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3D electrospinning used in medical materials

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Abstract: Electrospinning (ES) is an interesting and efficient technique for biomedical use. This is a method used for the fabrication of polymer fibers used in tissue engineering (TE). The electrospun nano- and microfibers biomaterial, called scaffolds, are also used for regenerative medicine. The aim of the present mini-review is to present methods used to fabricate 3D fibers by electrospinning and their applications in TE. Also, discussed here are issues regarding the electrospinning limitations and research challenges.

Keywords: Biomaterials; Electrospun; Three dimensional (3D) culture; Tissue Engineering.

Introduction

New frontiers in the application of techniques involving the production of medical products have been recently investigated. Electrohydrodynamic techniques, as electrospinning (ES), are very powerful tools for developing and producing materials with the structural features necessary for tissue engineering (TE) applications[1,2].

The conventional setup of an electrospinning process is illustrated in Fig. 1a. The equipment used for the electrospinning technique consists of a syringe with a needle attached to its tip, connected to an electrode, a hydrostatic pump, and an electrical source. In the syringe, a polymer solution is packaged and then, through the hydrostatic pump, this polymeric solution is directed to a metal collecting plate, which acts as a support for collecting the material produced[2,3]. When the applied voltage is increased beyond a critical value, where the electrostatic forces are balanced by the surface tension of the polymer solution drop at the tip of the capillary, Taylor's cone formation occurs (Fig. 1b)[4]. The potential difference between tip and metal collector induces stretching in a polymer solution from the apex of the Taylor cone. The electrospun process is divided into two stages: first, the drop geometry distortion occurs due to the action of the electric field and then a continuous jet formation occurs from the end of the drop[5]. Between the spinneret and the collector, the solvent is evaporated and fibers with smaller significant diameters than the spinneret are deposited in the collector[4]. Another monoaxial electrospinning process (with just one capillary) is emulsion electrospinning (Fig. 1c). An experimental setup with coaxial nozzle electrospinning (Fig. 1c) consists of two independents solutions simultaneously electrospun forming fibers with core-shell morphology[4,6].

In TE, the produced biomaterials are known as scaffolds. Scaffolds

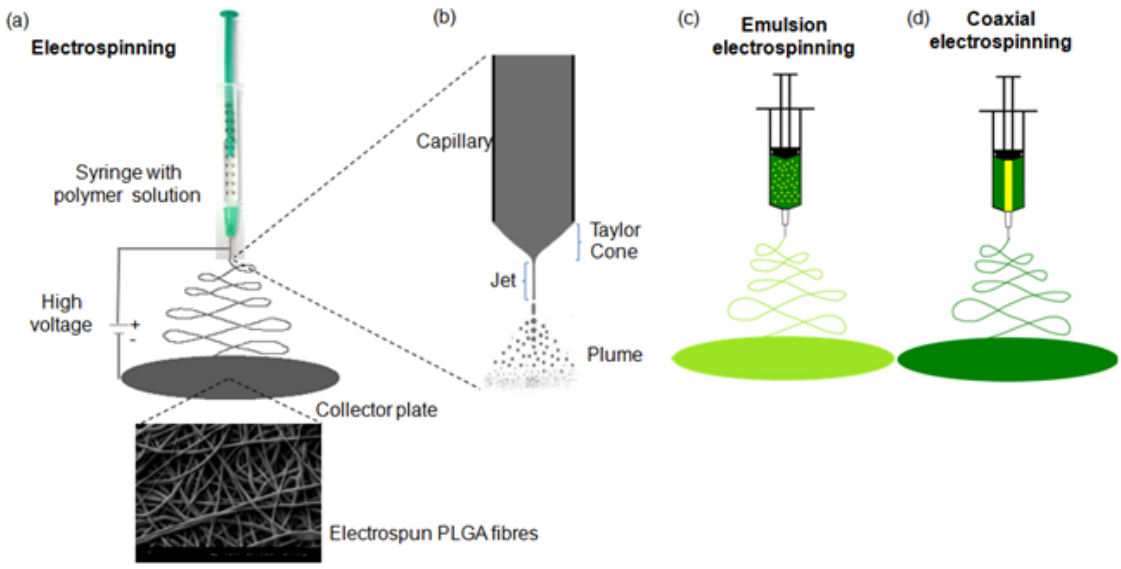

Figure 1 – Schematic diagram of the electrospinning processes for the production of nano- or microfibers. (a) The polymer solution is pumped into the syringe and passes through a spinneret.The nozzle is connected to a terminal of the power supply and a metal collector to the opposite terminal. The jets of polymer solution ejected from the capillary tube can form polymeric particles or filaments, depending on the physical properties of the polymer solution. (b) Schematic illustration of the Taylor cone (https://commons.wikimedia.org/wiki/File:Taylor_cone.jpg). (c) emulsion electrospinning and (d) co-axial electrospinning. Adapted, with permission, from Ref.[2].

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produced by ES technique are formed by nano– or microfibers, pretending to be similar to native extracellular matrix (EMC). Natural ECM where the cells are attached contain nanofibrous proteins and proteoglycans[7]. Thus, the scaffolds produced by ES have special characteristics for use in TE since they are biomimetic materials. In TE, the scaffolds are associated with cells and bioactive molecules, a strategy for a better cellular response on the surface of the biomaterial[8,9]. Some requirements of scaffolds for use in TE are illustrated in Fig. 2.

Several biomaterials are used for the production of medical structural supports by ES. Some examples are the FDA–approved synthetic polyesters poly(ε–caprolactone) (PCL) and poly(lactic–co–glycolic acid) (PLGA). PCL scaffold fibers produced by ES are shown in fig. 3a. In this case,1.2 ml was used of a polymer solution composed of 15% PCL in tetrahydrofuran:methanol (3:1) with the injection rate of 1.41 ml/h, distance of 15 cm between the tip and the plate and supply voltage of 20kV. Fig. 3b shows scaffolds of PLGA (18%) in acetone:hexafluoro (9:1) produced by ES. The procedure for producing PLGA scaffolds was similar to that used for producing PCL scaffolds except for a voltage of +13 kV and flow 1.74mL/h. When scanning electron microscopy (SEM) was realized in the PCL and PLGA electrospun fibers using a JSM–6060 – JEOL microscope, the density of the web uniform fibers was shown. The mean diameter (μm) and the standard deviation was of 1.35 ± 0.50 for PCL and 1.21 ± 0.25 for PLGA.

Additionally, natural polymers such as gelatin, collagen and chitosan, as well as compositions of the polymers can be formed by ES[10].

Research studies involving the use of ES have increased considerably over the last few years. The number of publications in the "PubMed" database (http://www.pubmed.gov) with the keywords "electrospinning" or "electrospun" in the field search Title/Abstract was 10,708 from 2001 until today. When the term "medical" or "tissue engineering" was added to ES searches, the number decreased to 254 and 2,920, respectively. Although in 2001 the numbers of articles were similar, over the years, research with ES has increased more than ES with TE, showing that this technique is being used for other purposes, mainly in batteries and filtration membranes studies. When the term "3D" (three–dimensional) was added to the search with ES and TE, the number fell to 375 articles (Fig. 4). Between these 375 papers, 54 correspond to reviews and 321 to original articles. The major applications of ES for TE are bone, soft tissue and vessels [2,10].

The traditional electrospun nanofibrous mats are two–dimensional (2D) and have some disadvantages regarding the lack of a 3D structure [3,11]. Although they have a high number of pores, the ES scaffolds have a small pore size (<10 μm) between the closely packed nanofibers[11–13]. These 2D biomaterials cause limited cell infiltration since the cells can only proliferate on the surface of the scaffolds, limiting the number of cells grown in and on the scaffolds[11].

Studies have shown that 3D biomaterials, when compared to 2D, serve as ideal scaffolds for cell delivery, providing a support structure and cell encapsulation, thus facilitating cell release at the target destination[11].

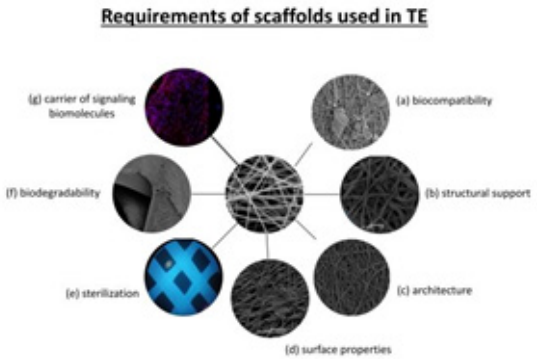

Figure 2 – Illustration of some requirements of scaffolds for use in TE. Adapted, with permission, from Ref.[8].

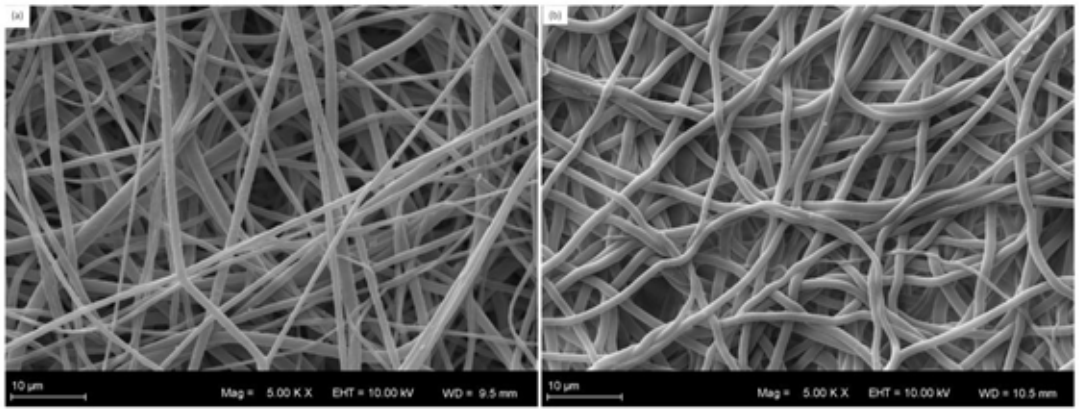

Figure 3 – SEM of fibers electrospun from different polymers. (a) PCL and (b) PLGA. Photographs kindly provided by the Stem Cell Laboratory archives, Universidade Federal do Rio Grande do Sul.

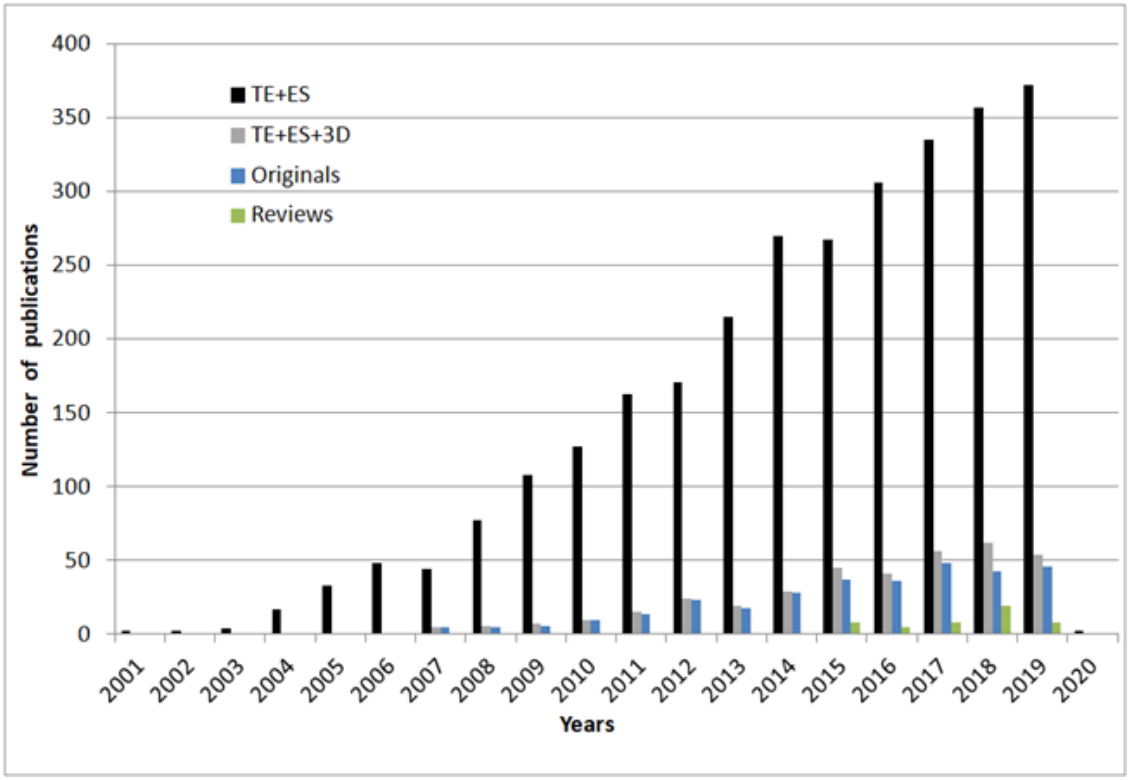

Figure 4 – The number of annual publications in the "PubMed" database from 2001 to November 2019. The black columns indicate the use of keywords "tissue engineering" and "electrospinning" or "electrospun", the gray columns indicate the addition of the keywords "3D", the blue columns indicate the originals and the green columns indicate the review papers (searches realized on 2nd November 2019).

3D electrospinning

In order to enhance the ideal pore size and a 3D structure in nanofibrous scaffolds to improve cell infiltration, some modifications in the ES method have been proposed. Some of the variations are the use of different salts and surfactants molecules, modifications on the collecting plate, the use of sacrificial elements, the gas foaming technique, extra metal as a positive electrode, the simple layer-by-layer ES, as well as the association between 3D techniques and electrospinning[3].

The use of sacrificial elements involves the use of soluble components that are mixed with insoluble polymers. The function of the soluble component (sacrificial element), that could be a polymer or particles, is to leave a bigger pore when it is removed by rinsing in water. Wang and colleagues fabricated an electrospun silk fibroin scaffolds with macropores and high porosity using electrospraying-generated poly (ethylene oxide) (PEO) microparticles as porogen. The scaffolds with a greater diameter allowed the cells to be infiltrated with up to 550 μm, while those on the control scaffolds remained on the surface[14]. Moreover, Zander and colleagues compared two methods to reach a bigger pore size, one fabricating aligned fibers by co-electrospinning of PCL with a sacrificial polymer PEO, and the other, fabricating a scaffold with various concentrations of PCL to obtain different fibers diameters and porosity. The last was the better method; however, a better infiltration only was achieved for the micron-sized fibers obtained from a 40 wt% PCL solution[15].

Another interesting method to improve the pore size is the cold-plate electrospinning technique (CPE), wherein humid conditions, polymer fibers are deposited in a cryogenic mandrel while the formation of ice crystals occur due to the condensation of humidity. Sheikh and colleagues produced 3D silk fibroin nanofibers with high porosity using the cold-plate technique and compared this technique to salt leaching electrospinning (SLE). Results showed that CPE can produce nanofibers with a higher porosity than those achieved using the TE and SLE techniques. Additionally, nanofibers had more regular pore architecture and consequently an intense cell infiltration, in contrast with the SLE, when there was a mild cell infiltration[16].

On the other hand, researchers have developed nanofibrous scaffolds with bigger pore size processing the electrospun scaffolds after its conventional production. In the study of Lee JB and colleagues, a 3D nanofibrous scaffold of poly-L-lactic acid (PLLA) was fabricated by subjecting a conventional PLLA electrospun scaffold, with densely packed nanofibers, to ultrasonication. The results showed highly porous scaffolds with over 98% porosity, which was dependent on the ultrasonic exposure time. The increase in the pore size and porosity allows a maximum 3D cell infiltration of 28% of the total depth of the scaffold in 7 days ***in vitro***[17].

The gas foaming technique is another way used to increase the pore size and porosity. This method takes advantage of gas at high pressure to produce harsh solvents instead of the often used particle-leaching methods[18]. Jiang and colleagues used this technique, where hydrogen gas bubbles generated from the sodium borohydride (NaBH4) hydrolysis reaction, expanded aligned electrospun nanofiber PCL mats in 3 dimensions, with an increased porosity of 84 to 99 %[19]. Hematoxylin-eosin (H&E) staining of cell-seeded scaffolds suggested that cells successfully infiltrated and proliferated throughout the bulk of expanded nanofiber scaffolds[19].

A simple way to produce 3D scaffolds with ES is to make rectangular scaffold mats which a glued forming a 3D conduit (Fig. 5a)[20]. In addition, a similar 3D conduit (Fig. 5b) can be produced by the electrospinning technique utilizing a cylinder grounded as a collection rotating mandrel[21-23]. This method involves the deposition of the fibers in the tubular collection, represented by "sa" and "pa" (Fig. 5b). The appearance of the scaffold (tubular conduit) is shown in Figure 5b. Sell and colleagues described the production of scaffolds for vascular grafts with a view towards the cardiovascular tissue engineering[21]. Lee et al developed this scaffold composite of PCL/collagen with adequate elasticity and burst pressure larger than with the PCL scaffolds alone, which is appropriate for vascular TE[21].

It is also possible to combine techniques such as ES and rapid prototyping. The 3D printing assists in the rapid fabrication of tissue engineering biomaterials for complex shapes and cells can be easily associated[11]. The association between 3D techniques and electrospinning is of great relevance, and electrospun nanofibers in association with 3D printed matrices have been developed (Fig. 5b)[8]. These scaffolds can be used for tissue engineering of cartilage and bone.

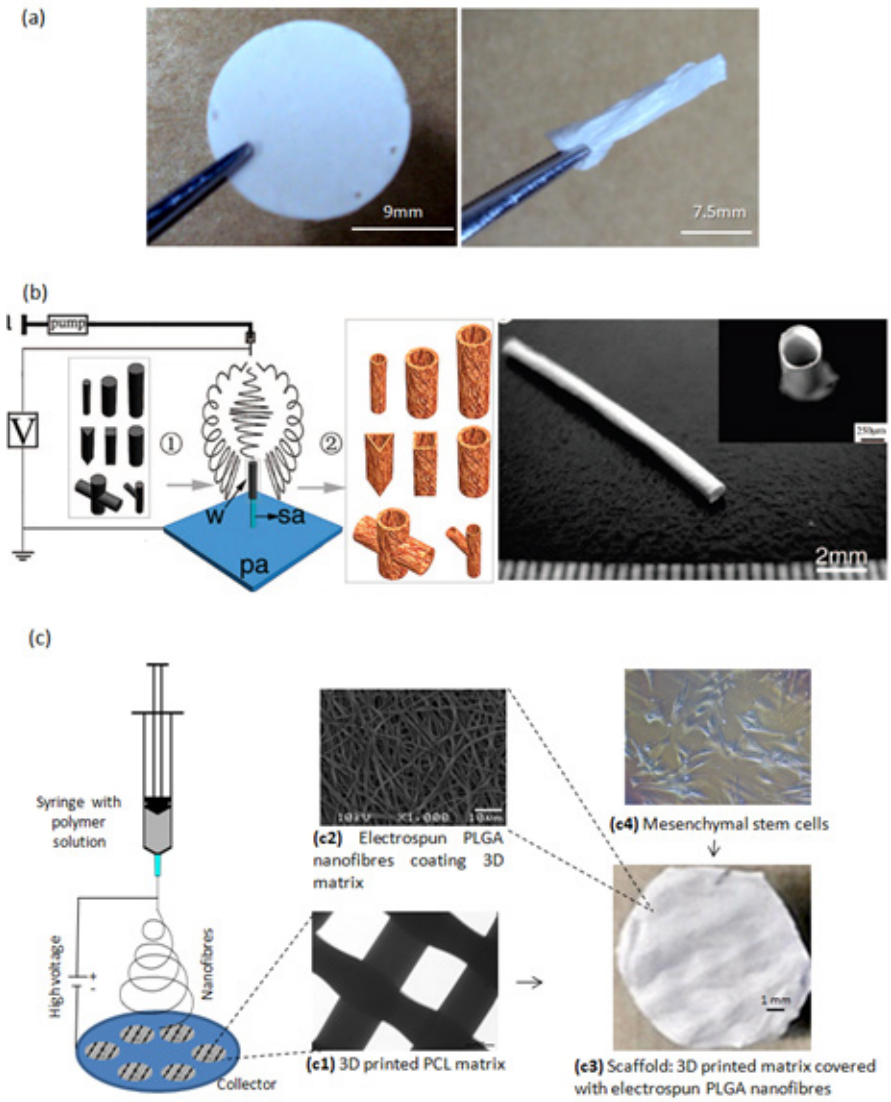

Figure 5 – 3D making scheme. (a) Production of 3D conduit. Adapted, with permission, from Ref. 20. (b) Fabrication of tubes by the electrospinning technique using 3D columnar collectors. 1: 3D columnar collectors. 2: fibrous tubes. (w: working collector; pa: plate assistant collector; sa: stick assistant collector) and an optical photograph of a tube fabricated using this method. Adapted with permission from Ref. 22. Copyright (2019) American Chemical Society. (c) ES at par 3D printed matrices. Adapted, with permission, from Ref.[8].

In a study with the laser–based 3D printing system, Lee and colleagues used stereolithography capable of fabricating 3D constructions through a layer assembly method. The association of this technique with electrospinning developed a 3D biomimetic neural scaffold associated with bioink to improve biocompatibility and mechanical properties[23].

In another study, hierarchical scaffolding supplemented with cellulose alginate hydrogel microfibers was developed to evaluate the effect of diameter and thickness on cell activity. The bioink used was associated with alginate solution MG63 cells and is overlaid with PCL nanofibers and 3D printed PCL microstructure by 3D printing. They concluded that the MG63–associated microfibers assisted in the process of proliferation, release efficiency and osteogenic differentiation, and are promising for soft tissue and hard tissue regeneration[24].

Lately, both 3D printing and electrospinning technologies have been associated to develop an efficient scaffold, capable of supporting the culture of tissues like skin. Miguel and colleagues produced a 3D skin asymmetric construct by using electrospinning and 3D bioprinting techniques. To develop the epithelium like a layer, a PCL/silk serin scaffold was electrospun and placed at the top of a dermis like layer formed by printing a layer by layer chitosan/sodium alginate hydrogel. Results showed that the epithelium layer, developed with the electrospun scaffold, provided a protective barrier against dehydration and potentially dangerous elements. On the other hand, the printed dermis exhibited an adequate porosity, wettability and biological properties for supporting cell adhesion, migration and proliferation[25].

Other 3D electrospun materials could be used in bone, skin and cartilage regeneration. On the skin, the biomaterials have crucial performance in body defence. They can be used as autologous and allogeneic keratinocyte grafts, and cellular biological matrix and there is also the possibility of including biological substances that can assist in the regeneration process. For bone use, calcium phosphate or growth factors associated with bone marrow or osteoblast–like stromal cells are used. Cartilage provides a structure to the body without bone stiffness, consisting mainly of chondrocytes incorporated into the non–vascularized extracellular matrix. One study used electrophilic silk matrices, which resulted in increased binding and proliferation of the chondrocytes[4].

Conclusions

The electrospinning to produce nonwoven fibers, from both natural and synthetic biomaterials, have been used in tissue engineering. Moreover, fibrous scaffolds confer better cell adhesion, proliferation, migration and differentiation for the cells.

These scaffolds produced by ES are formed by structures that mimic the fibrous structure of the ECM two–dimensionally making this technique

an effective and simple method for producing micro– and nanofibers with a wide variety of biodegradable polymers. Although there are many techniques for obtaining biomimetic 2D structures, the production of true 3D microenvironments for tissue regeneration is just beginning. There has been an increase in the number of annual publications in the field of electrospinning with applications in medicine. When the keyword 3D was added to research, the number decreased (about 10x less), indicating that the papers do not address the three–dimensional aspect. Between the papers that focus on TE + ES + 3D, most refer to original articles and 14% review the topic.

Electrospinning has been recognized as a versatile approach for obtaining variously shaped structures from a range of biomaterials for possible applications in tissue engineering and regenerative medicine. However, the dense bundling of fibers is a major setback, where the cells do not form a real three–dimensional environment. To this end, various strategies have been reported on electrospun scaffolding which thus improve cellular response. Key challenges include the development of ES–based 3D scaffolds that have an appropriater structure similar to native tissue to aid in cell adhesion and proliferation. The 3D approach offers advantages regarding the promotion of cell infiltration; increased cell numbers mimic better ECM and allows for the production of scaffolds which are more similar to living tissue. As each tissue and organ have different organizations, the fabrication of standardized scaffolds is not possible, and the optimization of each cell type is necessary.

Several electrospinning parameters affect the fibers, porous and consequently the formation of the 3D scaffolding structure, such as the solvent, polymer, processing additives, flow rate, applied voltage, needle size, spinner to the collector distance, ambient temperature and humidity. Some setups with electrospinning allow for the production of 3D scaffolds. For example, the use of layer–by–layer ES; the association between 3D techniques and electrospinning; an extra metal as the positive electrode or the modifications on the collecting plate; salts, surfactants and others sacrificial elements and gas foaming technique. The ES method with 3D collection is a versatile and extensively used technique for manufacturing fibrous tubes in biomedical applications.

It is necessary to fine–tune the process to create reproducible scaffolds with ideal fiber and pore sizes. Thus, innovative and alternative approaches in TE research and development are still seeking to understand the in vivo behavior of these materials, their rate of degradation and remodeling in situ, as well as their inflammatory and immunological responses.

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References

1. Huang Z–M, Zhang Y–Z, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Compos Sci Technol* **63**(15):2223–53 (2003). DOI: 10.1016/S0266–3538(03)00178–7.

2. Maurmann N, Sperling L, Pranke P. Electrospun and Electrosprayed Scaffolds for Tissue Engineering, in Cutting–Edge Enabling Technologies for Regenerative Medicine, Advances in Experimental Medicine and Biology, ed by H. J. Chun *et al.* Springer Nature Singapore, pp 79–100 (2018). DOI: 10.1007/978–981–13–0950–2.

3. Agarwal S, Burgard M, Greiner A, Wendorff J. In *Electrospinning : A Practical Guide to Nanofibers*. Ed by Walter de Gruyter GmbH, Berlin/ Boston 2016. 189 pgs (2016).

4. Zhang X, Reagan M, Kaplan D. Electrospun silk biomaterial scaffolds for regenerative medicine. *Adv Drug Deliv Rev* **61**: 988–1006 (2009). DOI: 10.1016/j.addr.2009.07.005.

5. Costa RGF, Oliveira JE de, Paula GF de, Picciani PH de S, Medeiros ES de, Ribeiro C, *et al.* Eletrofiação de Polímeros em Solução: parte I: fundamentação Teórica. *Polímeros* **22**:170–177 (2012). DOI: 10.1590/S0104–14282012005000026.

6. Hu W, Huang ZM, Liu XY. Development of braided drug–loaded nanofiber sutures. *Nanotechnology* 21(**31**):315104 (2010). DOI: 10.1088/0957–4484/21/31/315104.

7. Nagle AR, Fay C, Xie Z, Wang X, Wallace GG, Higgins MJ. Patterning and process parameter effects in 3D suspension near–field electrospinning of nanoarrays. *Nanotechnology* (2019). DOI: 10.1088/1361–6528/ab3c87.

8. Maurmann N, Pereira DP, Burguez D, Pereira FDADS, Neto PI, Rezende RA, *et al.* Mesenchymal stem cells cultivated on scaffolds formed by 3D printed PCL matrices, coated with PLGA electrospun nanofibers for use in tissue engineering. *Biomed Phys Eng Express* **3**:045005 (2017). DOI: 10.1088/2057–1976/aa6308.

9. Sousa RB De, Vieira EG, Osajimafurti JA *et al*. Recent advances in methods of synthesis and applications of bacterial cellulose/calcium phosphates composites in bone tissue engineering. *IJAMB* **1**:33–42 (2018). DOI: 10.25061/2595–3931/IJAMB/2018.v1i2.16.

10. Steffens D, Braghirolli DI, Maurmann N, Pranke P. Update on the main use of biomaterials and techniques associated with tissue engineering. *Drug Discov Today* **23**:1474–1488 (2018). DOI: 10.1016/j.drudis.2018.03.013.

11. Lin W, Chen M, Qu T, Li J, Man Y. Three–dimensional electrospun nanofibrous scaffolds for bone tissue engineering. *J Biomed Mater Res Part B* (2019). DOI: 10.1002/jbm.b.34479.

12. Dhandayuthapani B, Yasuhiko Y, Maekawa T, Kumar DS. Fabrication and characterization of nanofibrous scaffold developed by electrospinning. *Mat Res* **14**:317–325 (2011). DOI: 10.1590/S1516–14392011005000064.

13. Chen W, Xu Y, Liu Y, Wang Z, Li Y, Jiang G, *et al.* Three–dimensional printed electrospun fiber–based scaffold for cartilage regeneration. *Materials & Design* **179** (2019). DOI: 10.1016/J.MATDES.2019.107886.

14. Wang K, Xu M, Zhu M, Su H, Wang H, Kong D, *et al.* Creation of macropores in electrospun silk fibroin scaffolds using sacrificial PEO–microparticles to enhance cellular infiltration. *J Biomed Mater Res Part A* **101**(12):3474–81 (2013). DOI: 10.1002/jbm.a.34656.

15. Zander NE, Orlicki JA, Rawlett AM, Beebe TP. Electrospun polycaprolactone scaffolds with tailored porosity using two approaches for enhanced cellular infiltration. *J Mater Sci Mater Med* **24**(1):179–87 (2013). DOI: 10.1007/s10856–012–4771–7.

16. Sheikh FA, Ju HW, Lee JM, Moon BM, Park HJ, Lee OJ, *et al.* 3D electrospun silk fibroin nanofibers for fabrication of artificial skin. *Nanomedicine* **11**(3):681–91 (2015). DOI: 10.1016/j.nano.2014.11.007.

17. Lee JB, Jeong SI, Bae MS, Yang DH, Heo DN, Kim CH, *et al.* Highly Porous Electrospun Nanofibers Enhanced by Ultrasonication for

Improved Cellular Infiltration. *Tissue Eng Part A* **17**(21–22):2695–702 (2011). DOI: 10.1089/ten.TEA.2010.0709.

18. Lakhtakia A, Martín–Palma RJ, Carter P, Bhattarai N. Bioscaffolds: Fabrication and Performance. *Eng Biomimicry* 161–88 (2019). DOI: 10.1016/B978–0–12–415995–2.00007–6.

19. Jiang J, Carlson MA, Teusink MJ, Wang H, MacEwan MR, Xie J. Expanding Two–Dimensional Electrospun Nanofiber Membranes in the Third Dimension By a Modified Gas–Foaming Technique. *ACS Biomater Sci Eng* **1**(10):991–1001 (2015). DOI: 10.1021/acsbiomaterials.5b00238.

20. Dos Santos FP, Peruch T, Katami SJV, Martini APR, Crestani TA, *et al*. Poly (lactide–co–glycolide) (PLGA) scaffold induces short–term nerve regeneration and functional recovery following sciatic nerve transection in rats. *Neuroscience* **396**:94–107 (2019). DOI: 10.1016/j.neuroscience.2018.11.007.

21. Sell A, Michael J, Garg K, Wolfe P, Bowlin G. Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering. *Adv Drug Deliv Rev* **61**:1007–1019 (2009). DOI: 10.1016/j.addr.2009.07.012.

22. Zhang D, Chang J. Electrospinning of Three–Dimensional Nanofibrous Tubes with Controllable Architectures. *Nano Letters* **8**:3283–3287 (2008). DOI: 10.1021/nl801667s.

23. Lee SJ, Nowicki M, Harris B, Zhang LG. Fabrication of a Highly Aligned Neural Scaffold via a Table Top Stereolithography 3D Printing and Electrospinning. *Tissue Eng Part A* **23**(11–12):491–502 (2017). DOI: 10.1089/ten.TEA.2016.0353.

24. Yeo M, Kim G. Cell–printed hierarchical scaffolds consisting of micro–sized polycaprolactone (PCL) and electrospun PCL nanofibers/cell–laden alginate struts for tissue regeneration. *J Mater. Chem. B.* **2**:314–324 (2013). DOI: 10.1039/C3TB21163K.

25. Miguel SP, Cabral CSD, Moreira AF, Correia IJ. Production and characterization of a novel asymmetric 3D printed construct aimed for skin tissue regeneration. *Colloids Surfaces B Biointerfaces* **181**:994–1003 (2019). DOI: 10.1016/j.colsurfb.2019.06.063.