Review

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Electrospun Nanofibrous Mats: From Vascular Repair to Osteointegration

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Electrospinning is a versatile technique for generating a mat of continuous fibers with diameters from a few nanometers to several micrometers. The diversity of electrospinnable materials, and the unique features associated with electrospun fibers make this technique and its resultant structures attractive for applications in the biomedical field. This article presents an overview of this technique focusing on its application for tissue engineering. In particular, the advantages and disadvantages of using an electrospinning mat for biomedical applications are discussed. It reviews the different available electrospinning configurations, detailing how the different process variables and material types determine the obtained fibers characteristics. Then a description of how nanofiber based scaffolds offer great promise in the regeneration or function restoration of damaged or diseased bones, muscles or nervous tissue is reported. Different methods for incorporating active agents on nanofibers and controlling their release mechanisms are also reviewed. The review concludes with some personal perspectives on the future work to be done in order to include electrospinning technique in the industrial development of biomedical materials.

KEYWORDS: *Electrospinning, Tissue Engineering, Scaffolds, Drug Delivery, Osseointegration, Biomedical Materials, Nanofibers.*

CONTENTS

INTRODUCTION

In the last years nanostructured materials, including synthesis and characterization of nanoparticles, nanofibers, block copolymers, have received great attention by the scientific community.[1–3] Electrospinning is one of the most studied techniques since it is the only one that allows the obtainment of long and continuous fibers with diameters in the range of a few nanometers to microns. Furthermore, nowadays it is possible to control the morphology, diameter, and patterning of electrospun nanofibers over a wide range of values.[4–8] The interest in applying this technique for the development of biomedical materials in the field of tissue engineering arises from considering that from a structural viewpoint, almost all human tissues and organs are deposited in nanofiber-like structures, such as bones, muscles and tendons.

Non-woven membranes of electro-spun nanofibers are well known for their interconnected 3D porous structures and relatively large surface areas, which provide a class of ideal materials to mimic the natural extracellular matrix (ECM) required for tissue engineering.[9] In case of bone tissue, a key factor to improve regeneration is the incorporation of materials that promote osseointegration, such as hydroxyapatite and collagen.[10–23] On the other hand, in the case of muscles, besides the incorporation of bioactive

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groups, the orientation of mat fibers is a key goal to approach a similar structure to ECM.[24–26] The same idea is applied to tendon tissue engeniering.[27, 28]

In bony-prosthesis coatings, the application of electrospinning fiber mats has several advantages respect to conventional continuum coatings. As an example, physical nanostructures may provide successful forms of inhibitive interaction between bacteria and surfaces and consequently reduce bacterial growth.[29] Since the point of view of drug-delivery, the nanofibers based mat have a high surface area, maximizing drug release.

The aim of this work is to perform a detailed review of new developments in the area of electrospinning, describe the different morphologies that can be obtained and link them with the process and material variables.

In addition, a state of the art description of literature reports regarding the use of electrospinning mats in the field of regeneration or restoration of damaged or diseased

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tissue for the case of bones, muscles, tendons and neural tissues is provided. Finally the possible methods to introduce active agents, such as drugs, in nanofibers and the advantages and disadvantages of this type of structure from the point of view of drug-release are described.

ELECTROSPINNING PROCESS

Technique Description

General Description

The term "electrospinning," derived from "electrostatic spinning," has been used relatively recently (in around 1994), but its origin can be traced back to more than 60 years ago. It is an old technique, first observed in 1897 by Rayleigh, studied in detail by Zeleny around 1914 and patented by Formhals in 1934.[7] The popularity of the electrospinning process can be realized by the fact that over 200 universities and research institutes worldwide are studying various aspects of this process and the fibers it produces, and also the important number of patents published for its applications in recent years.[30–33]

Electrospinning is a broadly used technology that allows polymer fiber formation, with diameters from several micrometers to a few nanometers; through the use of electrical forces. These electrospun fibers are optimal candidates for a wide range of important applications in different areas such as drug carriers, tissue scaffolds, wound dressing, reinforcement materials, filters, protective clothing, electrodes, sensors, catalysts, etc.[34] In this technique a dissolved polymer is exposed to high voltage, generating an electric charge distribution within the viscous liquid. Under these circumstances two forces come into play: the electrostatic repulsion and the surface tension.

Four main elements are required for electrospinning: a spinneret (typically a hypodermic syringe needle), a high-voltage direct current power supply (5 to 50 kV), a syringe pump, and a grounded collector (Fig. 1). There are many different electrospinning setups in order to achieve different fiber morphologies, but typically, the positive electrode is attached to the spinneret filled with the polymer solution to be electrospun, while the collector is grounded. As the pump pushes the polymer through the syringe body, a droplet is formed at the needle tip. When electrical voltage is applied to the spinneret, the droplet becomes charged, and experiences an electrostatic repulsion. Under the influence of the electric field, it is stretched forming a conical shape structure known as the Taylor cone.[35] When the voltage exceeds a critical level, the repulsion force in the droplet overcomes the surface tension and, if the molecular cohesion of the liquid is sufficiently high, the polymer ejects from the spinneret and travels towards the collector plate as a jet. As the polymer jet moves through the air, solvent begins to evaporate producing a continuous fiber. After a fist nearly straight line path, the jet bends into a complex course, during which electrical forces stretches and thins it by large ratios. As the jet thins, it ultimately succumbs to one or more fluid instabilities which deform the jet as they grow. This whipping process elongates the fiber even more until it is finally deposited on the grounded collector.[36] If the polymer liquid to be electrospun is slightly viscous, the formed jet is not continuous and nanoparticles or a mixture of beads and fibers are obtained.[37–39]

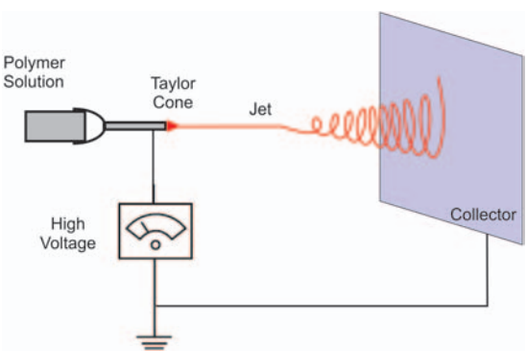

Figure 1. Schematic illustration of the basic setup for electrospinning.

The properties of the obtained fibers from the electrospinning process will depend on many variables. These can be classified into two groups: processing variables and materials variables. The processing variables include all the parameters related with the experimental setup, such us electrode shape, electrode material, electric field strength, electrode-ground distance, solution evaporation rate (pressure and temperature) and solution flow rate, among others. On the other hand, materials variables include solvent and polymer characteristics including chemical composition, polymer molecular weight, solution concentration and viscosity, surface tension, solution conductivity and charge density.[40]

Processing Variables

Collector geometry can influence mat morphology, such as fiber alignment and pattern. Collectors can be stationary or rotating-type disks or mandrels. In general, a randomly oriented web of fibers is collected on static targets, whereas aligned fibers are collected on high speed spinning substrates. Some collectors possess special geometries to affect particular fiber orientation or patterns. Figure 2 shows some of the usual collector configurations together with the typically obtained mats.

The simplest electrode shape involves a needle and a plane metal collector that can be continuous or patterned. Hong et al.[41] used a collector plate with different shaped voids on it to generate poly(caprolactone) fibers. They found that the size and the morphology of fibers are not affected by the geometrical patterns of the holes. However, fiber alignment is affected by the collector's void shape due to favorable rearrangement in the electrical field. Wang et al.[42] successfully prepared electrospun nanofiber mats with well-tailored architectures and patterns from

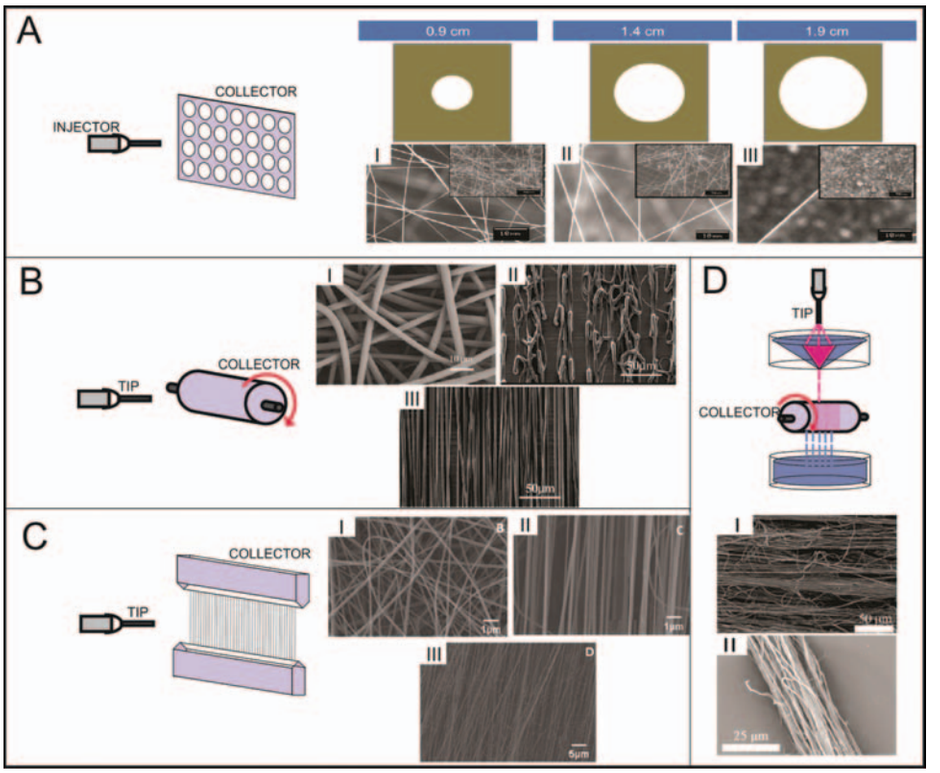

Figure 2. **Schematic diagram of various collector set-ups to obtain different fibrous assemblies. (A) Reprinted with permission from [41], J. K. Hong, et al., Analysis of void shape and size in the collector plate and polycaprolactone molecular weight on electrospun scaffold pore size. *J. Appl. Polym. Sci.* 128, 1583 (2013). © 2013, John Wiley and Sons. (B) Reprinted with permission from [44], Z. Liu, et al., Control of structure and morphology of highly aligned PLLA ultrafine fibers via linear-jet electrospinning. *Polymer* 54, 6045 (2013). © 2013, Elsevier. (C) Reprinted with permission from [46], K. T. Shalumon, et al., Fabrication of aligned poly(lactic acid)-chitosan nanofibers by novel parallel blade collector method for skin tissue engineering. *J. Biomed. Nanotechnol.* 8, 405 (2012). © 2012, American Scientific Publishers. (D) Reprinted with permission from [48], J. Wu, et al., Cell infiltration and vascularization in porous nanoyarn scaffolds prepared by dynamic liquid electrospinning. *J. Biomed. Nanotechnol.* 10, 603 (2013). © 2013, American Scientific Publishers.**

biodegradable poly(ε-caprolactone) using a stainless steel mesh as a template collector. It was found that the resulting polymer nanofiber mats had similar topological structures to that of the template stainless steel mesh.

In order to achieve fibers alignment different collector shapes are often used. One of the most used collectors consists of a rotating metal drum.[43] This simple set up allows the obtaining of a large area of aligned fibers. Liu et al.[44] obtained highly aligned poly(*L*-lactic acid) (PLLA) electrospun fibers controlling their arrangement and morphology by varying the winding velocity. When the winding speed is lower than the jet impinging speed, a force imbalance arises at the interface of PLLA electrified jet and conductive collecting drum, then the jet starts to perform a buckling process. If the winding speed increases to be very similar or higher than the jet impinging speed, the collecting drum generates a stretching force suppressing the longitudinal compressive forces on the jet surface so that linearly arranged PLLA electrospun fibers are obtained.

Another way to generate highly aligned nanofibers is using a parallel electrodes collector. The obtained materials are easily transferable to any substrate, but thick layers are not obtainable through this method. Nivison-Smith et al.[45] obtained parallel elastic fibers from synthetic elastin as a model of the arterial media using this electrospinning configuration and assessed the alignment of smooth muscle cells. Shalumon et al.[46] blended poly(lactic acid) with chitosan to fabricate electrospun nanofibers aligned using a collector made of parallel blades which increase the transversal electric field across the gap. Cell studies with human dermal fibroblasts showed the orientation of cells along the direction of fiber alignment, indicating that the prepared aligned nanofibers are a promising material for skin tissue engineering.

In order to have some control over the motion of the electrospinning jet, Teo et al.[47] proposed the use of knife edges to dominate the electrostatic field forces. This allows diagonally aligned fibers to be collected on a rotating tube, and then the creation of a laminate composite consisting of aligned fibers in different directions. This would result in a stronger tubular structure with widespread potential such as a blood vessel scaffold.

The fibers alignment and patterning are not the only objectives sought when reshaping the collector. In a typical electrospinning setup, the nanofibers are deposited on a solid substrate and the resultant nanofibrous structure is difficult to modify. However, a fluid system such as water could be a possible substrate for nanofiber collection as it can be manipulated after deposition. The main advantages of working with this configuration are the possibility to orient the fibers by movement on the water surface and to keep the fibers integrity as the adhesion between the nanofibers and the water surface is sufficiently weak. If the fluid which carries the nanofibers has a flow profile it could draw them into a yarn. Xu et al.[27]developed a tendon tissue engineering scaffold, poly(*L*-lactide-co-ε-caprolactone)/collagen nanoyarn network, that mimics the ECM of native tendon in terms of structure and inherent nanoscale organization using a water vortex in the collector arrangement. They found that the nanoyarn scaffold with 3D aligned microstructure, larger pore size, and higher porosity than random and aligned nanofiber scaffolds, exhibited native morphology and improved cellular proliferation. Wu et al.[48] also obtained porous nanoyarn scaffolds through dynamic liquid electrospinning. They obtained aligned nanoyarns of 24 μm, which were composed of a bundle of nanofibers. Histological analysis demonstrated that cells infiltrate throughout the nanoyarn scaffolds over a 10-day period, however, no cell infiltration was observed on the nanofiber scaffolds.

Not only may the collector shape be changed in the electrospinning set up, there is a wide range of spinneret configurations currently used to achieve different objectives such as hollow and core–shell nanofibers.[49] In the coaxial electrospinning setup a multiple solution feed system is used, which allows the injection of one solution into another at the tip of the spinneret. Here, the outer fluid acts as a carrier which draws in the inner fluid at the Taylor cone of the electrospinning jet.[50] It is important to notice that if the solutions are immiscible then a core shell structure is usually observed. Miscible solutions however can result in fiber porosity or a fiber with distinct phases due to phase separation during solidification.

Ji et al.[51] fabricated biodegradable core/shell structured poly(*L*-lactide acid)/chitosan nanofibers using a coaxial electrospinning setup. Their results indicated that the obtained nanofibers could be potential drug carrier for tissue engineering, as their surface area and pore size distribution exhibited higher capacity than pure poly(*L*-lactide) fibers. Viry et al.[52] combined coaxial and emulsion electrospinning to produce micro-structured core–shell fibers. An emulsion in which a drug was dissolved in water and emulsified in a polymer solution was electrospun coaxially as the core, while a polymer solution was electrospun as the shell. This technique provides a microstructured core which can be used as small drug reservoirs surrounded by a diffusive barrier producing a nearly linear release of small molecular weight drug over 18 days whereas classical core–shell fibers in comparison provided a linear release for 4 days followed by a steady state. Yu et al.[53] fabricated poly(acrylonitrile) (PAN) nanofibers coated with silver nanoparticles using a modified coaxial electrospinning process. The authors used PAN as the filament-forming polymer matrix, and a silver nitrate ($AgNO_3$) solution as sheath fluid. They found that the sheath $AgNO_3$ solution can take a role in reducing the nanofibers' diameters significantly. Besides, SEM and TEM images showed that silver nanoparticles, formed when $AgNO_3$ is *in situ* reduced were well distributed on the surface of PAN nanofibers and the antibacterial experiments demonstrated that these nanofibers show strong antimicrobial activities. Nguyen et al.[54] achieved a porous morphology on the outer layer of core/sheath structured composite nanofibers with a core of blended salicylic acid (SA) and poly(ethylene glycol) (PEG) and a sheath of poly(lactic acid) (PLA). They found that feed rates of the core and sheath strongly affect the stability of the core/sheath structure and the porous density of the obtained composite nanofibers significantly influences their SA release characteristics. At a lower ratio of feed rates of the core and the sheath, better stable core/sheath structures of nanofibers with higher porous density on the surface were formed resulting in a sustained release of SA over 5 days.

Multiple spinnerets are also used in order to combine different components. Tijing et al.[55] fabricated a hybrid, bimodal nanofibrous mat made of two polymeric nanofibers: polyurethane (PU) and silver nanoparticles (Ag NPs) *in situ* decorated poly(ethylene oxide) (PEO) utilizing an angled dual-spinneret electrospinning system. They found that the hybrid nanofibrous mat containing Ag NPs with an average size of 8 nm exhibited a strong antibacterial activity. Toncheva et al.[56] prepared PLA based fibrous materials containing diclofenac sodium (DS) and lidocaine hydrochloride (LHC) by dual spinneret electrospinning of two separate solutions each containing one drug only [(PLA/DS and PLA/LHC)] and compared with materials obtained by electrospinning of a common solution (PLA/DS/LHC) using a single spinneret. The dual spinneret approach allowed the ionic interaction between DS and LHC to be obviated and the drugs remained in the amorphous state regardless of the method of preparation of the fibrous materials.

One of the main drawbacks of electrospinning is that, compared to the popular industrial fibers spinning process, it is a very slow technique. Using multi-jet spinnerets could be a possible way to increase the electrospinning throughput. However, this set up has shown strong repulsion among the jets and this may lead to reduced fiber production rate and poor fiber quality. Some upward needleless electrospinning setups have good potential as suitable methods for manufacturing nanofibers with minimal dependences on the fluidic channel numbers.[57] Several configurations have been developed, such as two-layer-fluid electrospinning

setup,[58] bubble-electrospinning[59] and splashing needleless electrospinning.[60]

The injector-collector distance has important relevance, as it determines the flight path of the electrospun fibers, which in turn determines the extent of solvent evaporation and the potential gradient. As it was explained before, in the first part of the path the dominant force is the surface tension but in the latter portion the charge repulsion plays a dominant role as the whipping motion begins to thin down the fiber till it reaches the terminal diameter. The shortened flight path leads to a smaller distance over which the fibers can stretch and this causes a comparatively higher diameter and an increase in beds formation.[61] Bölgen et al.[62] investigated the tip–collector distance effect on diameter and morphology of poly(ε-caprolactone) (PCL) fibers obtained by electrospinning. Changing the separation between 5 and 20 cm, they found that, although the fiber size did not change significantly, homogeneously distributed beads started to form along PCL fibers when the tip–collector distance was reduced. Chowdhury et al.[63] studied the effect of experimental parameters on the morphology of electrospun Nylon-6 fibers, founding that for the larger used distance (11 cm) the evaporation of solvent was enhanced, leading to thinner fibers (900 nm) while for the shorter distance (5 cm) wet fibers with flatten cross-section were obtained.

Obviously, the applied voltage is determinant in the fibers formation. There exists a critical value beyond which any further increase in voltage leads to the ejection of a polymer jet from the apex of the Taylor cone. This critical value varies with the type of polymer solution and there is an optimum range of the applied voltage or the electric field strength for a given polymer-solvent system within which nanofiber formation is desirable. An electric field that may be weaker or stronger than this critical value will result in beaded morphologies or even inhibit polymer jet initiation. Zhang et al.[64] fabricated PAN fibers with tunable diameters by changing different variables. They found that when the voltage increased from 10 kV to 20 kV, fibers became more uniform and thicker with an average diameter of 300 nm, as compared with the diameters of 153 nm and 250 nm for fibers manufactured from 10 kV and 15 kV. They also observed that the shape of the beads changed from sphere to spindle with the increase of the applied voltage. However, Haghi et al.[65] reported a decrease in the fiber diameter when the applied electric field increased.

Zhu et al.[66] produced pure polyimide and Fe–FeO nanoparticles reinforced polymer nanocomposite fibers by electrospinning with optimized operational parameters. They investigated the effects of the applied electrical voltage on the fiber morphology and size distribution changing it from 10 to 15 kV. The fibers size distribution resulted uniform when the applied electrical voltage overcame the 12 kV. Besides, the incorporation of Fe-FeO nanoparticles enhanced thermal stability of the nanocomposites fibers, and produces an increment in glass transition and melting temperature, as compared with those of the pure polymer.

As it was explained before the solvent evaporation is one of the factors that determine the fibers diameter and morphology. This will strongly depend on the ambient conditions, such as temperature and pressure. Rodoplu et al.[67] obtained thin and non-beaded poly(vinyl alcohol) fibers, via controllable and repeatable process parameters. They found that, for a specific solution concentration, applied voltage, tip-collector distance and flow rate, the morphology of the fibers were "beaded" for the low temperature solutions (< 40 °C), but at higher temperatures of polymer solution (> 60 °C), the fibers became flat. The bead formation was eliminated at low temperature polymer solutions by changing the other electrospinning process parameters. Casper et al.[68] investigated of how humidity affects the surface of electrospun polystyrene fibers. At a humidity of less than 25%, the fibers were smooth and featureless. Increasing the humidity to 31–45% caused a visible difference in the surface morphology of the fiber, generating uniform, circular pores randomly distributed. When the fibers were electrospun under 50–72% humidity, the pores became abundant on the surface of the fiber leaving little space between adjacent pores, changing the circular shape due to the coalescence of smaller pores into larger, non-uniform shaped structures.

Finally, the flow rate at which the polymer solution is injected influences the diameter, porosity, and geometry of the electrospun nanofibers. Zargham et al.[69] obtained Nylon 6 fibers with different diameters using flow rates of 0.1, 0.5, 1 and 1.5 mL/h. At low flow rates, a small droplet was formed at the capillary tip which made various types of charged jets that reduced immediately due to the electric field. They observed that at 0.5 mL/h, the Taylor cone was more stable which resulted in the narrowest fiber diameter distribution. However, increasing the flow rate caused fibers to be collected without sufficient solvent evaporation, which in turn led to the formation of some defects, such as branched, splitting fibers, blobs and flattened web-like structures. Liu et al.[70] conducted a systematic study of the effects of polymer molecular weight, flow rate, voltage, and composition on the morphology of electrospun poly[(lactic acid)-co-(glycolic acid)] (PLGA) nanofibers. At the flow rate of 2 mL/h, non-uniform distribution of the PLGA nanofibers with an average diameter of 586 nm was observed, probably due to the instability of the jets. As the flow rate was decreased (1 and 0.5 mL/h), the fiber diameter distribution became homogeneous. Furthermore, the fiber diameter decreased with decrease in the flow rate (499 nm at 1 mL/h flow rate, and 465 nm at 0.5 mL/h flow rate). Haghi et al.[65] showed the trends in fiber diameters with the different process parameters involved. Figure 3 summarizes the behavior described by the authors.

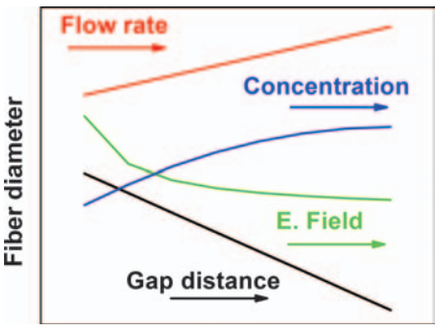

Figure 3. Dependence of nanofiber diameter with the different processing variables.

Materials Variables

Both polymer chemical composition and its molecular weight can influence the final properties of the electrospun nanofibers and moreover the possibility to perform them using this technique.

As it was explained before, humidity influences the morphology of electrospun fibers,[71] but the way in which it does it depends on different properties of the solution being electrospun. De Vrieze et al.[72] studied the influence of humidity and temperature on the formation and the properties of nanofibres using cellulose acetate (CA) and poly(vinylpyrrolidone) (PVP) as electrospun materials. They found that the trend in variation of the average nanofiber diameter as a function of humidity is different for CA and PVP, due to the variations in chemical and molecular interaction and their influence in the solvent evaporation rate. As the humidity increases, the average fiber diameter of the CA nanofibers increases, whilst for PVP the average diameter decreases. Yan et al.[73] electrospun tetracycline (Tet)-loaded PLGA nanofibers, due to their great potential as local drug-delivery systems. They explored the effects of the lactidyl/glycolidyl (LA/GA) unit ratio and molecular weight of PLGA on Tet entrapment efficiency and *in vitro* release kinetics, finding that the lower the molecular weight of PLGA was, the higher the GA content in PLGA was and the higher the resulting Tet entrapment.

Solution concentration directly affects the solution viscosity and therefore has an important influence in the obtained fiber properties. Nasouri et al.[74] investigated the effects of polymer solution properties, like polymer concentration, viscosity and Berry number, on electrospinnability of PAN/*N*,*N*-dimethylformamide solutions. Their study focuses on using curve fitting methodology and power law equation to analyze nanofibers morphology. The relation between the diameter of electrospun nanofibers (d) and solution concentration (C) resulted in the form: $d = 0.0326\ C^{3.45}$. Nasefa et al.[75] studied the effect of Nylon-6,6 solution's viscosity and concentration on the diameter and morphology of electrospun nanofibers. They found that there is a threshold viscosity required for the formation of a stable polymeric jet during electrospinning. With increasing viscosity, fiber diameter increased and more uniform fibers were formed.

Solvent selection has also a significant effect on fibers morphology, as different solvents may contribute different surface tensions or evaporation rates.[76,77] Qian et al.[76] processed poly(methyl methacrylate) (PMMA) nanofibers by means of electrospinning. Seven solvents were separately used to dissolve PMMA at a fixed concentration of 0.06 g/mL. As a result, ring-like, bead-like, ultrafine, and nano-porous nanofibers were generated, depending on the boiling points, molecular weight, and molecular structure of the solvents. For example, ring-like PMMA fibers were obtained due to the high boiling point (110 °C) and stereo-hindrance effect of toluene. Bead-like nanofibers were generated from PMMA/chloroform and PMMA/dichloromethane solutions. Moreover, two kinds of ultrafine nanofibers were produced through electrospinning of PMMA/1,1,1,3,3,3-hexafluoro-2-propanol, and PMMA/2,2,2-trifluoroethanol solutions. Figure 4 shows

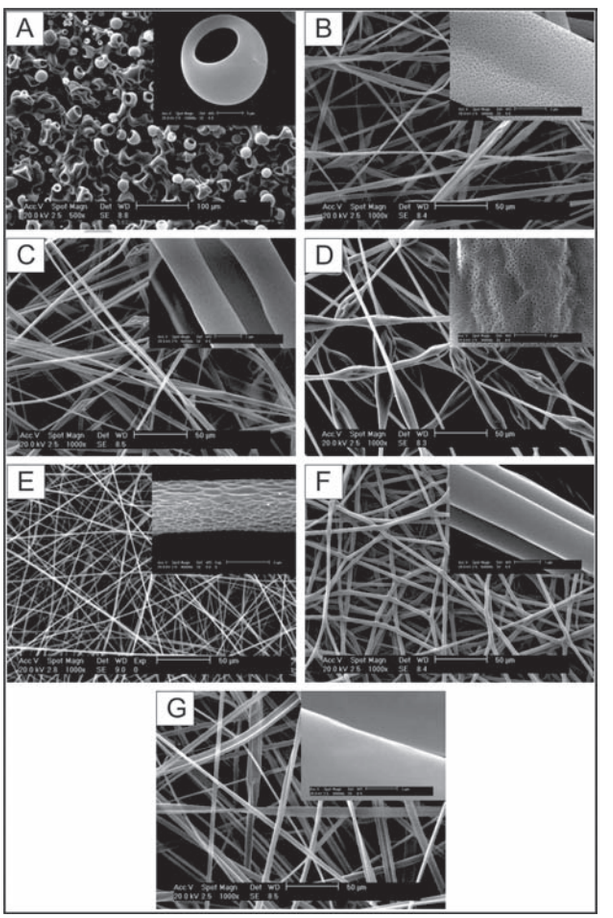

Figure 4. Morphology micrographs of electrospun PMMA nanofibres from (A) toluene, (B) CH_2Cl_2, (C) acetone, (D) $CHCl_3$, (E) TFE, (F) HFIP and (G) THF. These figures were adapted with permission from [76], Y.-F. Qian, et al., Electrospinning of polymethyl methacrylate nanofibres in different solvents. *Iran. Polym. J.* 19, 123 (2010). © 2010, Springer.

some of the fiber morphologies achieved by the authors using different solvents.

A mixture of solvents can also be used to obtain the polymeric solution. Two different solvent combinations from dimethylformamide (DMF), chloroform and dichloromethane, and different ratios were used by our group to obtain PMMA nanofibers. For the chloroform:DMF mixture, the effect of changing the solvents ratios was studied, finding that decreasing the DMF content generated porous in the fibers surface. Different morphologies can also be obtained by keeping the solvents ratio, but changing chloroform by dichloromethane. The porous on the fibers from the dichloromethane:dimethylformamide solution resulted more uniform than those from the chloroform:dimethylformamide mixture and the fiber shape was also modified. These effects could be caused by differences in the solvents evaporation due to their different boiling points, being 40 °C for dichloromethane, 61 °C for chloroform and 153 °C for dimethylformamide. Figures 5 and 6 reveal the mentioned effects on PMMA fibers.

Combining solvents and non-solvents is another way to obtain different morphologies. Qi et al.[78] fabricated fibers with micro- and nano-porous structure by electrospinning a ternary system of nonsolvent/solvent/poly(*L*-lactic acid). They used dichloromethane as solvent and butanol as nonsolvent, with a certain ratio. Since the solvent is more volatile than the nonsolvent, the composition of polymer fluid jet enters the two phase region of the ternary phase diagram, leading to different phase separated structures, and further evaporation of the residual nonsolvent would lead to porous fibers.

Finally, surface tension and solution conductivity could also be changed by adding different components to the polymeric solution.[39, 79, 80] Nartetamrongsutt et al.[39] added deionized water and ionic salts to a solution of PVP in ethanol in order to study their influence on electrospun fiber diameters and bead formation. The average fiber diameter could be reduced significantly, from about 700 nm to about 100 nm, by adding deionized water but the formation of beads increased. Further modification of the solution with ionic salts successfully reduced the formation of beads in the morphology of the electrospun

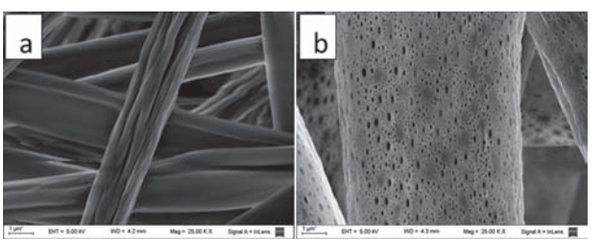

Figure 5. Effect of dimethylformamide percentage in the solvents mixture used to prepare the electrospinning solution on the obtained fibers morphology (a) chloroform:DMF (30:4) (v/v); (b) chloroform:DMF (30:1) (v/v).

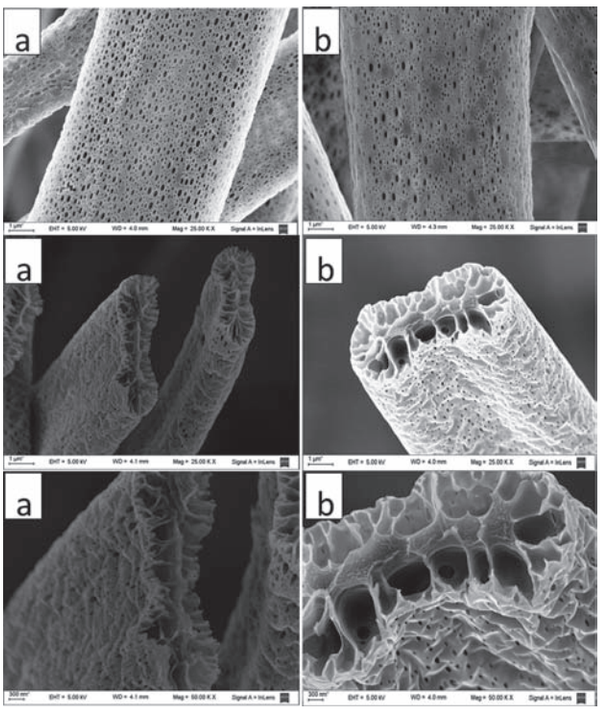

Figure 6. Dependence of fibers morphology with the solvent mixture used to prepare the electrospinning solution. (a) Dichloromethane:DMF (30:1) (v/v); (b) chloroform:DMF (30:1) (v/v).

fibers with average diameter smaller than 300 nm. The surface tension of a 10 wt% poly(vinyl alcohol)/water solution was changed by Jia et al.[81] through the addition of four different kinds of surfactants. Their results showed that the surface tension of the spinning solution decreased significantly when the surfactant content was less than 1%, while the viscosity and electric conductivity of the solution increased with the increasing of cationic and anionic surfactant content. The fiber diameter of poly(vinyl alcohol) mats remarkably decreased from 405 to 100 nm as the non-ionic surfactant content within the range of 1% (v/v) increased. Besides that, the surfactant content also had some influence on the thermal performance and inner structure of nanofibers. With the surfactant content increasing, both the heat of fusions and crystallinity increased significantly.

Polymers Used as Raw Materials for Mats Development

There is a wide range of polymers that are able to form fine fibers in the nanometer scale through the electrospinning process with biomedical applications. Electrospun nanofibers have been reported from various synthetic polymers, natural polymers or a blend of both.

Some of the typical natural polymers are collagen, chitosan, gelatin, casein, cellulose acetate, silk protein, chitin, and fibrinogen, among others; while synthetic

polymers, include poly(ε-caprolactone), poly(lactic acid), poly(glycolic acid) (PGA), polyurethane, the copolymer poly(lactide-co-glycolide), and the copolymer poly(*L*-lactide-co–caprolactone) [P(LLA-CL)], all hydrophobic biodegradable polyesters.

Naturally occurring polymers, normally exhibit better biocompatibility and low immunogenicity, compared to synthetic polymers, when used in biomedical applications. A strong reason for using natural polymers for electrospinning is their inherent capacity for binding cells since they carry specific protein sequences, such as arginine/glycine/aspartic acid.[82] The main drawback in manufacturing these polymers through the electrospinning technique is the possibility of partial denaturation. Collagen, for example, is a prominent biopolymer and is used extensively due to its excellent biological and physico-chemical properties for tissue engineering applications. However, it has been shown that the properties of leading natural biomaterial collagen are lost when it is electrospun into nanofibers out of fluoroalcohols.[83] Synthetic polymers often offer many advantages over natural polymers as they can be tailored to give a wider range of properties such as, necessary mechanical properties (viscoelasticity and strength), and desired degradation rate.

APPLICATIONS OF THE ELECTROSPINNING PROCESS TO THE DEVELOPMENT OF BIOMEDICAL MATERIALS

Advantages and Disadvantages of Using a Electrospinning Mat for Biomedical Applications

Electrospun fibers and mats provide several advantages such as high surface to volume ratio, very high porosity and enhanced physico-mechanical properties, as in this process, manipulation of the solution and process parameters can be easily done to get the desired fiber morphology and mechanical strength. In addition to these, the electrospun fibers are required in a small amount and the electrospinning process itself is a versatile process as fibers can be spun into any shape using a wide range of polymers.

For these reasons, electrospun nanofibers are broadly applied in biomedical applications, as tissue engineering scaffolds, in wound healing, drug delivery, in immobilization of enzymes, small diameter vascular graft implants, healthcare and in various researches that are ongoing.[84] Reviewing the number of patents, we can see that approximately two-thirds of all electrospinning applications are in the medical field.[7]

Another advantage of these nanostructured mats is the possibility to inhibit bacterial growth. Bacteria often form structured, multicellular communities, called biofilms, on surfaces in natural and anthropogenic environments.[85] In medical settings, biofilms are the cause of persistent infections, triggering immune response, release of harmful toxins and even obstructing catheters.[86] One method to combat bacterial biofouling is to modify the topographical structure of the surface in question, thereby limiting the ability of individual cells to attach to the surface, colonize, and form biofilms. The colonization process is governed by multiple cell-surface interactions which depend on surface energy, mechanical properties and topography.[29] Graham et al.[29] showed that controlling the surface structure, one can drastically limit initial bacterial attachment, thus reduce colonization and biofilm formation. Xu et al.[87] demonstrated that submicron surface textures may reduce the bacterial adhesion via a decrease in surface area that bacteria can contact, and subsequently inhibit biofilm formation.

However there are some disadvantages that need to be taken into account. Previous works with porous foams and sponges suggests that there exists an optimal pore size for cell infiltration.[88] The nanoscale pores obtained in the electrospinning mats is the none of the main obstacles limiting their application for tissue engineering. Cell-scaffold interaction is thus limited to the surface, as cells are unable to migrate through the small pores and populate the thickness of the scaffold. The small pores also prevent vascular ingrowth. This limits the thickness of the scaffold, as cells within the construct rely on diffusion from the vasculature for nutrient and waste transfer.

Although these factors limit the versatility of electrospun scaffolds in tissue engineering, scientists have proposed a number of methodologies to improve infiltration.[89] Most directly, cells have been electrosprayed into forming scaffolds. Stankus et al.[90] electrosprayed smooth muscle cells concurrently with electrospinning of a biodegradable, elastomeric poly(ester urethane)urea. In comparison with static culture, samples cultured in spinner flasks indicated 2.4 times more viable cells. Jayasinghe et al.[91] electrosprayed mouse lung fibroblasts directly with a biopolymer to form cell-bearing matrices, which resulted viable even when implanted subcutaneously into murine hosts. However issues of layering, sterility, and time (to produce thicker scaffolds) may limit this application.

On the other hand, Leong et al.[92] developed a technique that uses ice crystals as templates to fabricate cryogenic electrospun scaffolds with large three-dimensional and interconnected pores using poly(*D*,*L*-lactide). Manipulating the humidity of the electrospinning environment the pore sizes was controlled, ranging from 900 ± 100 μm^2 to 5000 ± 2000 μm^2. *In vivo* studies demonstrated improved cell infiltration and vascularization compared with conventionally prepared electrospun scaffolds.

The inclusion of sacrificial fibers or nanoparticles in a composite fibrous scaffold can also increase porosity and accelerate cell infiltration after their selective removal.[93] Phipps et al.[94] evaluated three separate techniques for their capability to increase the pore size of scaffolds made from polycaprolactone, collagen I and nanoparticulate hydroxyapatite: limited protease digestion, decreasing the fiber packing density during electro-spinning, and inclusion of sacrificial fibers of the water-soluble polymer

PEO. The PEO sacrificial fiber approach was found to be the most effective in increasing scaffold pore size. Furthermore, the use of sacrificial fibers promoted an increase in the cell infiltration into the scaffolds. Wag et al.[95] demonstrated that the use of PEO microparticles as porogen is a feasible and effective method for creating macroporous electrospun scaffold, which provide an alternative to address the limitation of cell infiltration associated with electrospun fibrous scaffold.

Another possible way to enhance the porous size of electrospinning mats is generating new holes once the scaffold is done. Valle et al.[96] patented a process to perforate biocompatible membranes by mechanical, thermal laser or ultraviolet radiation mean. They demonstrate that the perforated membranes could be used as a support for the *in vitro* growth of epithelial cells. Lee et al.[97] employed a femtosecond laser system to ablate and create microscale features on electrospun poly(*L*-lactide) nanofibrous scaffolds. Structured holes with diameters of 50, 100 and 200 μm and spacings of 50 and 200 μm between adjacent holes on the scaffolds were produced. Animal studies indicate that ablated scaffolds facilitate endothelial cell ingrowth as well as drastically increase M2 macrophage and overall cell infiltration, when compared with control (non-ablated) scaffold.

Applications in Tissue Engineering

Tissue engineering is a multidisciplinary field that combines medicine, biology, engineering and materials science fields for the development of biological substitutes and also for restoration, maintenance, or improvement of tissue function. Natural extra cellular matrix (ECM) (esta sigla ya se definio antes) forms a supportive meshwork around cells and provides anchorage to the cells, separating different tissues. It is made up of proteins and glycosamino-glycans which are carbohydrate polymers. Tissue engineering utilizes scaffolds to provide support for cells and to regenerate new extra cellular matrix which has been destroyed by disease, injury or congenital defects.[98, 99] The success of artificially recreating the extracellular matrix depends on the properties of the scaffolds. These scaffolds should be biocompatible, non-toxic, and have a desired degradation rate, high porosity, good mechanical properties and should not cause any foreign immune response. All of these properties are essential to facilitate and guide cell ingrowth and transport of gases, metabolites, nutrients, and signaling molecules, both within the scaffold and between the scaffold and the native local environment.

One of the purposes in scaffolds is that the cells recognize the biomimetic nanofibrous as self tissue and thus stimulate the healing and regeneration of the tissue. It was proved that cells are sensitive to minor changes in mineral content within nanofibers, for this attempt elucidating the sensing mechanism may lead to optimized scaffold designs.[100] With the goal of mimicking ECM, advancements in creating nanoscale materials such as nanofibrous scaffolds has become an important object of research in electrospinning field.[84, 101–103]

As it was described earlier, electrospinning technique could produce fibrous which have several nano- to micrometers diameter and proved to be a relatively simple and versatile method for forming non-woven fibrous mats. In addition, mats are highly porous and behave similar to the ECM in the body; therefore, scaffolds composed of polymers that are conducive to cellular attachment and present in the natural ECM would enhance the nanofiber efficiency making electrospinning technique an excellent candidate for use in tissue engineering.

Electrospinning can selectively process a variety of polymers. Biodegradable polymers are the most appropriate substrates for cells to attach, grow on, cell differentiation. These include both natural and synthetic materials, as well as hybrid blends of the two, which can provide an optimal combination of mechanical and biomimetic properties.[104, 105] For each individual application, scaffold can be controlled and optimized by varying the processing of the electrospun and solution parameters. The size scale of fibrous proteins found in the natural ECM can be easily mimic by electrospun fibers, since as we mentioned, this technique allows us to adjust fiber size. As another advantage of this technique, cell attachment has become improved due to the very high surface available of mat.[106] Cells could attach and organize around the fibers, when the diameters of fibrous scaffolds are smaller than the diameter of a cell. This is an important attribute when cell proliferation is desirable. According to Lord et al.[107] explored the influence of nanotopography on cellular response using a variety of surface topographies and cell types including osteoblasts,[108] fibroblasts,[109] macrophages,[110] neural[111] and endothelial cells.[112] Selected studies have been summarized in Lord's work,[107] where it is well established that different cell types will exhibit different behavior on different surface nanostructures, which are capable to modulate cellular responses.

It has been demonstrated that differences in nanofibers orientation affect cell affinity.[113, 114] In Nien's work[114] poly (ε-caprolactone)/poly(ethylene oxide)/chitosan (CS) fibers were fabricated in both aligned and random structures using electrospinning technique and their process parameters were optimized. These PCL/PEO/chitosan scaffolds were also used to investigate cell affinity. The results show that the aligned PCL/PEO/chitosan ultrafine fibrous mat had the capacity to induce cellular alignment and enhance cellular elongation. Doustgani[113] and co-workers investigated the biological effects of Mesenchymal Stem Cells (MSCs) on aligned and random nanofibrous. Their results reveal an improvement of the osteogenic differentiation of stem cells with fibers alignment, indicating these scaffolds potential applications as substrates for tissue engineering.

On the other hand, three dimensional scaffolds with higher porosity could be beneficial in allowing the delivery of nutrients and removal of metabolic products throughout

the scaffolds, as well as nutrient transport for cell survival. As it was explained before, scaffolds with small porous difficult cell infiltration into nanofiber mats, however many researchers have been examining possible ways to engineer nanofiber mats to combat these limitations.[115,116]

Another research on fibers alignment and porosity is the work of Wu[117] and co-workers, where blend collagen/poly(*L*-lactide-co-caprolactone) nanoyarn and nanofiber scaffolds were created by electrospinning to assess cell infiltration and vascularization, as well as guide cell behaviors. For this attempt pig iliac endothelial cells (PIECs) and MC3T3-E1 pre-osteoblastic cells were seeded on the surface of both electrospun nanoyarn scaffolds and nanofiber scaffolds up to 7 days. One of the results shows that nanoyarn scaffold possesses a higher porosity and higher average pore size, compared to that of randomly oriented nanofiber scaffold. This effect is reflected on cell infiltration, which is shown in Figure 7, where FE-SEM images showed that PICEs proliferated into multilayers and were oriented along the nanoyarns to form capillary like structures (A), while on the nanofiber scaffold, the PIECs were attached on the surface and expanded into a monolayer (B). In contrast, MC3T3-E1 preosteoblastic cells presented a linear phenotype and infiltrating the nanoyarn scaffold (C), whereas no infiltration was found on nanofiber scaffold and cells are randomly growing (D). According to the authors the cells experienced 3D culture patterns within the nanoyarn scaffolds and had more space to proliferate. In contrast, the nanofiber scaffolds just provided 2D surfaces for cell proliferation. In conclusion the study demonstrates, among other things, that cell growth pattern can be affected by the topography of scaffolds, showing the potential application for use in tissue engineering.

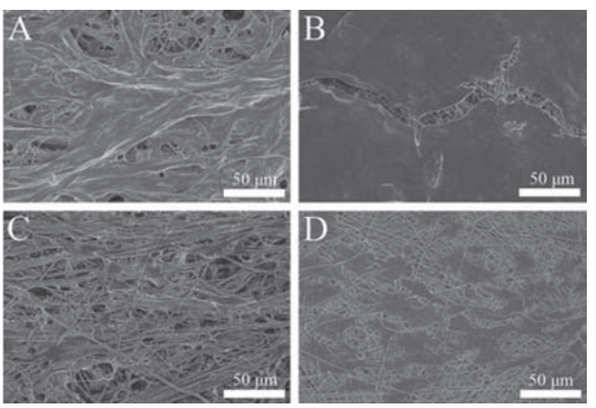

Figure 7. FE-SEM micrograph of PIECs (A) and (B) and MC3T3-E1 pre-osteoblastic cells (C) and (D) cultured on the naonyarn scaffolds (A) and (C) and nanofiber scaffolds (D) and (B) on the day 7. Cells exhibited an organized growth pattern on the nanoyarn scaffold, while on the randomly oriented scaffold, cells showed disorder growth pattern. Reprinted with permission from [48], J. Wu, et al., Cell infiltration and vascularization in porous nanoyarn scaffolds prepared by dynamic liquid electrospinning. *J. Biomed. Nanotechnol.* 10, 603 (2013). © 2013, American Scientific Publishers.

Bones Tissues

The general framework of the body is built up mainly of a series of bones, supplemented, in certain regions by pieces of cartilage. The bony part of the framework constitutes the skeleton. In the skeleton of the adult there are 206 distinct bones.[118] Bone is a hard and rigid tissue and is a dynamic organ that is constantly being remodeled; It preserves skeletal composition, structural integrity and regulates mineral homeostasis for a lifetime. Some of its functions include supporting the body, protecting organs, production of blood cell and storing nutrients. Besides, as is it an anisotropic structure, its mechanical properties differ in the different special directions.[119] Individual bones in the body can be formed from two types of bone tissue, compact bone and spongy bone. Compact bone forms the outer layer of all bones and most of the structure of the long ones; it is dense and looks smooth and homogeneous. Spongy bone has a porous structure; consistent of an irregular lattice of thin bone columns, where the spaces between them, are filled with the red bone marrow. The marrow also fills the cylindrical cavities of long bones and may have a different composition in each one.

During life, bone is permeated by vessels and enclosed in a fibrous membrane, called the periosteum, except in the regions where it is coated with articular cartilage. In Figure 8 a scheme of bone structure is shown. The extracellular matrix of bone is composed of collagenous fibers (type I) and calcium phosphate in the form of hydroxyapatite (HAp), with embedded cell components like osteoblasts and osteoclasts.

In the remodeling process, osteoclasts are the responsible for the removal of old or damaged bone while the replacement by new bone is made by osteoblasts. Bone restoration is limited and depends on fracture or defect size. Defects occur from trauma, tumor removal, and congenital deformities, in a wide variety of clinical cases. The artificial extracellular matrices (scaffolds) are critical in

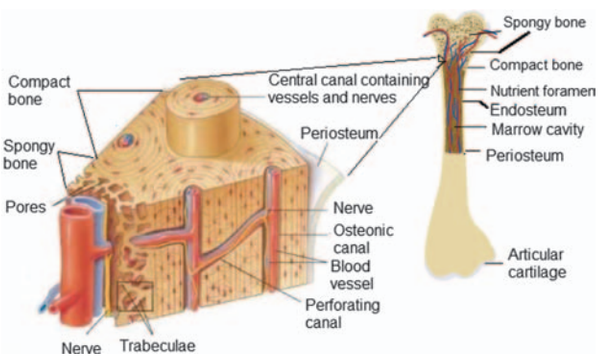

Figure 8. Schematic illustration of the bone structure.

fabrication technologies of implants and for tissue engineering. Cells grown on biodegradable scaffolds can be used to help repair damaged tissues in the body.[120, 121] For this attempt, scaffolds development has shown great promise in generating living alternatives for harvested tissues and organs for transplantation and reconstructive surgery.[122]

Scaffolds in Bone. Different kinds of materials are used for bone tissue engineering. Biodegradability is one of the most desired properties, as the scaffold should degrade with time and be replaced with newly regenerated tissues. This property eliminates the need of a second surgery to remove the implanted scaffold. Additionally, the degradation rate can be controlled, by using polymer blends[123] or incorporating different micro and nano particles into the scaffold.[124]

In bone tissue engineering natural biomaterials such as collagen, silk fibroin, and chitosan, and synthetic biopolymers such as polylactic acid, polycaprolactone, polyglycolic acid, and their copolymers are being used as scaffold. Sometimes elements that promote osseointegration can be added. HAp for example is a major inorganic component of bone and has been used for biomedical implant applications to enhance its biocompatibility, bioactivity, osteoconductivity and osteoinductivity.[20]

Osseointegration. Osseointegration occurs when bone cells attach themselves directly to the prosthesis without an interface between them. As we mentioned earlier hydroxyapatite and collagen are fundamental elements in extracellular matrix. The bone results in a highly complex mineral–organic composite material where HAp crystals are associated with the collagen framework in which they form apatite-reinforced collagen composites. Therefore, the collagen component can act as a matrix to embed the HAp particles, alleviating the brittleness of HAp, while the HAp used as a filler of the protein could improve the mechanical strength and biological performance of the collagen. Both materials are used as bone substitute for their ability to bond directly to living bone.[125, 126]

Most of the polymers used in electrospinning lack bioactive functional groups for bone bonding. In order to seek osteoconductivity, osteoinductivity and osteogenesis composited meshes have been performed.[127–133] In Tavaloki-Darestani's[21] work it was demonstrated that nanofibrous structures can be used as appropriate support for tissue-engineered scaffolds, and coating them with bioactive materials provides ideal synthetic grafts. The researchers fabricated PLGA nanofibrous scaffolds coated with type I collagen and nano-hydroxyapatite via electrospinning. Analyzing the ability for bone regeneration in a rat critical size bone defect, after 8 weeks of implantation, no sign of inflammation or complication at the site of surgery was found. To conclude, histological studies showed osseointegration to the surrounding tissue reaching the highest regeneration in rat calvarium by nanofibers coated simultaneously with collagen and HAp. Another similar work was performed by Lao et al.[22] in order to evaluate biological performance of PLGA scaffolds and PLGA with 5 wt.% HAp, revealing the higher bioactivity of the last scaffold.

The incorporation of an element like HAp does not only lead to a better osseointegration, but also modifies different properties of the matrix in which it was added.[23] As an example, in the study of Ito and co-workers[134] the development of a poly(3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) mat by electrospinning coated with HAp was reported. The resulting electrospun PHBV film was hydrophobic; however, after HAp deposition the surface became hydrophilic. This feature may be useful when controlling the degradation rate of the scaffold is desirable, since, the hidrophilicity property enhances the invasion of enzyme into the film and thus accelerates the biodegradation rate.

In a recent study, Remya and co-workers[15] incorporated HAp in polycaprolactone/polycaprolactone–polyethyleneglycol–polycaprolactone blend scaffolds with and without nanohydroxyapatite particles, to compare their physical and biological properties. They found a significant change in fiber morphology, porosity, surface wettability, and mechanical properties of electrospun PCL by the presence of both copolymer and HAp. Their results also proved that all scaffolds were non-cytotoxic, as they detected the presence of osteogenic-induced rabbit adipose-derived mesenchymal stem cells on all the scaffolds. In comparison a better performance was shown in cell viability and proliferation by the blend scaffold with HAp revealing, according to the authors, the potential of the cytocompatible scaffold for the fabrication of living bony constructs for tissue engineering applications.

To continue with synthetic polymers, several researches have demonstrated that PCL is a good candidate for bone tissue engineering.[135, 136] As we can see in the work of Shin et al.[137] mesenchymal stem cells have been successfully seeded onto PCL scaffolds showing the potential use of PCL in regenerative field.

On the other hand, natural polymers are one of the most attractive options for the preparation of three-dimensional porous scaffolds for bone tissue engineering. Chitin and chitosan have similar structures than glycosamine-glycans. Their antibacterial activity, chemical versatility, and inherent cellular interactions convert these polymers to appropriate choices for tissue engineering applications.[138, 139] After cellulose, chitin is the most abundant organic material produced by biosynthesis. Additionally, chitosan is a derivative of chitin and it is soluble in acidic solvents such as formic acid and others, while chitin is not.

Nanofibrous scaffolds of chitosan were prepared by electrospinning in Thien's[140] work. The fibers were treated with simulated body fluid (SBF) to encourage HAp formation, what happened after 6-day incubation. They examined the morphology and biocompatibility on the HAp/CS scaffolds. With this aim, scaffolds were applied to the

culture of rat osteosarcoma cell lines. The authors demonstrated that the biomimetic nanofibrous HAp/CS scaffolds could support and enhance the adhesion, proliferation, and particularly osteogenic differentiation. Therefore, the construction of nanofibrous scaffolds of HAp/CS seems to be an efficient strategy for functional bone repair and regeneration applications. Silk fibroin is a typical fibrous protein that has recently been studied due to its excellent biocompatibility, bio-absorbability, and low level of inflammatory potential.[141–145] Li and co-workers[11] developed a fibrous mat of silk fibroin with bone morphogenetic protein 2 (BMP-2) and/or nanoparticles of HAp (nHAp) using electrospinning. The scaffolds were used for *in vitro* bone formation from human bone marrow-derived mesenchymal stem cells (hMSC), which were cultured for up to 31 days under static conditions in osteogenic media (silk/PEO/BMP-2, silk/PEO/nHAP, silk/PEO/nHAP/BMP-2) and controls (silk/PEO, silk/PEO extracted). Comparing the systems, the results show that silk fibroin-based scaffolds not only supported hMSC growth and differentiation toward osteogenic outcomes, but they also were an efficient delivery system for BMP-2. According to the authors, electrospun silk-fibroin-based scaffolds are potential candidates for bone tissue engineering.

The most abundant natural, renewable, biodegradable polymer is cellulose.[146, 147] Cellulose is one of the longest studied polymers[148] but does not dissolve in common solvents, so it is common to use cellulose derivatives. Poly(vinyl alcohol) (PVA), is also commonly used in cellulose composites to facilitate electrospinning. Chalal at al.[149] uses modified cellulose (MC) to develop a suitable scaffold for bone tissue regeneration. According to the authors, the results showed that MC/PVA nanofibrous scaffold provides a beneficial frame for bone tissue engineering.

Several authors have also investigated composites with synthetic and natural biodegradable polymers such as polycaprolactone.[17, 150, 123] In the case of Zargarian and co-workers,[17] a nanofibrous composite scaffold of PCL/hydroxyapatite-chitosan/PVA was prepared by electrospinning to analyze the variations of the diameters due to changes in concentration of the spinning solutions and the ratios. In conclusion, the nanofibrous composite scaffold of electrospun PCL/HA-chitosan/PVA can potentially be used for the osteogenic differentiation of stem cells.

According to Cheng[14] and co-worker blending polymers is known to be a very effective way to produce new multipurpose advanced materials. In this work, the researches electrospun nanofibers of polycaprolactone-chitosan blends and studied the effect of type I collagen surface functionalization in regulating rat bone marrow derived stromal cells (rBMSCs) differentiation into osteogenic lineage. Four types of nanofibres matrices were employed for cell studies, pure PCL, PCL/CS, bovine serum albumin (BSA) modified PCL (BSA-PCL/CS) and collagen modified PCL/CS (COL-PCL/CS). The authors compared the collagen functionalized COL-PCL/CS nanofiber with control groups. The results showed that more than 90% of rBMSCs had attached COL-PCL/CS nanofibers during the first hour, which was the higher percentage of all nanofibers group. Besides, cell cultured on COL-PCL/CS showed significantly larger projected cell area than that of others at each time (as it can be seen in Fig. 9). The results also suggested that both chitosan blending and BSA modification promoted cell adhesion moderately, probably due to the increased hydrophilicity and adsorption of serum protein, respectively. Significantly improved rBMSCs adhesion, spreading, proliferation and osteogenic differentiation were observed on the functionalized collagen COL-PCL/CS nanofiber matrices as compared to control groups.

Developments of nanostructured fiber matrices and modification techniques can be especially useful in other scaffold systems for tissue engineering applications. Collagen has been widely used as a practical biomaterial in tissue engineering and offers great opportunities for fabricating artificial scaffolds to mimic bone grafts due to its excellent assembled structure, extensive occurrence in nature, and potential to completely degrade in biological environments. Until now, 28 types of collagen have been identified, type I is associated whit skin, tendon, vascular ligature, organs and bone. Collagen composites have been extensively studied by several authors applying different methods to incorporate collagen into nanofibers.[151] Since

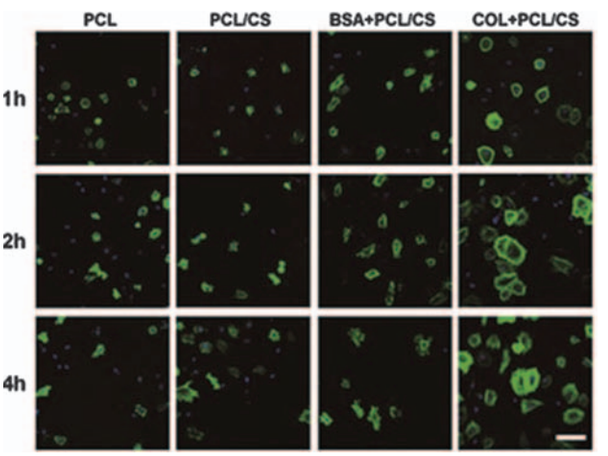

Figure 9. Collagen modified PCL/CS nanofiber promotes cell spreading during initial adhesion stage. At time points 1 h, 2 h and 4 h, rBMSCs were fixed and stained with fluorescence phalloidin (green) to visualize cytoskeleton during cell spreading. Nuclei were count-stained with DAPI (blue). Scale bar indicates a length of 50 μm. Reprinted whit permission from [14], Y. Cheng, et al., Collagen functionalized bioactive nanofiber matrices for osteogenic differentiation of mesenchymal stem cells: Bone tissue engineering. *J. Biomed. Nanotechnol.* 9, 1483 (2013). © 2013, American Scientific Publishers.

the hierarchical self-assembly of collagen–HAp composite is the naturally occurring process during bone generation and growth, collagen shows excellent biological properties in bone regeneration. In addition because the advantageous properties of both materials, collagen–HAp composite is an excellent scaffold for bone tissue; as it was reported in the work of Venugopal et al.[129] in which nanostructured collagen and collagen-apatite composites were produced by electrospinning and osteoblasts were cultured on the resulting mats. Proliferation of osteoblasts occurred on both nanofibrous scaffolds. However, osteoblasts cultured on composite nanofibrous scaffold showed normal rate of proliferation, higher level of mineralization and moderate increase in calcium and phosphorous activity through the activation of HAp.

As it was already mentioned, topographic and biochemical cues have been deliberately designed to recapitulate the native cell environment. The fiber mats obtained by the electrospinning technique have a high porosity and a good surface area-to-volume ratio that enhance cellular adhesion, migration, and proliferation.[106] Highly porous microstructure with interconnected pores and a large surface area is efficient to tissue ingrowth, it provides more structural space for the accommodation and attachment of cells, and enables the efficient exchange of nutrients and metabolic waste. However, the combination of pore size and interconnectivity required for optimal osteoconductivity has yet to be determined. Woodarda et al.[10] illustrate the importance of scaffold microporosity on bone ingrowth and on the mechanical behavior of HAp implant materials. It was reported that after implantation only the microporous scaffolds (2–8 μm) contained bone. Therefore, these results suggest that microporosity improves bone growth into scaffolds by increasing surface area for protein adsorption.

For long time titanium and its alloys were used for biomedical and dental implants due to their good mechanical properties, high corrosion resistance, and excellent biocompatibility.[152, 153] Titanium is not rejected by living organisms, possible due to the generation of titanium oxide on the surface of the metallic implant that leads to direct apposition of minerals on the bone-prosthesis interface and titanium osseointegration. Since this is a bioinert material, the research has been directed to the surface modification of these materials to improve bone implant contact and their biological properties.[154] A solution to this failure could be the application of a coating constituted of biomimetic apatite nanocrystals and collagen fibers.[129, 155] Recent studies concerning collagen coatings on titanium implants have demonstrated their effective role in stimulating cellular responses, increasing bone remodeling, and improving bone growth and bone implant contact.[156]

An alternative to improve osseointegration, is the modification of titanium implant surfaces.[157] Different authors proposed to alter the implant surface interaction with ions, biomolecules and cells. These interactions can favorably influence the molecular and cellular activities and also modify the integration process. However, sometimes osseointegration is not a desirable property. For example, in temporal prostheses, which are used for a limited period of time and then must be removed, osseointegration is an unwanted effect.

Bony-Prosthesis Coating. Systemic administration of antibiotics is mostly used to treat infections. However, it is not possible to reach a high drug level with this administration method, and in some cases local application becomes necessary. The application of a prosthesis having a coating containing the antibiotic is one possible way to locally introduce the drug.[158]

Total knee arthroplasty infection is a disastrous complication. A commonly used treatment is two-stage reimplantation. This procedure begins with the removal of the infected implant; continues with debridement of all infected and necrotic tissue, control of infection, and finally re-implantation of a new prosthesis. This methodology was first advocated by Insall et al.[159] However there have been some disadvantages in two-stage exchange arthroplasty, like poor mobility, limited stability, soft-tissue contractures and pain. To address this situation, antibiotic-loaded cement temporary spacers have been implantated. The use of spacers enables direct local administration of antibiotics, while preserving patient mobility and facilitates re-implantation surgery.[160]

Hsieh and co-workers[161] have shown that the use of antibiotic-loaded spacers in hip arthroplasty seems to offer great advantages, like a shorter hospital stay, less blood loss, a fewer postoperative dislocations among others. These spacers have been found to dramatically facilitate exposure at the second-stage procedure and are now being incorporated into all two-stage revision hip arthroplasties.[162]

However the use of continuum coating in prosthesis has presented some mechanical complications like fissure propagation and poor adhesion to the implant surface. Furthermore, a good diffusion of the antibiotic is necessary to produce a solid treatment of infection. It should be noted that in a continuum coating, the mayor proportion of the drug is contained inside the coating. Therefore, continuum coatings have a low efficiency in the delivery of drugs. This problem, among others, can be solved if the prosthesis coating is done by applying the electrospinning technique.

Nowadays, all kinds of drugs such as antibiotics can be incorporated into electrospun mats. Li et al.[163] prepared a mat of PLGA/ PEO loaded with gentamicin for coating titanium implants. The release of gentamicin showed an initial gentamicin burst followed by a slow release. The authors performed a bacterial adhesion study to determine whether this antibiotic coating can prevent bacteria from adhering and growing on the surface of the implant. In addition, they report a persistent antibacterial efficacy for 1 week and a significant reduction in the adhesion of

the staphylococcus aureus compared with bare titanium implants *in vitro*. This work shows an effective method to prevent infections by implantation.

Muscles

The muscles are connected with the bones, cartilages, ligaments, and skin, either directly, or through the intervention of fibrous structures called tendons or aponeuroses. Where a muscle is attached to bone or cartilage, the fibers end in blunt extremities upon the periosteum or perichondrium, and do not come into direct relation with the osseous or cartilaginous tissue. The muscles vary extremely in their form. In the limbs, they are of considerable length, especially the more superficial ones; they surround the bones, and constitute an important protection to the various joints. In the trunk, they are broad, flattened, and expanded, and assist in forming the walls of the trunk cavities. Hence the reason of the terms, long, broad, short, etc., used in the description of a muscle.[118]

There are three types of muscle tissue; smooth muscle, skeletal muscle and cardiac muscle. Figure 10 shows a schematic illustration of the basic structural characteristics of skeletal, smooth, and cardiac muscle.

Skeletal muscles are the only voluntary muscles tissue in the human body. The function of skeletal muscle is to create movement in the body by contraction; therefore, every physical action that a person consciously performs requires skeletal muscle. This contraction is controlled by the nervous system. Most skeletal muscles are attached to two bones through tendons which do not contract and they are made up of collagen fibers. The attachment that stays in place during movement is called the origin while the place on the moving bone that is connected to the muscle via tendons is called the insertion.

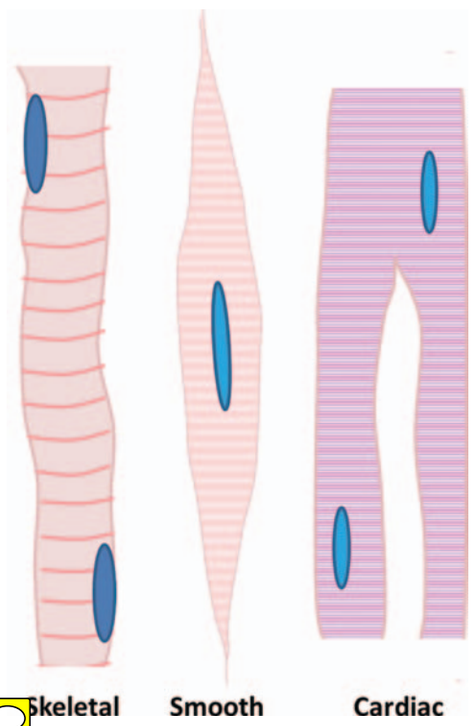

Figure 10. Schematic illustration of the basic structural characteristics of skeletal, smooth, and cardiac muscle.

Skeletal muscles are made up of thousands of cylindrical muscle fibers bound together by connective tissue through which blood vessels and nerves run. The fibers show a pattern of cross banding, which gives it the name of striated muscle.

Cardiac muscle is found in the heart and it cannot be controlled consciously. The heartbeat is generated within the heart itself; therefore, cardiac muscle is considered to be autorhythmic or intrinsically controlled. In a heartbeat all the fibers contract in a synchronous wave that sweeps from the atria down through the ventricles and pumps blood out of the heart.

Smooth muscle is found in all internal tissues and organs like the stomach, intestines, and vascular system. These muscles makes organs contract to move substances through them. This contraction tends to be slower than that of striated muscle, besides, is often sustained for long periods. Because they are controlled by the autonomic nervous system, it is known as involuntary muscle. It is made of single spindle-shaped cells and no striations are visible in contrasts with the banded appearance of cardiac and skeletal muscles.

Muscle cell is also known like myocyte which are formed in a process known like myogenesis. Myocytes are long, tubular cells that develop from myoblasts to form muscles, each of them contain myofibrils which are composed of repeating sections of sarcomeres. Those are the basic unit of a muscle and are referred as "striated" cells because they appear to have light and dark stripes when viewed under a light microscope (as it can be seen in Fig. 10). Striated muscle contracts and relaxes in short, intense bursts, whereas smooth muscle sustains longer or even near-permanent contractions. Myoblast and consequently myofibre elongation is thought to be essential for efficient contraction to take place.

There are various specialized forms of myocytes; cardiac, skeletal, and smooth muscle cells. Cardiac Myocytes are responsible for generating the electrical impulses that control the heart rate, among other things. These muscle cells are organized into a complicated lattice, and like skeletal muscle, they are oriented along a common axis. Each cell of the heart is invested with a basement membrane and interconnected to its neighbors by a complex matrix of collagen fibrils. The three dimensional pattern of the cell layers within the heart is critical for the orderly propagation of electrical signals and the coordinate contraction of the ventricular wall.

During lifetime many factors can produce injury to the body cells. Soft tissue damage occurs through direct or indirect trauma to muscles, ligaments, and joint capsules.

In the repairing process, most of the cells have the ability to repair and regenerate tissue, but a defect in this process can lead to cardiovascular, neurological, muscular or pulmonary diseases.

When muscle structure is irreversibly compromised or individual muscles have been ablated by surgical procedures or major injuries, the perspective of engineering new muscle fibers via satellite cells becomes an attractive difficult goal. Engineering muscle constructs *in vitro* would provide a valid alternative for tissue replacement in the enhancement of muscle regeneration.

Skeletal Muscle. As we already mentioned, the scaffold development for sustainable three dimensional growths of cells is of particular interest in the field of tissue engineering and regenerative medicine.

Numbers of attempts have been made so far to reconstruct skeletal muscle in the lab conditions.[164–166] To generate sufficient force for contraction, myofibers need to be packed parallel to each other, therefore, a key factor to overcome these problems is the design of appropriate scaffolds able to support cell fusion and the formation of long continuous muscle fibers.

Synthetic materials, such as PLGA, may create a biodegradable scaffold with the topography to introduce contact guidance to the cells in order for them to grow parallel to each other in one direction. Aviss and co-workers[25] developed electrospun highly aligned PLGA fibers. They showed that this highly aligned scaffold can provide the topographical cues for the alignment of murine myoblasts. However, it does not allow cellular infiltration thus it would only be used as a template to create and maintain the alignment of the myoblast cells.

Satellite cells are precursors to skeletal muscle cells. When muscle cells undergo injury they can migrate to enter the injured area and fuse with pre-existing damaged fibers, or fuse to form new myotubes. The repairing process ended once the proliferation potential of satellite cells is exhausted. Consequently there is no further regeneration and skeletal muscle is replaced by connective tissue.

Because of the function of satellite cells, researches has been focused their effort in developing a suitable environment for satellite cells to grow and differentiate.[167, 168] Amna et al.[169] developed Fe_3O_4/TiO_2 hybrid nanofibers by electrospinning founding that this composite showed a beneficial effect on the adhesion and propagation of satellite cells and could guide the spreading behavior of muscle.

Electroactive polymers as metal particles can be used to increase the conductivity of a polymeric scaffold for possible use in electrical stimulation techniques. These elements could improve the skeletal muscle cell elongation, orientation, fusion and striation. With this aim Sirivisoot et al.[169] studied the effects of electrically conductive materials made from electrospun single- or multiwalled carbon nanotubes with polyurethane to promote myoblast differentiation into myotubes in the presence and absence of electrical stimulation. The results showed cell differentiation in both conductive and nonconductive fiber scaffolds. However, multinucleated myotubes are more readily formed on carbon nanotube-containing scaffolds than on nonconductive scaffolds after electrical stimulation. To conclude, electrically conductive materials have the potential to aid in the engineering of functional skeletal muscle tissues.

Smooth Muscles. As we already mentioned, smooth muscles are tissues that contract without conscious control, found in the walls of the internal organs, such as the stomach, intestine, bladder, and blood vessels, excluding the heart.[170]

Recently the use of micro- and nanotechnology in vessel tissue engineering have gained greatly attention,[171] as well as scaffold design by different processes.[172, 173] Nanofiber based scaffolds offer great promise in regeneration of this type of tissues. In this context, different electrospinning mats have been designed and the proliferation and infiltration of smooth muscle cells (SMC) into them have been studied. An adequate scaffold for smooth muscle tissue regeneration must be elastic and malleable but maintain mechanical strength.[148, 175] Taking this into account, different polyurethaneures, such as poly(ester urethane) urea and poly(ether ester urethane) urea, have been employed for this porpoise.[115]

Nivison-Smith et al.[24] produced aligned and random fiber scaffolds from synthetic elastin (SE) as a model of the arterial media and seeded SMC on them. These scaffolds supported the attachment and orientated the growth of human vascular SMC *in vitro* and allowed for the expression of native SMC marker proteins. Xu et al.[176] also produced an aligned electrospun nanofibrous membrane by electrospinning, in this case using poly(*L*-lactid-*co*-ε-caprolactone) (75/25) copolymer. They found the smooth muscle cells attached and migrated along the axis of the aligned nanofibers and expressed a spindle-like contractile phenotype; the distribution and organization of smooth muscle cytoskeleton proteins inside SMC were parallel to the direction of the aligned nanofibers; the adhesion and proliferation rate of SMCs was significantly improved compared with the results obtained for a solvent cast polymer film with a homogenous plane surface.

However, as it was explained before, achieving high cellular density and infiltration by seeding cells on an electrospinning mat remains challenging. In order to overcome this limitation, Stankus et al.[177] electrosprayed vascular SMC concurrently with a biodegradable, elastomeric poly(esterurethane)urea. The electrosprayed SMC spread and proliferated in the microintegrated constructs similar to control unprocessed cells. The resultant scaffold was strong, flexible and anisotropic. The authors suggest the application of this novel material to fabricate high cell density elastic tissue mimetics, blood vessels or other cardiovascular tissues.

As it was mentioned before, another possible technique to improve cellular micro-integration is generating

new macropores on the electrospun mat. Lee et al.[178] utilized femtosecond laser ablation to produce microchannels inside electrospun polycaprolactone scaffolds for vascular wall engineering. Human coronary artery smooth muscle cells were seeded in proliferative medium. The authors confirmed the cell infiltration using confocal microscopy and scanning electron microscopy. Besides, the presence of F-actin filaments shows that the cells were adhered strongly to the scaffold and remained viable throughout the culture, validating the concept of "vascular wall engineering" producing intricate cell seeding through microchannels produced via femtosecond laser ablation was validated.

Cardiac Muscle. Heart failure following by an episode of myocardial infarction (MI) is a preeminent cause of death in the world. In patients who survive MI, scar tissue formation occurs and the damage to the heart wall is permanent. Therefore, the heart fails to pump sufficient amounts of blood to compensate the ventricles undergoing remodeling and hypertrophy; leading to congestive heart failure.

Due to limited ability of the cardiac cells to regenerate *in-vivo*, attempts are being made to restore the functionality of the heart affected from myocardial infarction.[179–182] For successful cardiac repair a critical step involved is the creation of tailor made 3-D matrices that act host to the cells (defined as cardiac patches) and should help in maintaining cellular viability, proliferation, differentiation and support cell integration. However, current cardiac tissue models are not yet able to stably maintain functional characteristics of cardiomyocytes for long-term culture and therapeutic purposes. Whit this aim Hussain et al.[183] fabricated bioactive 3-D chitosan nanofiber scaffolds using electrospinning and explored its potential for long-term cardiac function in the 3-D co-culture model. In order to enhance cell attachment and growth, fibronectin was incorporated onto the chitosan nanofibers by adsorption. Besides, neonatal rat cardiomyocytes were cultured on the chitosan nanofibers (in various culture conditions) to assess their viability, morphology, and function. The results demonstrate that the chitosan nanofibers retained their cylindrical morphology in long-term cell cultures and exhibited good cellular attachment and spreading in the presence of adhesion molecule, fibronectin. Besides, the results suggest that cardiac co-culture model (cardiomyocytes-fibroblasts or cardiomyocytes-endothelial cells co-culture) is a promising system for the maintenance of long-term survival and function of cardiomyocytes.

A co-culture system was also used in Ravichandran's work,[26] where Poly (glycerol sebacate)/collagen (PGS/collagen) core/shell fibers were fabricated by core/shell electrospinning technique, with core as PGS and shell as collagen polymer; with the desire to be a potential cardiac patch material for myocardial infarction. Collagen nanofibers were also fabricated by electrospinning for comparison with core/shell fibers. They found that the PGS/collagen core/shell had mechanical properties comparable to that of native heart muscle with a young's modulus of 4.24 MPa, while in collagen nanofibers it was comparatively higher (30.11 MPa). Cardiac cells and mesenchymal stem cells (MSCs) co-culture system was characterized for cell proliferation and differentiation of MSCs into cardiomyogenic lineage. The authors have reported that direct cell-to-cell contact between MSC and adult cardiac cells is necessary for the differentiation of MSC into cardiac cells. Finally the results evidence cell proliferation significantly increased on PGS/collagen core/shell scaffolds compared to collagen fibers. However, even though the implanted stem cells survived and regenerated the infarcted myocardium, the site myocardial infarction is a poor environment for cell growth. To increase cell viability, some factors to improve such an infertile environment are desirable.

As described before, electrospun fibrous scaffolds provide both flexibility and guidance for cardiac cells growth and MSC differentiation into cardiac lineage and thus can be successfully applied to obtain structurally and functionally competent cardiac tissue constructs.[184, 185] Since the fibrous scaffolds are highly porous, cells can easily penetrate deep within it. It is known that fiber diameter is directly related to the pore size in electrospun scaffolds. This is a significant parameter for homogeneous cell delivery. Balguid[186] and co-workers evaluate how cell delivery can be optimized by tailoring the fiber diameter. Their results suggest that the optimal electrospun scaffold geometry is not generic and should be adjusted to cell size.

Tendons

Tendons are strong bands of fibrous connective tissue and are capable to resist high tensile loads. They are glistening, fibrous cords, varying in length and thicknesses, sometimes round, sometimes flattened, devoid of elasticity and consist almost entirely of collagen.

Tendons work together with muscles to move bones, transmitting forces generating by muscles. For every muscle there are two tendons and each of them has to points of unions, where the tendon attaches to muscle and where attaches to bone. Besides, tendons are very sparingly supplied with blood vessels.

Injuries to tendons involve either a tear of some of the fibers or a complete rupture. In this case it takes more time to heal because tendons require less blood supply than muscles to function. Also tendons are slightly elastic; therefore, they are more susceptible than muscles to inflammation. Chronically injured tendons can occur anywhere, but especially around joints such as the shoulder, knee, elbow, etc.

Tissue engineering affords the opportunity to improve tendon injuries. Several approaches are under investigation, including the use of nanofibrous scaffolds for regeneration of the tendon.[187, 189] Rotator cuff tears are one

of the most common causes of shoulder pain and disability among adults. Whit the aim of improve repair of rotator cuff tears Chainani et al.[190] investigated the use of tendon-derived extracellular matrix (TDM)-coated electrospun multilayered scaffolds compared to fibronectin (FN) or phosphate-buffered saline (PBS) coating for use in rotator cuff tendon tissue engineering. Multilayered poly(*e*-caprolactone) scaffolds were prepared by sequentially collecting electrospun layers onto the surface of a grounded saline solution into a single scaffold. Scaffolds were then coated with TDM, FN, or PBS and seeded with human adipose-derived stem cells; to evaluate the benefit to coating the scaffolds. The results indicate that multilayered electrospun scaffolds incorporated with TDM show increased levels of collagen accumulation as compared to FN- or PBS-coated scaffolds. This provides a novel means for tissue engineering of the rotator cuff; however the exact mechanism of action of TDM is under investigation.

Another strategy for tendon tissue engineering is to use aligned fibers because they could mimic tendon tissue better than nonaligned scaffolds. This idea was developed by Xu and co-workers[27] who propose a novel electrospun nanoyarn network that is morphologically and structurally similar to the ECM of native tendon tissues. The authors developed a nanoyarn, random nanofiber, and aligned nanofiber scaffolds of a synthetic biodegradable polymer, poly(*l*-lactide-co-ε caprolactone), and natural collagen I complex by electrospinning technique. These scaffolds were characterized in terms of fiber morphology, pore size, porosity, and chemical and mechanicalproperties for the purpose of culturing tendon cells (TCs) for tendon tissue engineering. Morphologic results showed in the case of nanoyarn scaffold, that the yarn was twisted by many nanofibers similar to the structure and inherent nanoscale organization of tendons. Besides, the scaffold contained 3D aligned microstructures with large interconnected pores and high porosity. The presence of collagen in the three scaffolds was revealed, while the mechanical properties of the scaffolds; indicated desirable properties for tissue regeneration. Further, the results revealed that TC proliferation and infiltration, were significantly enhanced on the nanoyarn scaffold compared with that on the random nanofiber and aligned nanofiber scaffolds. The results demonstrates that electrospun P(LLACL)/collagen nanoyarn is a novel, 3D, macroporous, aligned scaffold that has potential application in tendon tissue engineering.

Neural Tissues

The nervous system coordinates the voluntary and involuntary actions of the body and transmits signals between its different parts. This system is classified into the central nervous system (CNS), which includes the brain and spinal cord, and the peripheral nervous system (PNS), including the cranial nerves arising from the brain, the spinal nerves arising from the spinal cord, and sensory nerve cell bodies. Peripheral nerves innervate muscle tissue, transmitting sensory and excitatory input to and from the spinal column.[191]

At the cellular level, the nervous system is composed of two cell types: neurons and neuroglia. Neurons are the basic functional unit of the nervous system. They relay information about external environment from sensory organs to the brain, allow control over skeletal muscles, and ensure necessary actions such as heart beat and bowel movements continue without any conscious effort. These cells contain a cell body, where the nucleus is located, small processes called dendrites, and a long process called axon. An electrical signal is detected by the dendrites and transmitted down the axon, to an effector; which may be another neuron, a smooth muscle, a skeletal muscle or many other possibilities. Neuroglia, or supporting cells, such as Schwann cells and oligodendrocytes, act to line and protect neurons and increase the speed and efficiency of information transmission. While neurolgia cells have some capacity for cell division, neurons cannot divide by mitosis. However they can regenerate a severed portion or sprout new processes under certain conditions.[192]

The physiology of the nervous system presents unique challenges to bioengineering research addressing nerve injuries. The organization and intricacy of the central and peripheral nervous system pose special criteria for the selection of a suitable scaffold to aid regeneration. The scaffold must have sufficient mechanical strength while providing an intricate network of passageways for axons, Schwann cells, oligodendrocytes, and other neuroglia to populate. If neural regeneration is to occur, these intricate passageways must not be impeded by macrophages, neutrophils, or other inflammatory cells. Therefore it is imperative that the scaffold does not illicit a severe immune response.

Peripheral Nervous System Injury and Regeneration. The most severe injury of the PNS is a complete nerve transection. After a nerve is severed, the distal portion begins to degenerate as a result of protease activity and separation from the metabolic resources of the nerve cell bodies. Although new axonal sprouts usually emanate to achieve regeneration, functional re-innervation requires that axons extend until they reach their distal target, and in humans, axon regeneration occurs at a rate of about 2–5 mm/day; thus significant injuries can take many months to heal.[193] For this injury type, treatment typically consists of either direct end-to end surgical reconnection of the damaged nerve ends, which is applicable only for small defects repair, or the use of an autologous nerve graft, that is harvested from another site in the body and is used to span the injury site. In the PNS, the tissue bioengineering challenge is to find an alternative to the autologous nerve graft and thus eliminates the need for two surgeries and the removal of tissue from the patient. Thus, researchers have focused on developing alternative treatments to the nerve graft, especially for larger defects, and improving recovery rates and functional outcome.

There has been much research dedicated to the use of synthetic scaffolds to bridge peripheral nerve injuries.[194, 195] Hollow polymeric tubular nerve conduits have been developed with the objective of bridging two injured nerve stumps and forming a protected lumen for the formation of a fibrin cable that enables Schwann cells to migrate from each severed nerve stump. The Schwann cells form longitudinally aligned strands (bands of Büngner) that guide axonal regeneration. Although the use of these conduits has demonstrated a marked ability to aid in peripheral nerve regeneration, they still leave much to be desired. Regenerated sections of the nerve do not have the same axon density as a native nerve, and there has been very little success reported in repairing gap defects greater than 2 cm.[196] It has been hypothesized that these failures are due to lack of three dimensional support, contact guidance, and biochemical signals.[197] To address these issues, researchers have begun investigating various luminal fillers for the conduits. Griffin et al.[198] developed a novel biocompatible polymer fiber mats that can be stacked within the lumen of a nerve guidance conduit through the electrospinning technique. They fabricated aligned poly(lactic-co-glycolic acid)/bioactive polyanhydride fibrous substrates with fiber diameters of 600 ± 200 nm. Schwann cells and dissociated rat dorsal root ganglia demonstrated elongated and healthy proliferation in a direction parallel to orientated electrospun fibers with significantly longer Schwann cell process length and neurite outgrowth when compared to randomly orientated fibers. Kim et al.[199] reported the successful bridging of a 17 mm nerve gap in rats using electrospun fibers. Regenerated axons re-innervated muscles, and reformed neuromuscular junctions. Electrophysiological and behavioral analyses revealed that, but not randomly oriented constructs facilitated both sensory and motor nerve regeneration, significantly improved functional outcomes. These, together with many other recent publications,[200, 201] are promising indications that electrospun fibers have a place in nerve conduits designed to bridge larger gaps.

Central Nervous System Injury and Regeneration. On the other hand, the CNS does not have the capacity of peripheral nerves to regenerate appreciably in their native environment. For CNS injury, and particularly spinal cord injury, clinical treatment is unpromising. If bone fragments exist near the site of injury, then surgery may be performed to reduce any risk of secondary injury and anti-inflammatory drugs can be administered to reduce swelling and secondary injury.[202] Unfortunately, there is currently no treatment available to restore nerve function. In this context, the CNS is a great challenge for new therapies.

After a spinal cord injury, the activation of microglia, recruitment of *T*-lymphocytes, disruption of the blood-brain barrier, and influx of macrophages play an important role in the secondary damages occurring.[203, 204] However, growth factors and protease inhibitors released by macrophages and *T*-lymphocytes promote regeneration.[204] These contradicting roles suggest that the response to trauma can be optimized.

Studies suggest that a physical barrier can protect intact spinal tissue from the immune system following and injury. Liu et al.[205] evaluated the potential application of electrospun collagen nanofibers for spinal cord injury treatment *in vitro* and *in vivo*. They seeded rat astrocytes, (typical secretors of regeneration inhibitory molecules) and dorsal root ganglias on collagen-coated glass cover slips (substrate controls), and randomly oriented or collagen fibers to evaluate scaffold topographical effects on astrocyte behavior and neurite outgrowth, respectively. As in above-mentioned findings, the cell growth was found to be oriented by the fibers alignment. Furthermore, when cultured on collagen nanofibers, astrocyte proliferation was suppressed as compared to cells on 2D controls. Besides, the authors formed collagen scaffolds into spiral tubular structures, and demonstrated the feasibility of using electrospun nanofibers for the treatment of acute SCI using a rat hemi-section model. At days 10 and 30 postimplantation, extensive cellular penetration into the constructs was observed regardless of fiber orientation. However, scaffolds with aligned fibers appeared more structurally intact at day 30.

Chow et al.[206] demonstrated the potential of electrospinning to generate an aligned matrix that can influence the directionality and growth of axons. The authors electrospun polydioxanone to fabricate matrices possessing either aligned or randomly oriented fibers. Besides, rat dorsal root ganglia were cultured on these matrices for 10 days to evaluate alignment influence on neuritic outgrowth. The results show a directional growth that mimicked the fiber alignment of the underlying matrix.

Active Agent Incorporation

As it was mention in the last sections, electrospun mats are often employed as a delivery mean for different active agents.[207] Employing different techniques, such as blending, surface modification, and coaxial process, bioactive molecules can be incorporated into the electrospinning nanostructures. By careful selection of materials and processing conditions, desired encapsulation efficiency as well as preserved bioactivity of the therapeutic agents can be achieved.[80, 116]

Drug Delivery Systems and Release Control

One of the main areas of research in electrospinning biomedical applications is drug delivery, where the fibers help to encapsulate the therapeutic agent. Different alternatives to drug delivery systems have been widely investigated and reported by different researchers.[208] As it was explained before, due to its large aspect ratio, nanofibers have a huge surface area, increasing the liberation rate compared with a continuous material, where most of the drug is contained inside the bulk and less than the 10% of

the drug is in fact delivered.[209–211] In Figure 11, a diagram showing the idea is presented.

Many drugs have been incorporated into polymer electrospinning fibers, such as antibiotics, anti-inflammatories, immunosuppressive and antiproliferatives. In particular, polymer nanofibers provide a useful pathway for delivery of poorly water-soluble drugs, which compounds for oral delivery present one of the most frequent challenges to formulation scientists in the pharmaceutical industry.[212, 213] When administered orally, these drugs have shown to be slowly and unpredictably absorbed since their bioavailability is largely dependent on the dissolution process in gastrointestinal tract. Electrospun nanofibers may provide novel approaches in this field. Yu et al.[214] prepared novel solid dispersions of poorly water-soluble drugs with special microstructural characteristics using electrospinning process and compared the electrospun nanofibrous material with those prepared from three traditional solid dispersion processes such as freeze-drying, vacuum drying, and heating drying. *In vitro* dissolution tests demonstrated that the electrospun nanofibers released 93.8% of the drug content in the first 2 minutes. Electrospun nanofiber-based solid dispersions showed markedly better dissolution-improving effects than the others, mainly due to their huge surface area, high porosity resulting from web structure, and the more homogeneous distribution of acetaminophen in the nanofiber matrix. Lin et al.[215] studied the controlled release behaviour of the water-insoluble drug nifedipine from electrospun polycaprolactone-based polyurethane nanofibers. *In-vitro* drug release studies revealed that a self-assembly process of nifedipine particles might be achieved within the body of the nanofibers. The electrospun nanofiber resulted an ideal drug carrier compared with a spin-coated film and could achieve controlled release of drug.

On the other hand, localized inoculation of medicines can significantly reduce the systemic absorption of the drug and prevent/reduce any side effects. Compared to systemic drug delivery the local administration of drugs is considered to be more effective, since the pathogen-specific drug can be placed directly in the focus of infection achieving effective concentrations. For effective elimination of pathogenic bacteria, the antibiotic agent has to be available in adequate concentrations for a sufficiently long period of time, overcoming the minimum inhibitory concentration (MIC). Reise et al.[216] electrospun PLA fibers loaded with 0.1–40% (w/w) of metronidazole finding that concentration influenced fiber diameters and thus w/w surface areas. All fiber mats released 32–48% of their total drug content within the first 7 days and showed excellent cytocompatibility, suggesting their potential clinical applicability for the treatment of periodontal diseases.

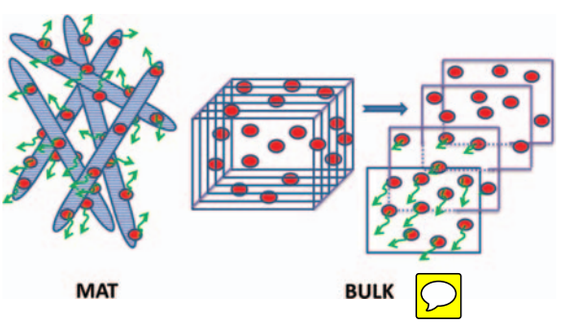

Figure 11. Schematic illustration showing a comparison between the drug release in an electrospun mat and in a bulk material.

The release of the drug is dependent on the degradation of the polymer fibers and its diffusion through it, and thus can be properly controlled. Porous nanofibers, for example, generate a completely different drugs diffusion pathway than continuous ones.[217]

The way in which the agent is incorporated to the fiber will also be determinant in its availability and release rate. Drug loading of electrospun matrices for drug delivery can be achieved by several techniques including post-spinning modifications, electrospinning of drug/polymer blends and core–shell electrospinning.

Drug-Incorporation Techniques

Post-Spinning Modifications. Post-spinning modifications allow the incorporation of different drugs, mixtures and concentrations to a common polymer matrix, avoiding the exposure of the drug to the electrospinning process and preventing the loss of drug. Bölgen et al.[218] slowly dropped an ornidazole solution in absolute alcohol and propylene glycol onto poly(caprolactone) electrospun nonwoven membranes. The fibrous matrices adsorbed the entire drug solution due to their unique ultra-high surface area. However, a burst release of 80% of the loaded ornidazol from PCL matrices was observed within 3 h, limiting the applicability of the process to circumstances that require high initial drug concentrations.

Our research group also applied an antibiotic on an electrosinning mat. The used drug was gentamicin sulfate and it was sprayed on a highly porous PMMA mat. One of the problems involved in this technique is the solvent selection for the antibiotic solution, as it must solubilize the drug and, at the same time, successfully wet the fibers. In this context, spraying gentamicin sulfate requires a mixture of solvents, as the drug is completely soluble in water, but the PMMA mat is hydrophobic. According to this, a mixture of water and ethanol was employed. However, if high alcohol content was used in the solution, the antibiotic could not be completely dissolved and fibers with drug agglomerates on their surface were obtained, as it is shown in Figure 12.

Drug/Polymer Blends. Most publications studying drug loaded fibrous matrices prefer direct electrospinning of drug/polymer blends. Much of the interest in using drug/polymer blends for electrospinning arise from the possibility to achieve sustained drug release as compared to more instant release typical of adsorptive drug

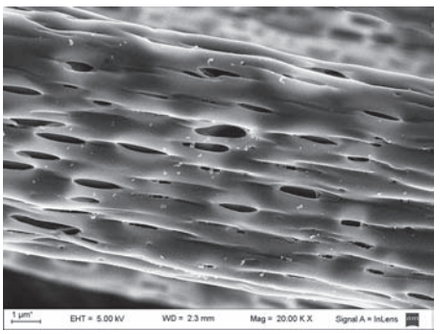

Figure 12. Agglomerated drug on the electrospun mat as a result of the solvent used.

loading resulting from a post-spinning modification. Natu et al.[219] determined the effect of drug solubility in polymer, drug state, drug loading and fiber composition on fiber morphology, drug distribution and release kinetics. They obtained bicomponent fibers of two semi-crystalline copolymers, poly(ε-caprolactone) and poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) by electrospinning loaded with different concentrations of acetazolamide and timolol maleate (below and above the drug solubility limit in polymer). They found that the release was more sustained when the drug, in amorphous form, was encapsulated inside the fibers. Moreover, timolol maleate was released faster than acetazolamide, indicating that drug solubility in polymer influences the drug elution. Besides, the authors show that fiber composition also controlled drug release. The high loadings fibers showed higher extent of burst and shorter periods of release (almost 90% of drug released after 2 days) than low drug content fibers (around 50% of drug released after 52 days), suggesting that loading and drug encapsulation in either crystalline or amorphous form are interrelated and control the release rate, especially in the burst stage. The fiber composition also controlled drug release, since release was slower from PCL fibers than from bicomponent fibers regardless of the drug type. Finally, the authors modeled the release data finding a three stage release mechanism implied: a dissolution stage (mainly produced by crystalline drug that was not properly encapsulated), a drug desorption coupled to diffusion stage, followed by polymer degradation control stage.

The drugs exposure to organic solvents, electric charge and mechanical stress experienced when electrospinning drug/polymer blends may affect therapeutic agent. Many authors successfully electrospun drug loaded polymer fibers where the drug functionality seemed to be completely unaffected.[220–222] Although, many proteins and growth factors experience denaturation when exposed to the electrospinning process, there exist different approaches to overcome this drawback. Nie et al.[223] electrospun a water/oil emulsion consisting of a bone morphogenetic protein 2 [BMP-2] solution as aqueous phase and an oil phase containing PLGA dissolved in dichloromethane. The authors analyzed BMP-2 by Fourier transform infrared spectroscopy once it was released from such fibers and found indications for denaturation. However, in the presence of hydroxyapatite (HAp) in the formulation and presumably resulting from a mutual interaction, BMP-2 was protected from denaturation. Different growth factor, including NGF, EGF, BMP-2, transforming growth factor β3 (TGF-β3) and insulin-like growth factor I, also retained bioactivity as a consequence of the interaction with silk fibroin.[224, 11]

Core–Shell Fibers. As it was explained in Section Processing Variables, two different polymer solutions may be electropun by utilizing a co-axial setup of an inner and an outer capillary tube. This allows the localization of the drug either in the core or in the shell of electrospun fibers in order to better control their release kinetics. Usually, a drug that is embedded in the core is released through aqueous pores or upon degradation of the shell, both against the concentration gradient. In the latter case, the shell structure can provide temporal protection of drugs in the core and defer drug action until shell degradation starts. A main advantage described in several studies using core–shell electrospun fibers is the successful prevention/reduction of initial burst phenomena due to the barrier formed by the shell. Tian et al.[225] successfully controlled release of vascular endothelial growth factor through the emulsion electrospun core–shell structured nanofibers of poly(l-lactic acid-co-ε-caprolactone). Sohrabi et al.[226] also achieved sustained drug release in poly(methyl methacrylate)–Nylon 6 core/shell nanofibers with ampicillin encapsulated.

In some cases the core material did not even need to be electrospinnable by itself. In fact, the necessary fiber forming function may be assumed by the shell polymer solution alone allowing the processing of low-viscosity core solutions loaded with, e.g., enzymes or gentamycin sulfate in aqueous formulations.[227]

CONCLUDING REMARKS

Electrospun nanofibers have been proposed as materials for the development of biomimetic scaffolds for the regeneration of bones, muscles or nervous tissues or as a coating for bony prosthesis. In particular, the possibility of developing scaffolds from the electrospinning of different biodegradable polymers or mixtures of them has been investigated, and even the advantages of fillers addition to promote osseointegration has been studied.

The development of tissue engineering methodologies depends on the improvement of the design and fabrication techniques of nanostructure scaffolds as well as a profound understanding of the material structure-related biological processes.

In the review the great interest that electrospinnig technique has generated among researchers in the biomedical area due to electrospun mat properties was shown: high

surface area, high porosity, ability to have interconnected pores, ability to control the Young modulus, possibility to control nanofiber morphology, diameter, and patterning. However, it is interesting to note that very few commercial products have yet been developed by this technique. In our personal view, this is mainly due to the following reasons: the scarce amount of material produced per unit of time compared to other methods which in turn increases the final cost, the small pore size in the mat that often prevents adequate cellular integration and vascularization of the new tissue, and the fact that the high surface area of the mat leads to an abrupt release of the drug during the first hours being very difficult to keep it stable for a long time. Therefore, in our opinion, the previous three mains aspects should be addressed in the near future in order to allow electrospinning technique as a simple and powerful tool for industrial developments in the biomedical material field.

ABBREVIATIONS

ECM, Natural extracellular matrix
PLLA, Poly(*L*-lactic acid)
PAN, Polyacrylonitrile
SA, Salicylic acid
PEG, Poly(ethylene glycol)
PLA, Poly(lactic acid)
PU, Polyurethane
PEO Poly(ethylene oxide)
DS, Diclofenac sodium
LHC, Lidocaine hydrochloride
PLGA, Poly[(lactic acid)-co-(glycolic acid)]
CA, Cellulose acetate
PVP, Poly(vinylpyrrolidone)
PMMA, Poly(methyl methacrylate)
PCL, Poly(ε-caprolactone)
PGA, Poly(glycolic acid)
P(LLA-CL), Poly(*L*-lactide-co–caprolactone)
PIECs, Pig iliac endothelial cells
Hap, Hydroxyapatite
PHBV, Poly(3-hydroxybutyrate-co-3-hydroxyvalerate
DMF, Dimethylformamide
CS, Chitosan
ESF, Eri silk fibroin
PVA, Poly(vinyl alcohol)
MC, Modified cellulose
SE, Synthetic elastin
SMC, Smooth muscle cells
MI, Myocardial infarction
MSCs, Cardiac cells and mesenchymal stem cells
PGS, Poly(glycerol sebacate)
TDM, Tendon-derived extracellular matrix
FN, Fibronectin
PBS, Phosphate-buffered saline
FN, Fibronectin
PNS, Peripheral nervous system
CNS, Central nervous system
TC, Tendon cells.

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