. Coaxial Electrospinning Formation of Complex Polymer Fibers and their Applications. *Chempluschem* **2019**, *84*, 1453–1497. https://doi.org/10.1002/cplu.201900281.
80. Lu, Y.; Huang, J.; Yu, G.; Cardenas, R.; Wei, S.; Wujcik, E.K.; Guo, Z. Coaxial electrospun fibers: Applications in drug delivery and tissue engineering. *WIREs Nanomed. Nanobiotechnol.* **2016**, *8*, 654–677. https://doi.org/10.1002/wnan.1391.
81. Saudi, A.; Amini, S.; Amirpour, N.; Kazemi, M.; Zargar Kharazi, A.; Salehi, H.; Rafienia, M. Promoting neural cell proliferation and differentiation by incorporating lignin into electrospun poly(vinyl alcohol) and poly(glycerol sebacate) fibers. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2019**, *104*, 110005. https://doi.org/10.1016/j.msec.2019.110005.

82. Liu, J.J.; Wang, C.Y.; Wang, J.G.; Ruan, H.J.; Fan, C.Y. Peripheral nerve regeneration using composite poly(lactic acid-caprolactone)/nerve growth factor conduits prepared by coaxial electrospinning. *J. Biomed. Mater. Res. Part A* **2011**, *96A*, 13–20. https://doi.org/10.1002/jbm.a.32946.
83. Xu, H.; Gao, Z.; Wang, Z.; Wu, W.; Li, H.; Liu, Y.; Jia, S.; Hao, D.; Zhu, L. Electrospun PCL Nerve Conduit Filled with GelMA Gel for CNTF and IGF-1 Delivery in Promoting Sciatic Nerve Regeneration in Rat. *ACS Biomater. Sci. Eng.* **2023**, *9*, 6309–6321. https://doi.org/10.1021/acsbiomaterials.3c01048.
84. Zhang, Y.; Zhang, Z.; Wang, Y.; Su, Y.; Chen, M. 3D myotube guidance on hierarchically organized anisotropic and conductive fibers for skeletal muscle tissue engineering. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2020**, *116*, 111070. https://doi.org/10.1016/j.msec.2020.111070.
85. Onoe, H.; Takeuchi, S. Cell-laden microfibers for bottom-up tissue engineering. *Drug Discov. Today* **2015**, *20*, 236–246. https://doi.org/10.1016/j.drudis.2014.10.018.
86. Jana, S.; Levengood, S.K.L.; Zhang, M. Anisotropic Materials for Skeletal-Muscle-Tissue Engineering. *Adv. Mater.* **2016**, *28*, 10588–10612. https://doi.org/10.1002/adma.201600240.
87. Batalov, I.; Jallerat, Q.; Kim, S.; Bliley, J.; Feinberg, A.W. Engineering aligned human cardiac muscle using developmentally inspired fibronectin micropatterns. *Sci. Rep.* **2021**, *11*, 11502. https://doi.org/10.1038/s41598-021-87550-y.
88. Kobayashi, M.; Lei, N.Y.; Wang, Q.; Wu, B.M.; Dunn, J.C. Orthogonally oriented scaffolds with aligned fibers for engineering intestinal smooth muscle. *Biomaterials* **2015**, *61*, 75–84. https://doi.org/10.1016/j.biomaterials.2015.05.023.
89. Nardone, G.; Oliver-De La Cruz, J.; Vrbsky, J.; Martini, C.; Pribyl, J.; Skládal, P.; Pešl, M.; Caluori, G.; Pagliari, S.; Martino, F.; et al. YAP regulates cell mechanics by controlling focal adhesion assembly. *Nat. Commun.* **2017**, *8*, 15321. https://doi.org/10.1038/ncomms15321.
90. Teo, B.K.K.; Wong, S.T.; Lim, C.K.; Kung, T.Y.S.; Yap, C.H.; Ramagopal, Y.; Romer, L.H.; Yim, E.K.F. Nanotopography Modulates Mechanotransduction of Stem Cells and Induces Differentiation through Focal Adhesion Kinase. *ACS Nano* **2013**, *7*, 4785–4798. https://doi.org/10.1021/nn304966.
91. Von Erlach, T.C.; Bertazzo, S.; Wozniak, M.A.; Horejs, C.-M.; Maynard, S.A.; Attwood, S.; Robinson, B.K.; Autefage, H.; Kallepitis, C.; Del Río Hernández, A.; et al. Cell-geometry-dependent changes in plasma membrane order direct stem cell signalling and fate. *Nat. Mater.* **2018**, *17*, 237–242. https://doi.org/10.1038/s41563-017-0014-0.
92. Iranshahi, K.; Defraeye, T.; Rossi, R.M.; Müller, U.C. Electrohydrodynamics and its applications: Recent advances and future perspectives. *Int. J. Heat Mass Transf.* **2024**, *232*, 125895. https://doi.org/10.1016/j.ijheatmasstransfer.2024.125895.
93. Wang, Z.; Li, S.; Wu, Z.; Kang, Y.; Xie, S.; Cai, Z.; Shan, X.; Li, Q. Pulsed electromagnetic field-assisted reduced graphene oxide composite 3D printed nerve scaffold promotes sciatic nerve regeneration in rats. *Biofabrication* **2024**, *16*, 035013. https://doi.org/10.1088/1758-5090/ad3d8a.
94. Jensen, B.N.; Wang, Y.; Le Friec, A.; Nabavi, S.; Dong, M.; Seliktar, D.; Chen, M. Wireless electromagnetic neural stimulation patch with anisotropic guidance. *NPJ Flex. Electron.* **2023**, *7*, 34. https://doi.org/10.1038/s41528-023-00270-3.
95. Liu, Z.; Cai, M.; Zhang, X.; Yu, X.; Wang, S.; Wan, X.; Wang, Z.L.; Li, L. Cell-Traction-Triggered On-Demand Electrical Stimulation for Neuron-Like Differentiation. *Adv. Mater.* **2021**, *33*, 2106317. https://doi.org/10.1002/adma.202106317.
96. Guo, W.; Zhang, X.; Yu, X.; Wang, S.; Qiu, J.; Tang, W.; Li, L.; Liu, H.; Wang, Z.L. Self-Powered Electrical Stimulation for Enhancing Neural Differentiation of Mesenchymal Stem Cells on Graphene-Poly(3,4-ethylenedioxythiophene) Hybrid Microfibers. *ACS Nano* **2016**, *10*, 5086–5095. https://doi.org/10.1021/acsnano.6b00200.
97. Hu, W.; Wei, X.; Zhu, L.; Yin, D.; Wei, A.; Bi, X.; Liu, T.; Zhou, G.; Qiang, Y.; Sun, X.; et al. Enhancing proliferation and migration of fibroblast cells by electric stimulation based on triboelectric nanogenerator. *Nano Energy* **2019**, *57*, 600–607. https://doi.org/10.1016/j.nanoen.2018.12.077.
98. Choe, G.; Han, U.G.; Ye, S.; Kang, S.; Yoo, J.; Cho, Y.S.; Jung, Y. Effect of Electrical Stimulation on Nerve-Guided Facial Nerve Regeneration. *ACS Biomater. Sci. Eng.* **2023**, *9*, 3512–3521. https://doi.org/10.1021/acsbiomaterials.3c00222.
99. He, L.; Xiao, Q.; Zhao, Y.; Li, J.; Reddy, S.; Shi, X.; Su, X.; Chiu, K.; Ramakrishna, S. Engineering an Injectable Electroactive Nanohybrid Hydrogel for Boosting Peripheral Nerve Growth and Myelination in Combination with Electrical Stimulation. *ACS Appl. Mater. Interfaces* **2020**, *12*, 53150–53163. https://doi.org/10.1021/acsami.0c16885.
100. Huang, J.; Xue, S.; Buchmann, P.; Teixeira, A.P.; Fussenegger, M.; Huang, J.; Xue, S.; Buchmann, P.; Teixeira, A.P.; Fussenegger, M. An electrogenetic interface to program mammalian gene expression by direct current. *Nat. Metab.* **2023**, *5*, 1395–1407. https://doi.org/10.1038/s42255-023-00850-7.
101. Argentati, C.; Morena, F.; Tortorella, I.; Bazzucchi, M.; Porcellati, S.; Emiliani, C.; Martino, S. Insight into Mechanobiology: How Stem Cells Feel Mechanical Forces and Orchestrate Biological Functions. *Int. J. Mol. Sci.* **2019**, *20*, 5337 https://doi.org/10.3390/ijms20215337.
102. Elosegui-Artola, A.; Andreu, I.; Beedle, A.E.M.; Lezamiz, A.; Uroz, M.; Kosmalska, A.J.; Oria, R.; Kechagia, J.Z.; Rico-Lastres, P.; Le Roux, A.L.; et al. Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores. *Cell* **2017**, *171*, 1397–1410.e1314. https://doi.org/10.1016/j.cell.2017.10.008.