3D biomaterials produced by near-field electrospinning and melt electrowriting

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Abstract

Near-field electrospinning and melt electrowriting are attractive techniques that can be used to produce polymeric nano- or microfibres and build three-dimensional (3D) shapes that can be used in biotechnology and biomedicine. Preferred patterns can be designed due to the possibility to define nozzle and collector movements. Opposite to conventional electrospinning, near-field electrospinning enables formation of very fine fibres assembled in structures with much larger pore sizes, tailored according to the requirements of cells, which makes such scaffolds highly interesting for cell culture, tissue engineering applications and similar biomedical and biotechnological applications. In addition, this technique is relatively simple, reproducible and inexpensive. Melt electrowriting can be used to draw microfibres from a solution or a melt through an electrostatic field allowing precise deposition with high accuracy, leading to highly porous scaffolds that facilitate homogeneous cell distribution. This review provides an overview of new theoretical and experimental findings related to near-field electrospinning and melt electrowriting for applications in biotechnology and biomedicine, such as printing scaffolds for tissue engineering and cell culture, producing wound dressings, and others. Near-field electrospinning and melt electrowriting processes are briefly explained, and the most relevant polymers for biomedical applications are presented. Finally, recent challenges and suggestions for future research directions are given.

Keywords: 3D porous scaffolds; biomedicine; nanofibers; three-dimensional shapes; biomedicine.

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1. INTRODUCTION

Fibrous scaffolds have been used in biotechnology and biomedicine for decades [1-3]. Electrospinning is usually applied to produce polymeric nanofibers for biotechnological and other applications [4-8]. This electrostatically driven process enables spinning of polymers, polymer blends, and polymers with embedded nanoparticles to create nanofiber mats with arbitrarily oriented fibres [9]. Oriented nanofiber bundles are advantageous for many applications [10,11] and can be produced by several techniques, such as a fast-rotating collector electrospinning, spinning on the gaps between grounded electrodes, structuring the electric field by dielectric or conductive materials, magnetic field-assisted electrospinning, centrifugal electrospinning, or post-processing to draw the fibres [9,12-15]. However, certain randomness is always present due to the bending instability of the jet travelling to the collector, resulting from the repulsive forces between surface charges that are necessary for jet stretching and correspondingly small fibre diameters [9].

Near-field electrospinning differs regarding the distance between the needle, through which the polymer solution is inserted into the strong electric field, and the collector is much smaller than in common electrospinning, typically below 10 mm [16-18]. At this distance, the jet is still stable, so that highly aligned and complex patterns can be written by producing nano- or microfibres [9].

As an alternative, melt electrowriting has emerged, which combines additive manufacturing with electrohydrodynamical stabilization of the molten jet, resulting in a possible printing resolution around 1 µm [19].

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The possibility to create highly aligned fibres by near-field electrospinning (NFES) and melt electrowriting (MEW) makes these techniques promising for tissue engineering and other biomedical and biotechnological applications. In the next sections, both processes as well as the most common biomedical polymers are briefly described, followed by the most recent applications reviewed in detail, and an outlook on overcoming recent challenges in utilization of these technologies.

2. NEAR-FIELD ELECTROSPINNING

Near-field electrospinning is a technique quite similar to the more common far-field electrospinning, where usually a syringe containing a polymer solution is placed opposite to a collector, with a strong electric field applied in-between (Fig. 1a). This leads to the formation of a so-called Taylor cone at the end of the needle from which a jet is initiated that is only stable for a short distance, before it becomes instable and shows a whipping action, which leads to stretching and thinning of the polymeric jet before it reaches the substrate [20]. In a homogeneous electric field, the resulting nanofibers are thus more or less arbitrarily positioned on the substrate. In NFES the nozzle-to-collector distance and voltage are strongly reduced (Fig. 1b), resulting in much better-controlled patterning (Fig. 1d) than in the common far-field electrospinning process (Fig. 1c) [20]. The nanofiber thickness depends not only on the polymers used, but also on the voltage and other spinning parameters, where low voltages of a few hundred Volts result in fine fibres of a few tens of nanometres in diameter [20,21]. It should be mentioned that even the length of the needle can modify the NFES results, which is rarely mentioned in the literature [22,23].

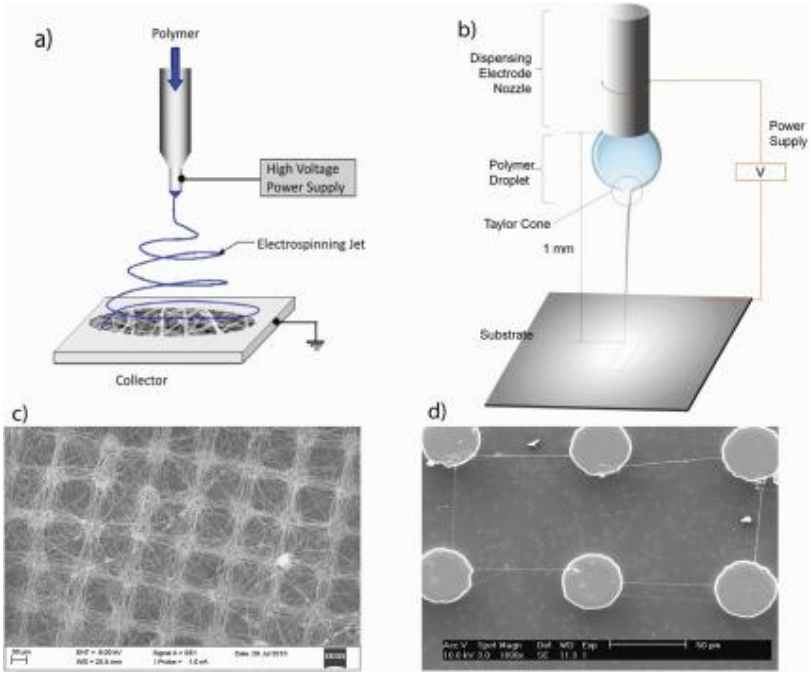

Figure 1. Far-field electrospinning (FFES) (a, c) compared with low-voltage near-field electrospinning (NFES; b, d). The fibres are chaotically deposited on carbon 3D posts in FFES (c), whereas NFES allows more controlled patterning (d). From [20], originally published under a CC BY-NC-ND 4.0 license.

3. MELT ELECTROWRITING

Melt electrowriting can be regarded as a hybrid technology, combining extrusion 3D printing with electrospinning. Figure 2a shows the principle of this technique, while Figure 2b depicts an exemplary scaffold printed from poly(caprolactone) (PCL) using melt electrowriting [24]. In addition to common planar substrates, MEW is also often performed on cylindrical rotating substrates to prepare tubular scaffolds [25]. Interestingly, MEW printers have recently been made available at low costs by an open-source device called MEWron, thus making MEW possible for many research groups to obtain microfibres with a diameter range of around 1.5 to 100 µm, depending on the material and device

configuration [24,26]. Important parameters, influencing the printing results and fibre diameters, are voltage and pressure, collector speed, nozzle diameter and the temperature of the molten polymer, besides the environmental parameters temperature and relative humidity which have also significant impacts [27,28]. In addition, the substrate should be conductive to support well-aligned fibre placement [29].

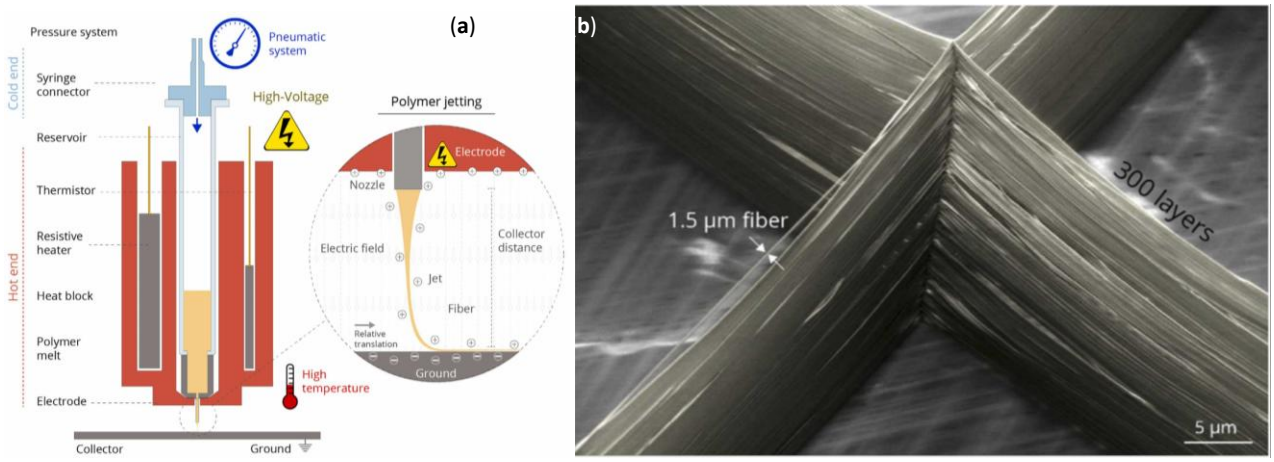

Fig. 2. Melt electrowriting (MEW): (a) Schematic including the main components and working mechanism of MEW; (b) poly(caprolactone) square scaffold with 500 µm interspace, 1.5 µm fibres and 300 layers. From [24], originally published under a CC-BY 4.0 license

4. BIOMATERIALS USED FOR NEAR-FIELD ELECTROSPINNING AND MELT ELECTROWRITING

Several polymers have been successfully used in near-field electrospinning and melt electrowriting.

Poly(caprolactone) (PCL) is often used in NEFS, but also biopolymers like alginate, chitosan, collagen, gelatine as well as poly(g-benzyl-l-glutamate) (PBLG), poly(L-lactic acid) (PLLA), polydioxanone (PDO), polymethyl methacrylate (PMMA), poly(ethylene oxide) (PEO), poly(2-ethyl-2-oxazoline) (PEtOx), poly(2-ethyl-2-oxazine) (PEtOzi), and various other polymers, copolymers and polymer blends [30].

PCL is also most often used in MEW due to its biocompatibility, good thermal stability and printability, either solely or blended with other polymers [31,32]. In addition, different other polyesters can be used, such as poly(hydroxymethylglycolide-*co*-*ε*-caprolactone) (pHMGCL) or thermoplastic elastomers (TPEs) as well as water-soluble polymers like poly(2-ethyl-2-oxazoline) (PEtOx), hydrogels like poly(2-ethyl-2-oxazine) (PEtOzi) or non-biodegradable polymers like polypropylene (PP) [31]. Mixing these polymers with different fillers, such as hydroxyapatite (HA), bioactive glass, metal or metal-oxide nanoparticles, reduced graphene oxide (rGO) etc. can be used to improve cell adhesion and proliferation, mechanical or electrical properties, reduce degradation or modify hydrophobicity of the material [32].

As this wide range of potential materials, combined with the high degree of freedom offered by the selected structures, which are built from nano- and microfibres with a wide range of diameters, shows, near-field electrospinning and melt electrowriting enable a wide variety of applications in biomedicine, biotechnology, and similar fields [33]. A selection of the most recent interesting applications is given in the next sections.

5. BIOMEDICAL APPLICATIONS OF NEAR-FIELD ELECTROSPINNING

Several studies of near-field electrospinning to prepare scaffolds for tissue engineering can be found in literature. Poly(vinyl alcohol) (PVA) blended with chitosan was used in NFES, with a distance of 3 mm between the needle and the collector under a voltage of 2 kV [34]. It was shown that higher collector speeds were advantageous for well-defined fibre placement and thus suggested to use this strategy for obtaining tissue engineering scaffolds.

PDO was used for preparation of near-field electrospun small-diameter vascular graft scaffolds with micron-sized polymer fibres [35]. These tubular scaffolds had similar mechanical properties as native vessels and expanding pores, to support transmural endothelialization. Comparing NFES with far-field electrospinning to prepare PDO scaffolds, the same research group showed a higher neutrophil innate immune response of DNA extrusion to form neutrophil

extracellular traps due to the possibility to tailor the pore sizes and mechanical properties [36]. Other researchers prepared PDO near-field electrospun bioresorbable vascular grafts whose fibrous architecture aimed to mimic the arterial extracellular matrix with the native vessel properties regarding tensile strength, suture retention, and burst pressure, which was best achieved by fibre alignment angles of 15°/75° [37].

Using partly branched PCL microfibres prepared by NFES on a valley-shaped collector, Qavi and Tan tried to mimic the arteriole-capillary-venule structure by spinning stem-branch-stem fibrous structures [38]. Concentrating on relatively thin fibres only, Davis *et al.* [40] used NFES of gelatine to prepare fibres with a diameter of 2.3 µm [39], which is similar to gelatine fibres prepared with far-field electrospinning.

Besides tissue engineering, other biomedical and biotechnological applications of NFES can be found in the literature. NFES was applied to prepare medical wound dressings based on PCL blended with collagen type I in different ratios and added erythromycin, used to prevent wound infections due to Gram-positive and Gram-negative bacteria [41]. Good mechanical properties, thermal stability, antibacterial, slow-release and moisturizing functions of this wound dressing were reported.

PCL was functionalized with collagen and the natural drug usnic acid [42]. The authors found that the electrospun fibre structures were hydrophilic due to the addition of collagen and inhibited the growth of Gram-positive and Gram-negative bacteria due to the usnic acid, as shown in Figure 3. In addition, crosslinking the collagen with genipin resulted in improved mechanical properties, thermal stability and drug release performance.

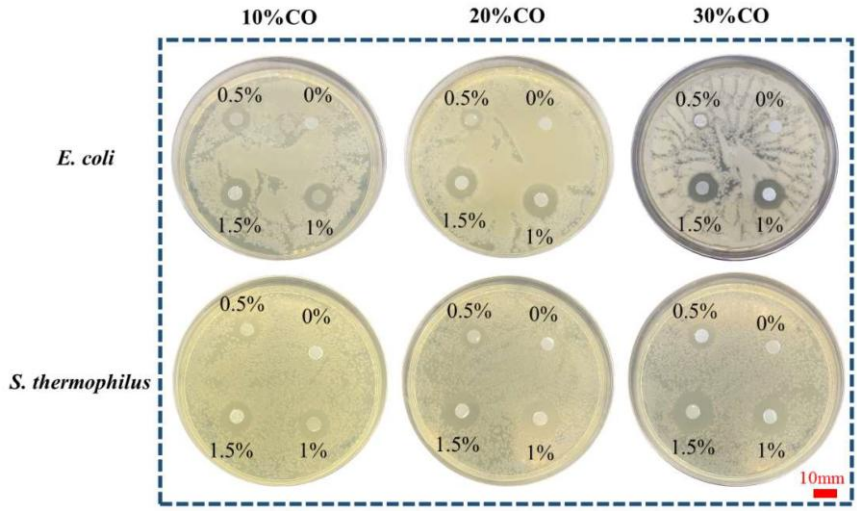

Fig. 3. Effect of composite fibres with different contents of collagen (CO) and usnic acid (the number in the culture dish is the acid content) on antibacterial properties against E. coli and S. thermophilus. From [42], originally published under a CC-BY 4.0 license.

6. BIOMEDICAL APPLICATIONS OF MELT ELECTROWRITING

Similar to the aforementioned MEWron device, a custom-made MEW device was used to prepare PCL box-structure and triangular scaffolds by applying a temperature of 73 °C, a pressure of 1.2 bar in the syringe and an acceleration voltage of 6 kV, while the nozzle-collector distance was 4 mm, and the collector was rotated at the rate of 400 mm min^{-1} [43]. By varying the scaffold design, the authors could tailor the scaffolds especially for osteoblasts or for keratinocytes and connective tissue, so that a bilayer scaffold combining the optimum designs could be produced by connecting these surface layers by a casted PCL film. A custom-made MEW device was also used by Eichholz and Hoey, who showed significant differences in the morphology, spreading and cytoskeletal tension of human skeletal stem cells depending on the architecture of 10 µm PCL fibres [44].

Tubular melt-electrowritten PCL scaffolds were used to investigate the influence of these scaffolds, applied blank or seeded with ovine bone marrow mesenchymal stem cells, on ovine tibial segmental defects in merino-cross sheep [45]. The authors reported a positive impact of seeded scaffolds, while also mentioning the problems of up-scaling from *in vitro* and small animal models to *in vivo* application on surgically relevant scales. By filling PCL melt electrowritten scaffolds with

nano-HA, Chen *et al.* [46,47] reported improved bone marrow mesenchymal stem cell proliferation as well as high structural regularity and fibre conformity combined with good mechanical stability.

Gwiazda *et al.* [48] investigated the influence of fibre orientation on cell alignment. Comparing different PCL matrices with three different patterns assembled of 20 µm fibres, human mesenchymal stem cells seeded on these scaffolds were found to align spontaneously along these patterns. Large cellularized bone-ligament-bone constructs with complex geometries were thus proposed for increased mechanical resilience and elasticity. In another study, MEW was combined with fused deposition modelling (FDM) 3D printing to prepare PCL scaffolds with aligned and orthogonal fibres, partly coated with collagen [49]. Human adipose-derived stem cells (hADSCs) were found to proliferate on the obtained scaffolds (Fig. 4), while the aspect ratio of cells on the aligned fibres was significantly higher than that on orthogonal fibres. Besides, osteogenic and tenogenic differentiation of hADSCs was found depending on HA addition in different regions.

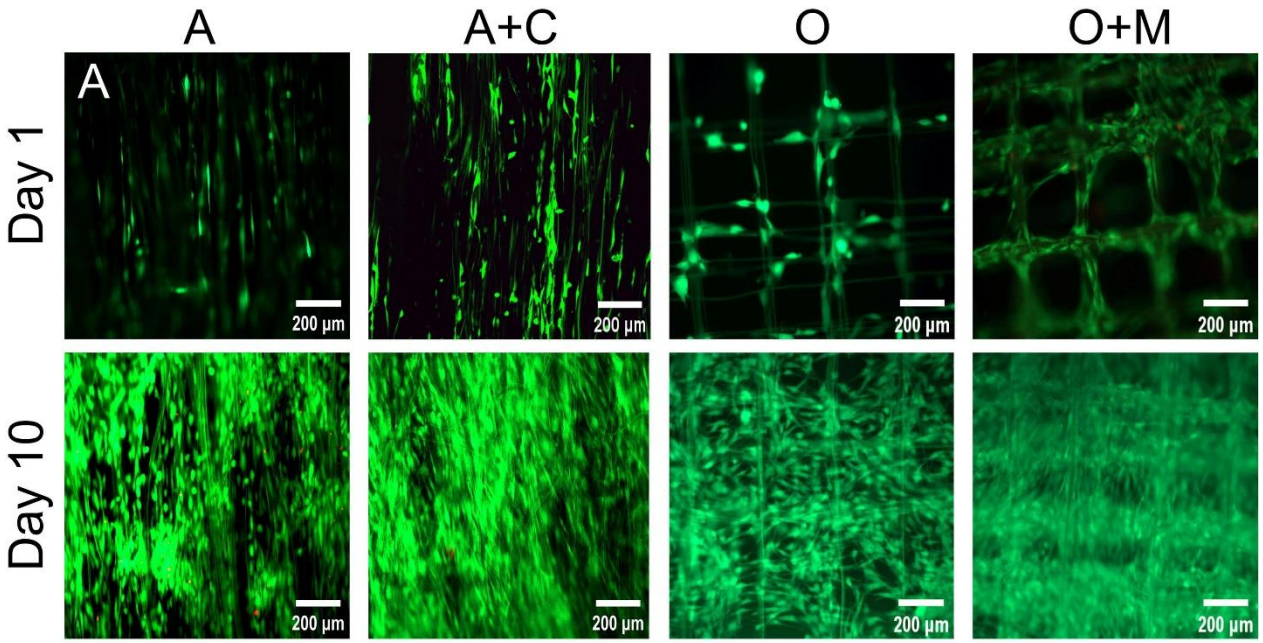

Fig. 4. Live/dead staining of human adipose-derived stem cells on aligned (A), collagen-coated aligned (A+C), orthogonal (O), and mineral-coated orthogonal (O+M) PCL scaffolds, taken on day 1 and day 10. From [49], originally published under a CC-BY-NC 3.0 license

While PCL is most often used for melt electrowriting, some authors also applied other materials. PLLA electrowritten scaffolds with 40 µm fibre diameter and 200 µm pore size were successfully tested *in vitro* for bone tissue engineering [50]. Combining PCL with PLA led to slightly increased viability of L929 mouse murine fibroblast and human umbilical vein endothelial cells compared to pure PCL and PLA scaffolds [51].

Finally, it should be mentioned that the relatively low voltages used in MEW allow even for printing of polymer-DNA composites [52] or of cell-laden polymers, which is sometimes described as electrohydrodynamic bioprinting. This technique was for example reported for printing of alginate-based bioinks with C2C12 cells [53] or human chondrocytes [54].

7. CONCLUSION AND OUTLOOK

Near-field electrospinning and melt electrowriting are recently developed techniques to prepare nano- and microfibres and place them at well-defined positions. The possibility to tailor pore sizes and geometries, independent of fibre diameters, is supportive for cell culture and tissue engineering applications.

Nevertheless, there are still challenges to overcome. On the one hand, while several polymers have already been investigated for NFES or MEW, nowadays especially MEW is mostly performed with PCL or PCL blends, suggesting that more research is needed on the use of other polymers. Additionally, the large number of custom-made setups for NFES and MEW reduces the reproducibility of studies [36]. The situation is even more complicated by sometimes missing information such as the needle length in NFES or environmental parameters. Also, the fibre throughput in these

techniques is still slower than in common far-field electrospinning and several attempts have been made to speed up the fibre production. Finally, freely available programs to translate CAD data of diverse shapes into instructions for NFES or MEW devices are still missing [55].

We hope that this review can help gaining more interest in developing these techniques further.

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3D biomaterijali proizvedeni elektropredenjem u bliskom polju i elektropisanjem rastopljenim materijalom

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(Mini pregledni rad)

Izvod

Elektropredenje u bliskom polju (engl. near-field electrospinning) i elektropisanje rastopljenim materijalom (engl. melt electrowriting) predstavljaju atraktivne tehnike koje se mogu koristiti za proizvodnju polimernih nano- ili mikrovlakana i izradu trodimenzionalnih (3D) struktura koje nalaze primenu u biotehnologiji i biomedicini. Poželjne strukture se mogu projektovati zahvaljujući mogućnosti definisanja kretanja dizne i kolektora. Za razliku od konvencionalnog elektropredenja, elektropredenje u bliskom polju omogućava formiranje veoma finih vlakana koja se organizuju u strukture sa znatno većim porama, prilagođene zahtevima ćelija, što ove nosače čini izuzetno zanimljivim za kulture ćelija, inženjerstvo tkiva i slične biomedicinske i biotehnološke primene. Pored toga, ova tehnika je relativno jednostavna, ponovljiva i jeftina. Elektropisanje rastopljenim materijalom može se koristiti za izvlačenje mikrovlakana iz rastvora ili topljenog polimera kroz elektrostatčko polje, omogućavajući precizno nanošenje sa visokom tačnošću, što dovodi do veoma poroznih nosača koji olakšavaju ravnomernu raspodelu ćelija. Ovaj pregledni rad pruža uvid u nova teorijska i eksperimentalna saznanja vezana za elektropredenje u bliskom polju i elektropisanje rastopljenim materijalom, sa primenama u biotehnologiji i biomedicini, kao što su štampanje nosača za inženjerstvo tkiva i kulture ćelija, izrada obloga za rane i druge primene. Procesi su ukratko objašnjeni, a zatim su predstavljeni i najrelevantniji polimeri za biomedicinske primene. Na kraju, izloženi su aktuelni izazovi i predlozi za buduće pravce istraživanja.

Ključne reči: 3D nosači, biomedicine