Electrospun nanostructured scaffolds for tissue engineering applications

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Despite being known for decades (since 1934), electrospinning has emerged recently as a very widespread technology to produce synthetic nanofibrous structures. These structures have morphologies and fiber diameters in a range comparable with those found in the extracellular matrix of human tissues. Therefore, nanofibrous scaffolds are intended to provide improved environments for cell attachment, migration, proliferation and differentiation when compared with traditional scaffolds. In addition, the process versatility and the highly specific surface area of nanofiber meshes may facilitate their use as local drug-release systems. Common electrospun nanofiber meshes are characterized by a random orientation. However, in some special cases, aligned distributions of the fibers can be obtained, with an interconnected microporous structure. The characteristic pore sizes and the inherent planar structure of the meshes can be detrimental for the desired cell infiltration into the inner regions, and eventually compromise tissue regeneration. Several strategies can be followed to overcome these limitations, and are discussed in detail here.

Tissue engineering and regenerative medicine are commonly defined as being an interdisciplinary field that aims at the development of biological substitutes that restore, maintain or improve tissue function or a whole organ [1]. Efforts in this have been directed to produce biocompatible scaffolds that physically support cells and provide conditions for cell adhesion and growth, mimicking the native extracellular matrix (ECM) of tissues [2]. Those scaffolds can be obtained from different materials, including biodegradable polymers, ceramics or composites containing both polymer and ceramic phases. Generally, those systems are aimed at being resorbed under physiological conditions. The degradation kinetics of ideal scaffolds should follow the tissue growth kinetics in such a way that the material is completely degraded when the tissue is fully regenerated [1]. Moreover, appropriate cytocompatibility, porosity, pore size, surface properties and mechanical stability have been defined as being critical requirements [3–5].

The cellular response to a biomaterial is believed to be enhanced when the morphology of the scaffold mimics the architecture of the native tissue. This is typically thought of as being associated with the material topography and the highly specific surface area, which are also characteristics of nanofiber meshes [6–11]. This hypothesis increased the interest for nanofibrous scaffolds that can closely mimic the surface structure and morphology of native

ECMs of many tissues. Different techniques, such as self-assembly [12], phase separation [13] and electrospinning [14], have been used to develop nanofibrous scaffolds. In this review, we intend to provide an updated overview of the current state of the art on the applicability of fibrous scaffolds produced by electrospinning in tissue engineering. The simplicity of this technique, its cost–effectiveness and versatility to produce nanofiber meshes from many polymers commonly proposed for tissue engineering applications, helps in understanding why electrospinning is currently the most-used technique for producing scaffolds. Most of the published works use electrospinning to produce random fiber meshes that may have important limitations for cell migration and colonization of the inner regions of the meshes, eventually compromising its effectiveness for some tissue-engineering applications.

Electrospinning technique

Conventional electrospinning involves drawing a polymer solution droplet, dispensed by a syringe pump, from a capillary. The solution undergoes extensional flow and deposits into a collector by the application of an external electrostatic field. The process starts by the application of a strong electric field to a droplet of the polymer solution in the tip of the capillary. When the intensity of the electric field generates a sufficient stress in the droplet to overcome its surface tension, a

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tiny solution jet is ejected in the direction of the collector. Before reaching the collector, the solvent partially evaporates and the jet of solution is subjected to intensive extensional strain, leading to the deposition of long and thin fibers, eventually at the nanoscale. The most typical morphology obtained corresponds to a randomly aligned and porous nonwoven mesh [15–18].

Processing parameters

The submicrometer diameter of the fibers in the nonwoven meshes produced by electrospinning have a high surface area:volume ratio, which raised the interest of these structures for biomedical applications [19–21]. The properties of the obtained meshes depend on various parameters involved in the deposition process, namely the type of polymer and its molecular weight, the nature of the solvent used, the solution concentration, the solution viscosity, applied voltage, distance between the tip of the capillary and the collector and collector type [10,14,22–26]. Several works have demonstrated that electrospun fibers' diameters can be varied using solutions with different polymer concentrations, and thus tuning the solution viscosity [10,23,27]. In general, the diameter of electrospun fibers increases proportionally with the polymer concentration [10,27]. The porosity of the meshes can also be controlled to some extent by adjusting both the solution properties and the above referred operating parameters [26,28]. The alignment of the fibers in the mesh structure is also an important aspect to be tailored. Static flat collectors, the most commonly used, cause the deposition of randomly oriented fibers. When dynamic collectors are used, such as rotating mandrels with controlled rotary speed, some degree of alignment of the fibers may be obtained within a tubular structure. Recent studies have shown that some cell types elicit specific responses to aligned fibers, preferring to grow along oriented regions of nanofiber meshes [10,22,29–31].

Electrospun materials commonly used in TE

In the literature, several procedures have been proposed for the electrospinning of fibers from different materials currently used in tissue engineering, such as synthetic [23,27,32,33] and natural polymers [29,34–36], polymer blends [37–40], polymer composites [41–43] and ceramics [44–46]. Since most synthetic biodegradable or bioresorbable polymers consist of polyesters, volatile organic solvents are typically used in their processing.

The possible presence of residual solvents in the electrospun fibers cannot be excluded, which may eventually compromise their use in biological experiments. In the case of natural-origin polymers, a stabilization process of the nanofiber structure may be needed before performing biological experiments. Typical examples include chitosan nanofibers, produced from a solution of chitosan in trifluoroacetic acid [36,47], or silk fibroin nanofibers, produced from a solution of silk fibroin in formic acid [48,49]. A survey of the polymers commonly used in tissue engineering and already processed by electrospinning, including the solvents used herein and the resulting range of fiber diameters, are presented in **Table 1**.

The analysis of **Table 1** allows one to conclude that a broad list of solvents have been used for each material and a wide distribution of fiber diameters may be obtained, ranging from 40 nm to a few microns. It should also be highlighted that the meshes are typically characterized by a heterogeneous distribution of fiber diameters.

Applications in tissue engineering

Tissue engineering strategies frequently propose the use of synthetic 3D ECMs, such as scaffolds, for the regeneration of human tissues. Many concepts are also based on the use of cells isolated from a small tissue biopsy. The synthetic ECMs are intended to provide a temporary template for cell seeding, proliferation and, when using progenitor cells, differentiation. The constructs are used to develop a tissue precursor *in vitro*, to be transplanted into the patient to promote the formation of functional neotissue. It is critical that this neotissue will structurally integrate within the host tissues. Among the several types of scaffolds proposed for tissue engineering, electrospun meshes seem to have specific advantageous properties and limitations, which will be reviewed in the following section.

Extracellular matrices & electrospun nanofibers

The ECM of human tissues is a dynamic and hierarchically organized structure composed of polysaccharides (such as glycosaminoglycans) and proteins (such as collagen and proteoglycans) synthesized by the adjacent cells [39,50,51]. In this complex structure, the collagen fibers provide strength to the tissue and, more importantly, have many cell-adhesive peptide moieties for cellular anchoring. The hydrated gel composed of proteoglycans and other proteins fills the extracellular space, creating an appropriate microenvironment for tissue

Table 1. Polymer nanofibers and solvents that are most commonly used.		
Polymer	**Solvent**	**Fiber diameters (nm)**
PLA	Chloroform/DMF [94], HFP [95], dichloromethane/DMF [10,11]	800–3000 [94], 250–1250 [10], 235–3500 [11]
PGA	HFIP [27,95,96]	220–880 [27]
PLGA	Tetrahydrofuran/DMF [32,97], HFP [95]	500–800 [97], ~760 [32]
PCL	Chloroform/methanol [23], methylene chloride, chloroform/DMF [98], tetrahydrofuran/DMF [33], methylenechloride/DMF [99]	2000–10000 [23], 500–1200 [99]
PHBV	TFE [100], chloroform [101]	100–2000 [100], 1000–4000 [101]
P(LLA-CL)	Dichloromethane/DMF [90]	~470 [90]
Collagen	HFP [14,29,102,103]	50–300 [29], 100–1200 [14], 300–375 [103]
Chitosan	Acetic acid solution [104], TFA/dichloromethane [36]	~130 [104], ~300 [36]
Silk fibroin	Water [34,105], formic acid [49]	~575 [34], 30–120 [49], ~700 [105]
Hyaluronic acid	Hydrochloric acid solution [106,107]	49–74 [106], 57–83 [107]
Gelatin	HFP [95,108], TFE [35]	77–485 [108]
Fibrinogen	HFP [109,110]	80–700 [109]
Chitin	HFP [111]	40–640 [111]

DMF: N,N-dimethylformamide; HIFP: Hexafluoroisopropanol; HFP: 1,1,3,3,3-hexafluoro-2-propanol; PCL: Polycaprolactone; PGA: Poly(glycolic acid); PHBV: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); P(LLA-CL): Poly(L-lactic acid)-co-poly(ε-caprolactone); TFA: Trifluoroacetic acid; TFE: 2,2,2-trifluoroethanol.

maintenance and remodeling in response to appropriate stimuli, while allowing for the diffusion of nutrients, metabolites and signaling molecules. Those components interact together to form an interconnected nano- or micro-ranged fibrous network bounded to the membranes of cells. Tissue ECMs act as a scaffold to support and hold cells together, to control the tissue's structure and to regulate cellular functions such as adhesion, migration, proliferation, differentiation and, ultimately, tissue morphogenesis [52]. The ECM also serves as a storage depot and a controlled-release system of growth factors and signaling molecules.

The ECM interacts with the adjacent cells both mechanically and chemically, remodeling the architecture of the tissues. The structure of different collagen types determines their function as structural elements of the connective tissues [53]. Tendon ECM is composed of parallel and aligned collagen fibrils, while those found on the skin are mesh-like. In most connective tissues, the matrix macromolecules are secreted by fibroblastic cells into the extracellular space. In specialized types of connective tissues, such as cartilage and bone, cells of the fibroblast family (chondrocytes and osteoblasts, respectively) are responsible for ECM deposition. The matrix either becomes calcified into the hard and tough structures of bone and teeth, or can form the transparent matrix of cornea. ECM can also adopt the cord-like organization that gives tendons their tensile strength and elasticity.

In native tissues, the diameter of collagen fibrils ranges between 30 and 300 nm. Electrospun nanofibers, with diameters between 300 and 1000 nm, can provide appropriate micro-environments for cell attachment, proliferation and differentiation [2,54]. The versatility of the process allowing the use of homo- and co-polymers, blends of polymers and even polymer compositions with inorganic materials or other additives also allow obtaining functionally active meshes [23,27,32–46]. The ultra-thin fibers produced by electrospinning, having a high surface area, follow the structure of native ECM [55]. The obtained meshes have high porosity with interconnected pores and, in association with their high surface area, maximize the opportunities for cell–synthetic ECM interactions.

Limitations of the electrospinning process include the insufficient control over the fibers' diameters and the mesh morphology, leading to nonuniform nanofibrous structures. The size of the pores is also, in many cases, insufficient for allowing cell migration into the inner regions of the meshes.

In vitro & in vivo applications

Electrospun polymeric nanofibers have been proposed as scaffolds for tissue engineering of skin, cartilage, bone, peripheral nerve system, heart, blood vessels, ligament/muscle, kidney and liver. In general, the electrospun nanofibrous

matrices support cell adhesion, phenotype maintenance, proliferation and differentiation of stem cells. In most of these studies, biodegradable polymer materials such as polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA) or its copolymers were used. A survey of electrospun scaffolds produced from different biodegradable polymers to target different tissues is summarized in **Table 2**.

Cell lines or primary cells taken from their biological context do not adequately model the *in vivo* tissue behavior. The difficulty to mimic the *in vivo* microenvironment in cell culture systems justifies the interest in electrospun nanofiber meshes. However, the merits of electrospun nanofiber meshes as adequate scaffolds still need to be demonstrated upon implantation in animal models.

The performance of electrospun polyurethane (PU) nanofiber mesh as a wound-healing device was examined *in vivo* using a pig model [56]. Histological results showed that the epithelialization rate is increased and the obtained dermis structure in wounds covered with electrospun nanofibrous membrane is improved. In addition, this mesh as a wound dressing demonstrated controlled evaporative water loss and excellent oxygen permeability, and allowed fluid drainage from the wound. Furthermore, the mesh inhibits exogenous microorganism invasion into the wound. Another *in vivo* study, using a rat model subjected to midline celiotomy, examined the effect of using electrospun, nonwoven, bioabsorbable polylactic-co-glycolic acid (PLGA)-based membranes impregnated with antibiotics (Mefoxin®, cefoxitin sodium) as antiadhesion membranes [57]. Results showed that the electrospun PLGA/PEG-PLA membranes impregnated with 5 wt% Mefoxin completely prevented cecal adhesions (0%) in rats at the site of the injury. The performance of antibiotic (Biteral®, ornidazol) loaded PCL membranes to prevent postsurgery abdominal adhesions and to improve healing was recently studied [58]. The rat model underwent defects on the abdominal walls of the peritoneum. Capillaries were formed predominantly at the edges of the antibiotic-loaded PCL membrane in which sutures were applied.

Another *in vivo* study aiming at studying bone regeneration proposed PCL scaffolds obtained by electrospinning seeded with mesenchymal stem cells (MSCs) [59]. The cell/scaffold construct was cultured with osteogenic supplements in a rotating bioreactor for 4 weeks, before implantation in the omenta of rats during 4 weeks. The results showed ECM formation throughout the constructs, mineralization and type I collagen expression. The authors concluded that bone grafts with bone-like appearance could be developed from electrospun nanofibrous scaffolds. Electrospun silk fibroin (SF) membranes were tested as bone periodontal regenerative implants [60]. This study used calvaria defects in New Zealand White rabbits and a complete bony union across the defects was observed after 8 weeks. At 12 weeks, the defect had completely healed with new bone and without any evidence of an inflammatory reaction. These results strongly suggest that the SF membrane can be useful as a solution for guided bone regeneration.

In the regeneration of a nerve conduit, PLGA (10:90) fibers were collected over a Teflon® tube of 1.27 mm diameter and implanted into a rat sciatic nerve [61]. The porous nanofibrous scaffold allowed the diffusion of nutrients into the lumen, facilitating nerve regeneration and, simultaneously, acting as barrier to undesired scar-tissue infiltration.

Potentialities of the electrospinning process
Development of hybrid polymeric matrices

Nature tends to assemble structures with a minimum quantity of materials and with maximum functionality. Indeed, natural ECM consists of less than 1% solid materials, and yet contributes significantly to the mechanical and functional properties of tissues. By understanding the hierarchical tissue organization from the molecular level up to macroscopic scale will likely guide us to new designs of the synthetic ECMs for use in regenerative medicine [62].

A critical issue in tissue engineering is to learn how to engineer biomaterials that help in recapitulating the early events of morphogenesis that lead to the formation of the hierarchical organization of the ECM and drive the cells to build fully functional adult tissues. Recently, in our group, an innovative use of the electrospinning technique was proposed to produce nanofibers on starch-polycaprolactone (SPCL) microfiber meshes combining nano- and microfibers in the same 3D scaffold architecture [63,64]. The micro–nanofibrous architecture was composed of electrospun nanofibers randomly deposited over a wet-spun mesh structure produced from microfibers, with a refined structure resembling nanobridges connecting the microfibers. The concept

Table 2. Polymer nanofibers as tissue engineering scaffolds.

Cultured cells	Scaffold material	Potential application in tissue engineering
Fibroblasts	PLGA [97], PLGA–chitin nanoparticles [112], PLGA–dextran [113], EVOH [114], polyamide [115], silk fibroin [49]	Skin
Keratinocytes	PLGA and PLGA–chitin nanoparticles [112], silk fibroin [49,116], collagen type I [102]	
Chondrocytes	PCL [117,118], SPCL [118], collagen type II, chitosan/PEO [119,120]	Cartilage
Fibrochondrocytes	PCL [22,72]	
Mesenchymal stem cells	PCL [22,33,121]	
Osteoblasts	PCL–CaP [93], PCL–CaCO$_3$ [122], PCL–HA [42], PNmPh [123], chitosan/PEO [120], PCL [33,124], PLLA [125], PCL–gelatin [35]	Bone
Neural stem cells	PLLA [10,126,127]	Nerve
Cardiomyocytes	PCL coated with collagen type I [128], PLLA, PLGA, PEG–PLA [129]	Cardiac
Arterial smooth muscle cells	PLA [130], P(LLA-CL) [30,131,132], EVOH [114], PLGA–collagen [133], PLGA–collagen type I–elastin [134], PCL, collagen type I [14] and III [135]	Vascular
Vascular endothelial cells	P(LLA-CL) [131,132,136], PLLA [11], PNmPh [123], PET–gelatin [92], P(LLA-CL)–collagen type I [90], PLGA–collagen [133], PLGA–collagen type I–elastin [134]	
Myoblasts	DegraPol® or PEU [137]	Ligament/Muscle
Ligament fibroblasts	PU [69]	
Porcine bone marrow stromal cells	PLGA [138]	
Kidney cells	Polyamide [115]	Kidney
Hepatocytes	PCLEEP–PAA–AHG [139], PPC [140]	Liver

EVOH: Poly(ethylene-co-vinyl alcohol); P(LLA-CL): Poly(L-lactic acid)-co-poly(ε-caprolactone); PCL: Polycaprolactone; PCL–CaP: PCL nanofibers coated with calcium phosphate; PCL–CaCO$_3$: PCL nanofibers with calcium carbonate; PCLEEP–PAA–AHG: Poly(ε-caprolactone-co-ethyl ethylene phosphate) grafted with poly(acrylic acid) and covalently conjugated with galactose ligands; PCL–HA: PCL nanofibers with hydroxyapatite; PEG–PLA: Poly(ethylene glycol)–poly(lactic acid); PET: Poly(ethylene theraphtalate); PEU: Polyesterurethane; PLGA: Poly(lactic-co-glycolic acid); PLLA: Poly(L-lactic acid); PNmPh: Poly[bis(p-methylphenoxy) phosphazene]; PEO: Poly(ethylene oxide); PPC: Poly(propyl carbonate); PU: Polyurethane; SPCL: Starch–PCL.

was to provide a dual structure aiming at facilitating the adhesion of two different cell populations. Indeed, the unique architecture that is generated supports and guides osteoblast-like cells (SaOs-2 cell line), bone marrow stromal cells (BMSCs), human umbilical vein endothelial cells (HUVECs) and microvascular endothelial cells (HPMEC-ST1.6R cell line). It was observed that endothelial cells have a distinctive preference for nanofibers, all other cell types preferred attaching to microfibers. These results showed that the micro–nanostructures are interesting candidate scaffolds for vascularized tissues such as bone.

Incorporation of biologically active factors
Drug-release systems can be very useful in the context of tissue engineering. Tissue engineering scaffolds would be greatly enhanced if they were designed with the capacity to locally release molecules, such as growth factors, enabling cell-guiding activity when seeded at the surface of the scaffolds. Thus, a controlled and local release of biologically active factors would significantly improve the efficacy of the tissue-engineering scaffolds and would probably enable the use of much lower quantities of those expensive proteins [62].

Many strategies can be used to control the release of proteins and growth factors from scaffolds. When biodegradable polymers are used, a common approach is to load the growth factors on the material and use the combined effects of diffusion and erosion to mediate the release kinetics. In the case of diffusion, the surface area and wettability are important parameters controlling the release kinetics. Nanofiber meshes inherently have an appropriate structure to maximize surface area. The other main

parameter, hydrophilicity, is less important in the case of nanofiber meshes than in compact structures because of the porosity. In addition, the hydrophilicity can be optimized by using surface-modification methods.

The electrospinning processing, being solvent based, allows the mixing of drugs and bioactive agents before the production of the electrospun nanofibers. However, the solvent needs to be harmless for the loaded bioactive agent, and must not compromise its functionality. Depending on the chemical interactions between drug and polymer carrier, different modes of interaction may be explored [65]:

• Drug as particles or inclusions trapped in the nanofiber structure;

• Drug and its carriers in nanofibers, resulting in a nonwoven nanofiber mesh with two types of fibers;

• Blend of drugs and carrier materials integrated into one mesh of composite nanofibers;

• Carrier material electrospun into a tubular form in which the drug particles are encapsulated.

Nanoscale drug-release systems can be tailored to tune the release kinetics, to regulate local distribution and to minimize toxic side effects, thereby enhancing the effectiveness of the bioactive agent released [62]. Electrospinning also allows control of the fiber diameter, to some extent, and control the release of kinetics by the diameter of the fibers, both in diffusion- and in degradation-controlled release. Moreover, the electrospinning process, being based in solvents, does not involve high temperatures, which is particularly useful for heat-sensitive drugs. Furthermore, it enables minimizing the initial burst release and the possibility of delivering uniform and highly controlled doses of bioactive agents at the wound site by tuning the surface properties of the nanofiber [66]. A survey of the electrospun nanofiber meshes proposed as drug-delivery systems are listed in **Table 3**.

In summary, the analysis of **Table 3** suggests that many studies explored the loading of antibiotics onto nanofiber meshes, but only a few reported the loading of antitumor or growth factors or other specific drugs. The materials that have been proposed as nanofiber drug carriers are restricted to the group of biodegradable synthetic polymers.

Nanofiber alignment & co-electrospinning
The nanofiber-based meshes more frequently reported in the literature are nonwoven and randomly aligned. Those structures may be desirable for some tissue applications. However, some human tissues have typically preferential orientations and frequently highly aligned structures, with a precisely defined architecture. This common observation leads to particular interest in aligned fiber orientations in the meshes to be used as scaffold for specific tissues. It may be hypothesized that controlled orientation of nanofibers may be required to create scaffolds to use in targeting specific tissue-engineering applications. The fiber orientation may influence cell attachment and growth and also provide stimulation for the spatial distribution of cells, guidance, cell-mediating activity and gene expression [67].

Considering the conventional electrospinning setup, a few variables have a critical role in determining the nanofiber orientation; the type of collector used is very important. Initial attempts to produce oriented electrospun nanofibers were based in high-speed rotating cylinders as collectors [68]. Using this method, the extent of fiber alignment achieved is limited. Many studies have explored nanofiber alignment in electrospinning through the use of rotating belts or cylinders as collectors [10,22,30,69–72]. Studies using these aligned meshes suggest that some cells interact with the nanofibrous scaffolds and may show preferential grow in the direction of the fiber orientation. Using a radically different method, by varying the geometrical configuration of electrically conductive collectors, it was demonstrated that the orientation of electrospun nanofibers could also be obtained without the rotation of the collector [73]. The collector in this case consisted of two conductive strips separated by a gap of variable width (up to several centimeters). Using this method, long electrospun fibers could be uniaxially aligned [74–76]. Another significant progress in collecting parallel-aligned electrospun nanofibers was obtained using a novel approach to position and align individual nanofibers over a tapered and grounded wheel-like bobbin [77]. Recently, another method was described consisting of a fiber bundle with a diameter in the micron range with aligned nanofibers between two parallel steel blades [78]. A similar. structure, composed of aligned nanofibers, was also reported, involving two grounded circular disks equidistant from the spinneret, with rotation of one of those collector discs [79].

The setup for electrospinning typically involves a single capillary as the spinneret, and thus allows the generation of fibers with a particular composition in each fabrication run. The

Table 3. Nanofiber meshes as drug-delivery systems.		
Incorporated drug	**Scaffold material**	**Potential application as drug-delivery system**
Tetracycline hydrochloride	PEVA, PEVA/PLA, PLA [21]	Antimycotic
Mefoxin® (cefoxitin)	PLA [141], PLGA [26], PLGA/PLA/PEG-b-PLA (80:5:15) [20]	Antibiotic
Cefazolin	PLGA [142]	
Itraconazole	HMPC [143]	
Gentamycin sulfate	PCL [144]	
Biteral (ornidazol)	PCL [58]	
Rifampin (rifadin)	PLLA [145]	
Paclitaxel	PLLA [145], PLGA [146]	Antitumor
Ibuprofen	PLGA, PEG-g-CHN [147]	Anti-inflammatory
Paracetamol	PDLLA [148]	Analgesic and antipyretic
Heparin	PCL [149], PEO-LMWH, PLGA [150]	Anticoagulant
Resveratrol (phytoalexin)	PCL [144]	Antioxidant
Bone morphogenetic protein-2	Chitosan [151]	Growth factor
Human nerve growth factor	PCLEEP [152]	
Bovine serum albumin	PVA [153], PCL/PEG(shell)-dextran/BSA(core) [86]	Protein
Plasmid DNA	PLA-PEG, PLGA [154], LEL [155]	DNA

HMPC: Hydroxypropylmethylcellulose; LEL: Poly(lactide)-b-poly(ethylene glycol)-b-poly(lactide); PCL: Polycaprolactone; PCLEEP: PCL/ethyl ethylene phosphate; PEO–LMWH: Poly(ethylene glycol) functionalized with low-molecular-weight heparin; PEG-b-PLA: Poly(ethylene glycol)-b-PLA; PEG-g-CHN: Poly(ethylene glycol)-g-chitosan; PEVA: Poly(ethylene-co-vinyl acetate); PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); PLLA: Poly(L-lactic acid); PVA: Poly(vinyl alcohol).

nanofibers have a solid inner structure and a smooth surface. Core/shell or hollow nanofibers can also be fabricated by co-electrospinning of two different polymeric solutions. The solutions can be selected to be immiscible and forced to flow through a spinneret composed of two coaxial capillaries [80–85]. These structures have particular interest as drug-delivery systems, since the release kinetics can be fine tuned by the properties of the polymer in the shell or by its thickness [86]. The use of a natural polymer in the shell of core–shell nanofibers could also improve the cytocompatibility of synthetic polymers (in the core of the composite nanofiber). Using this method, strong inflammatory reactions could be avoided and the mechanical properties of natural-based nanofibers could be improved. It is also possible to speculate that the fabrication of hollow nanofibers with multiple walls by using more complex spinnerets composed of more than two coaxial capillaries may be technically feasible [17]. Recently, the encapsulation of viable cells into poly(dimethyl siloxane) fibers obtained by coaxial electrospinning technology was reported [87].

Conclusion

Numerous studies reported the use of electrospun fiber meshes in tissue engineering. However, some technical barriers remain uncrossed and many possible configurations of the process were not fully exploited. Despite the high level of porosity and high specific surface area of the nonwoven fiber meshes, the pore size is usually too narrow to allow cell migration through the inner regions of the fiber-mesh scaffolds. This is the most serious limitation of these structures, and may compromise its use in the regeneration of tissues. Variations in the electrospinning setup or in the deposition pattern may be valuable strategies to control porosity. Strategies already suggested in the literature include the use of porogen agents such as salt particles [88] or chemical blowing agent [89]. Most biological studies with electrospun nanofiber meshes show that cells tend to stay at the surface of the meshes. This behavior is observed even when the pore size is sufficiently large to allow cells to migrate into the inner regions of the mesh scaffolds. Coating with cell-affine materials such as collagen was proposed to facilitate cell ingrowth into the core of meshes [90,91].

Other limitations of the electrospun nanofiber meshes in tissue engineering is the typical 2D thin structure. Fibrous meshes are generally obtained as planar sheets, which may limit the applicability of these structures to the regeneration of layered tissues. During processing, the time of deposition may be increased in order to produce 3D structures. However, in practice this is not feasible, since this way it is progressively more difficult to control the fiber-deposition process. By complementing or associating electrospinning with other techniques, it may be possible to obtain macroporous structures with tissue-scale motifs, this being a promising strategy to produce scaffolds that combine good mechanical properties and adequate topography for cell fixation. In our understanding, much more effort is required to produce 3D stable macroporous structures, exploring the advantages of electrospun fiber meshes and avoiding their limitations. The production of mesh structures together with well-controlled properties and architecture of individual fibers, such as alignment, would enable the production of structures that would have a huge impact in the tissue-engineering field.

Appropriate biomaterials tuned for specific cell types also have unsolved challenges. As previously mentioned, different cell types behave and react according to the fiber composition [11]. Efforts to improve cell attachment may include bulk modification [34,42] or surface activation [8,27,90,92,93] of the fiber meshes (Figure 1). Both strategies have been followed to improve interactions of specific cell types with the surface of fiber meshes. Eventual residual solvent in the meshes is another subject that is not sufficiently discussed and that might considerably affect the cell viability and the efficacy of these meshes as supports for tissue engineering.
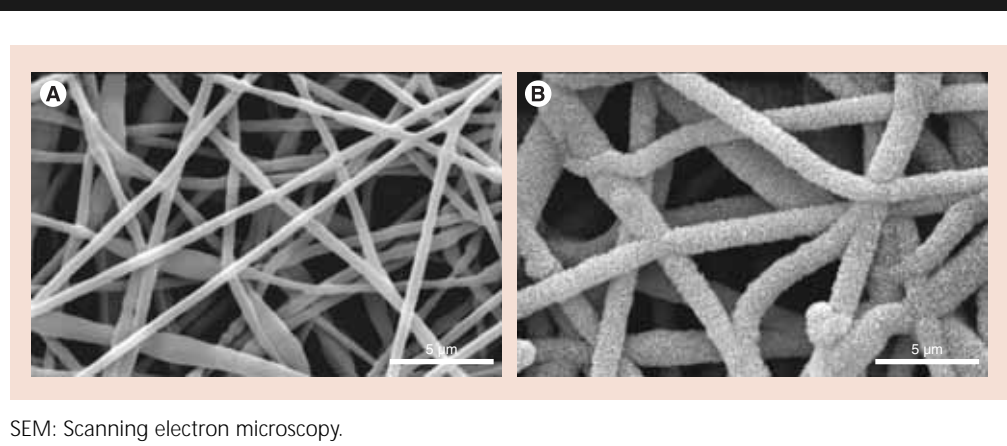
Future perspective

Most of the electrospun fibers proposed for tissue engineering are obtained from synthetic materials. More efforts should be devoted to the development of natural-origin polymers (e.g., chitin, chitosan, alginate, starch, hyaluronic acid and dextran), so that a better biological compatibility and performance can be achieved.

It is unclear, at this stage, as to what extent the aggregation and conformation of polymer chains are affected by the electrospinning process. Those changes are mainly related to the solvent used. The solvents have a crucial role since they are expected to solvate the polymer molecules, thus forming the electrified solution jet. A systematic study regarding the influence of the type of solvent and polymer concentration on the polymer-chain conformation and, consequently, in the properties of the nanofiber meshes, is needed.

A number of authors successfully encapsulated drugs into electrospun fibers by mixing or dissolving the drugs in the electrospun polymeric solution. However, the encapsulation of proteins is yet to be studied in detail, despite their biochemical importance as signaling agents for tissue engineering applications. Controlling fiber orientation of the tissue nanofibrous meshes is of major relevance and a challenging task in tissue engineering scaffolding. Regarding the *in vivo* testing, only a few studies were published and long-term performance of as-spun or modified fibers is yet to be published.

Figure 1. SEM micrographs of electrospun poly(ε-caprolactone) nanofiber meshes before (A) and after biomimetic coating (B).

SEM: Scanning electron microscopy.

Executive summary

Biodegradable nanofiber meshes

- More than 100 different polymers were already processed by electrospinning. The biodegradable and bioresorbable polymers, either synthetic or of natural origin, and considered as having adequate properties for tissue engineering and regenerative medicine, are the largest group of materials processed by this technique.

Synthetic extracellular matrix analogues

- Electrospun nanofibers have morphology and fiber diameters comparable with those found in the extracellular matrix (ECM) of human tissues. It is hypothesized that the similar morphology of the electrospun nanofiber meshes to those found in ECM and the high surface area provide improved microenvironments for cells to regenerate tissues.

In vitro & in vivo *studies*

- The properties of nanofiber meshes were shown to stimulate cell attachment, proliferation, maturation and differentiation. Thus, electrospun nanofibers were used in studies with different cell types, including cell lines or primary cultures, progenitor or terminally differentiated cells.
- *In vivo* studies demonstrated the applicability of electrospun nanofiber meshes as wound dressings, postsurgery anti-adhesion abdominal membranes, bone and periodontal regenerative implants and nerve conduits for neuronal tissue regeneration.

Hybrid polymeric matrices

- Combination of electrospun nanofibers with wet-spun microfibers was proposed by our research group. The interest of those micronanostructured scaffolds for bone-tissue engineering was demonstrated by cultivation of osteoblasts and endothelial cells.
- Combining electrospinning with other polymer-processing techniques allows one to obtain macroporous structures containing tissue-scale differentiated features, which are promising for scaffolding using specific cocultures of cells.

Drug-release systems

- Electrospun nanofibers have adequate properties for the release of biologically active agents, namely the small fiber diameter and the high specific surface area are characteristics that encourage the use of nanofiber meshes for this purpose.
- Antibiotics are the class of drugs most commonly incorporated in electrospun nanofibers. Fewer studies validated the incorporation of growth and differentiation factors.

Fiber alignment

- Much attention has been given recently to the production of parallel-aligned nanofibers in a mesh-like structure.
- Cell-biology studies demonstrated that fiber orientation can induce cell guidance and patterning, enabling targeting of the regeneration of specific complex tissues.

Core/shell or hollow nanofibers

- The production of core/sheath or hollow electrospun nanofibers was successfully achieved by the use of a coaxial spinneret.
- These nanostructures have particular interest in drug-delivery systems, and also as cell carriers and whenever biocompatibility needs to be improved.

Microporosity

- Microporosity is one of the most serious limitations to the use of electrospun nanofiber meshes. Typical pore sizes are a few micrometers in diameter, not allowing cell migration into the inner regions of the meshes.
- Some valid strategies to overcome this weakness include the use of porogens, such as salt particles or chemical blowing agents, but this key problem still needs major research effort.

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