

Electrospinning of hydrogels for biomedical applications

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The field of biomedical applications for hydrogels requires the development of nanostructures with specific controlled diameter and mechanical properties. Nanofibers are ideally candidates for these advanced requirements, and one of the easiest techniques that can produce one-dimensional nanostructured materials in fibrous form is the electrospinning. This technique provides extremely thin fibres with controlled diameter, highly porous microstructure with interconnected pores; extremely versatile allowing the use of various polymers for tailoring various applications requirements and it is a simple cost-effective method on preparation of scaffolds. In this section, we will discuss recent and specific applications with a focus on their mechanisms. As such, we conclude this section with a discussion on perspectives and future possibilities on this field.

Keywords Electrospinning; Hydrogel; Tissue engineering; Drug delivery; Wound healing

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1. Introduction

Electrospinning is an easy technique for the production of polymer fibres in a nanoscale from a variety of materials in one-dimensional, two-dimensional and three-dimensional (Li and Xia 2004; Greiner and Wendorff 2007). This technique provides extremely thin fibres with controlled diameter, highly porous microstructure with interconnected pores; extremely versatile allowing the use of various polymers for tailoring various applications requirements. The ability to produce nanofibers composites of blend polymers, composites with metals and/or ceramics with high surface area than regular fibres, it provides various areas of applications such as nanocatalysis, tissue engineering, biomedical, pharmaceutical and environmental engineering (Bhardwaj and Kundu 2010). One of the limitations of electrospinning is their low production rate which several groups are trying now to overcome this limitation by producing multiple spinnerets arranged in an ordered position such as circle or lines (Zhou et al. 2009; Persano et al. 2013).

Fields of applications on electrospinning largely studied are drug delivery, tissue engineering and wound healing. One of the main reasons on these applications is that polymeric scaffolds produced by electrospinning have the advantage of mimic the natural extracellular matrix (ECM). However, nanofibers produced by electrospinning on tissue engineering have irregular distribution of cells and poor migration at the interior of the scaffold on normal behaviours (Bhardwaj and Kundu 2010). Furthermore, the increase in packing density of nanofibers produced by electrospinning limits cells to enter on the inner part of the fibres. Several techniques have been performed to modify the fibres parameters to overcome these issues (Li et al. 2014) which will be discussed on this chapter.

Although not yet deeply discussed, hydrogels nanofibers can overcome the limitations of polymeric nanofibers. Hydrogels are hydrophilic crosslinked polymers structured in a three dimension that can swell under aqueous conditions but not dissolve instantly. Hydrogels swell to a greater or lesser extent in water due to hydrophilic power of the group chains. On lower hydrophilicity, the polymer will swell in water, but with a further increase in hydrophilicity the polymer becomes water soluble (de Lima et al. 2015). Hydrogels nanofibers have the advantage of combine both techniques and it is being widely investigated in wound healing (Fogaça and Catalani 2013; Choi et al. 2015) since they can maintain a healing environment, adequate humidity on the region of the wound, and absorb the exudate with exchange of minimal pain. In addition, their inherent abilities help on diffusion of extended release of drugs, in particular on the case of hydrophobic drugs (McKenzie et al. 2015).

The focus on this chapter will be treated, along with the fundamentals on electrospinning, on the polymers employed for the application intended, modifications of parameters on fibres for tissue engineering. Finally, a focus on incorporation of drugs onto nanofibers and the diverse applications with novel techniques have been investigated with a perspective for future prospects.

2. Basic of electrospinning

Electrospinning process is a simple and very controllable technique which produces fine fibres using electrostatic forces. These fibres are either produced by polymer melt or solution with fibres diameters in the nanometre scale with a large surface area. A typical electrospinning apparatus usually involves syringe (attached with the emitter) with feed pump, a grounded collector and power supply with high voltage as described in figure 1

(Bhardwaj and Kundu 2010). The process of electrospinning involves on applying high voltage in the emitter and collector, charging the polymeric solution. When this polymer reaches the electric emitter, the droplet that is being held by its surface tension deforms once the electric field reaches an important value to a cone formation labelled as “Taylor cone”, the polymeric solution is then expelled in a jet shape from the emitter and accelerated towards the collector which is of opposite polarity. During the travelling towards the collector, the solvent is evaporated in the air and dried fibres forms at the collector. However, it is essential to adjust the electrospinning parameters due to instability of polymeric jet to obtain uniform nanofibers (Greiner and Wendorff 2007).

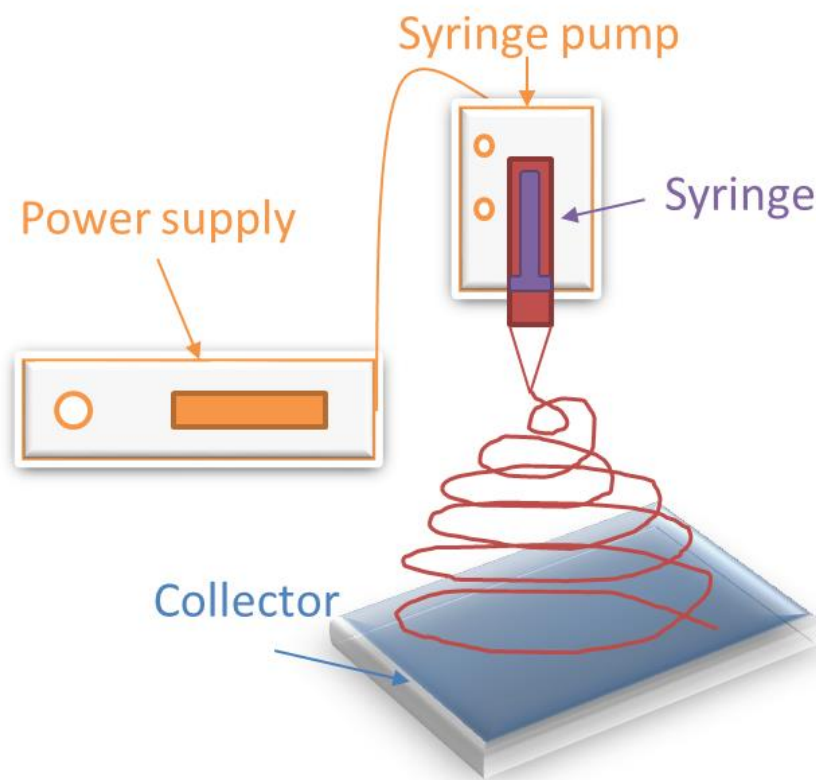


Figure 1. Schematic drawing of basic setup for electrospinning.

2.2 Parameters effect on electrospinning

The process parameters are important when aiming to obtain continuous non-beaded fibres and manipulating improves it when designing nanofibers, some of these processes can be governed by the solution such as surface tension, concentration, viscosity, molecular weight and conductivity. Alternatively, processing parameters of the electrospinning apparatus also plays important roles which are flow rate, voltage, type of collector and collector-emitter (tip) distance. Finally, the ambient when producing nanofibers affects the morphology and structure.

2.3 Parameters of the solution

2.3.1 Concentration

Concentration on electrospinning is crucial for fibre formation because it cannot form until a minimum required. According to Li and Wang (2013) there are four critical concentrations that affects the morphology of fibres: very low – nanoparticles are formed, there is electrospray rather than electrospinning due to limited viscosity and surface tensions (Deitzel et al. 2001). At slightly increasing on concentration, fibres and beads mixture occurs (Eda and Shivkumar 2007) and with a suitable concentration evenly nanofibers are obtained (Fong et al. 1999). Finally, in case of high concentrations, “helix-shaped” microribbons can be observed (Yang et al. 2004).

2.3.2 Molecular Weight (M.W.)

The polymeric solution chains entanglements are reflected on molecular weight which is named as solution viscosity and it affects the morphology of fibres. On electrospinning, is essential to aim for higher molecular weight since there are enough chains entanglements to stabilize the jet and form nanofibers (Bhardwaj and Kundu 2010). An equation was

obtained by (Gupta et al. 2005), for the exact transition from beads to fibres as it will be discussed in section 2.2.

2.3.3 Viscosity

As another crucial parameter, viscosity can also determine the fibre morphology (Larrondo and St John Manley 1981). The balance of viscosity is crucial since low viscosity excludes the formation of continuous fibres and just beads are normally obtained; whereas high viscosity difficult the jet formation of polymers on the emitter. For melt electrospinning, the variation of polymer viscosity varies depending on the spinning (Bhardwaj and Kundu 2010). The viscosity is also connected to the concentration and molecular weight parameters. Various works investigated the effect of viscosity onto electrospinning polymeric solutions (Geng et al. 2005; Kim et al. 2010; Binulal et al. 2014).

2.3.4 Surface Tension

Surface tensions values might be different for different solvents. Higher values of surface tension might inhibit the electrospinning generating unsteady droplets (Moses et al. 2001). A lower surface tension values on the other hand strongly depends of the other parameters, for example, electrospinning can be formed on low electric field with low values of surface tension (Rogina 2014). According to Bhardwaj et al., the surface tension determines the boundary of nanofibers condition on electrospinning if all other variables are held constant.

2.3.5 Conductivity/surface charge density

With a few exceptions, polymers are polyelectrolyte and this ability highly influences the jet formation. Usually, higher values of conductivity of the solution decreases the nanofiber diameter and lower conductivity results in non-uniform fibres with beads (Chuachamsai et al. 2008). Increasing on conductivity can be achieved by adding ionic salts on the polymeric

solution like NaCl or KH_2PO_4 (Li and Xia 2004) resulting in fibres with decreasing diameters and beadless fibres (Huang et al. 2006).

2.4 Processing parameters

2.4.1 Voltage

Voltage is one of the most important parameters since electric jets released from the emitter only occurs after a voltage threshold. Higher voltage on polymers for electrospinning influence fibre diameter but it depends on the concentration and the emitter to collector distance (Deitzel et al. 2001; Li and Wang 2013).

2.4.2 Flow Rate

Flow rate affects the velocity of the jet and the amount of polymer transferred. Lower flow rate is generally required since it leads to more time for polarization and evaporation of the polymeric solution (Yuan et al. 2004). Higher values of flow rate may induce the formation of bead fibres with thick diameter due to short drying period before reaching the collector (Zhang et al. 2005).

2.4.3 Type of collector

Collectors are conductive substrate that collects the charged fibres during the electrospinning process. The simplest collector is aluminium foil, but it is difficult to remove the fibres on this type of collector, so different arrangements have been performed such as, conductive paper, gridded bar, rotating rod and wheel. Such collectors can also contribute to the alignment of fibres which are useful in tissue engineering (Vaquette and Cooper-White 2011).

2.4.4 Distance between the collector and emitter (tip)

The distance between the emitter and collector also affects the fibre diameter and structure. Similar to the voltage parameter condition, minimal required distance is necessary so that fibres dry before it reaches the collector, dryness from the solvent, otherwise beaded fibres will be formed (Ki et al. 2005).

2.5 Ambient parameters

As explained before, the ambient affects the morphology of the nanofibers. Humidity seems to have a great impact in some polymers in which after a critical value it can dissolve in the cross points of the structure and fuse together forming a dispersal layer (Yao et al. 2013). The temperature on the other hand, shows that lower temperatures let to a slower evaporation of the solvent and fibres could not be formed. The average diameter of the nanofibers has a complex behaviour effect on higher temperatures (De Vrieze et al. 2009) since the formation of nanofibers on electrospinning depends on the solvent and rigidity of the polymeric chains.

2.6 Theoretical foundation for electrospinning polymers capable of physical gelation.

The process formation of fibres via electrospinning are affected by various parameters such as, flow rate (Uchko et al. 1999), voltage (Yuan et al. 2004; Zhang et al. 2005), polymer molecular weight (M.W.) (Casper and Stephens 2004; Tao and Shivkumar 2007) and concentration (Fong et al. 1999; Deitzel et al. 2001; Eda and Shivkumar 2007; Felice et al. 2015). To tailor these parameters to obtain a window for range of fibres formations is not straight forward, since these processes are inter-dependent on each other. For these reasons, an integrated approach must be used to identify the range of fibres formation. For

gel polymers, the nanofibers formation is dependent of the entanglements of the structure of the gel and the solution (Shenoy et al. 2005a). For this reason, a semi-empirical method, described by Shenoy et al. (2005)a can presume the shift over electro-spraying to -spinning based on the amount of chains entanglements - Physical link of polymeric chains which are similarly to chemical crosslinks (Shenoy et al. 2005b).

The equation (1) correlates the polymer M.W. to its entanglement solution M.W. Due to preparation method of polymers, normally the average M.W. is considered.

$$(n_e)_{soln} = \frac{M_w}{(M_e)_{soln}} = \frac{\phi M_w}{M_e} \quad (1)$$

For polymers solutions, its entanglement value is dependent of the solution concentration and the polymer volume fraction Φ , described in equation (2)

$$\phi_p = \frac{\rho_s W_p}{\rho_s W_p + (1 - W_p) \rho_p} \quad (2)$$

where ρ_p and ρ_s denote the density of polymer and solvent and W_p is polymer weight concentration of the solution.

With these equations It is possible to obtain a single value (n_e) which corresponds to viability of formation of fibres in electrospinning based on their polymeric chain entanglements (Shenoy et al. 2005a; Husain et al. 2016).

The number of chains entanglements, due to variations of polymer solution concentration (C) and molecular weight, causes morphology transitions on most electrospinnable polymers (Felice et al. 2015). This variation of chains entanglement leads to alteration of viscosity (Bock et al. 2012) and can be used as a determinant for solution regimes of

polymers (Gupta et al. 2005). The different solution regimes, particles to fibre, can be defined by the Berry equation (3) (Hager and Berry 1982) which correlates the polymer concentration with the intrinsic viscosity:

$$B_e = [\eta]C \quad (3)$$

Where B_e is the berry number, $([\eta] = \lim_{c \rightarrow 0} \frac{\eta_{sp}}{C})$ the intrinsic viscosity; η_{sp} is the specific viscosity and C is the polymer concentration.

The solution regimes are classified according to the critical overlap concentration (C^*) and the chain entanglement concentration (C_e) described as diluted, semi-diluted unentangled and semi-diluted entangled also represented in Fig 2 (Gupta et al. 2005). Chain overlap is absent in dilute system and thus, chain entanglement does not exist, limiting the system with significantly weak entanglements (Shenoy et al. 2005a). When the concentration of the solution is equal to the concentration inside a single macromolecular chain, the critical concentration overlap occurs, $A \rightarrow B$ process in fig 2.

As the concentration increases, the chain reaction overlap occurs but it is not enough to produce a degree of entanglement. Although some entanglements are observed, the chain entanglements within the drop formed on electrospray is not enough to stabilize the particle structure formation and these are not optimum as it leads to inferior and non-reproducible morphology since they lose their shape when impacting over the collector showing a beaded and non-continuous fibre formation (Husain et al. 2016). For entangled systems, $B_e > 4$ or C_e , is generally accepted as the formation of pure fibres and the effect is due to the increase in entanglements chains so the jet formation can be stabilize through jet

breakup inhibition because of the increased surface tension (Felice et al. 2015; Husain et al. 2016).

In practice, electrospun beaded fibres occur unexpectedly, but it was reported in literature (Taepaiboon et al. 2006; Husain et al. 2016). Formation of beads, as explained before, usually are dependent of the parameters of the polymeric solution which includes the polymer structure, concentration and salt content (Li and Wang 2013).

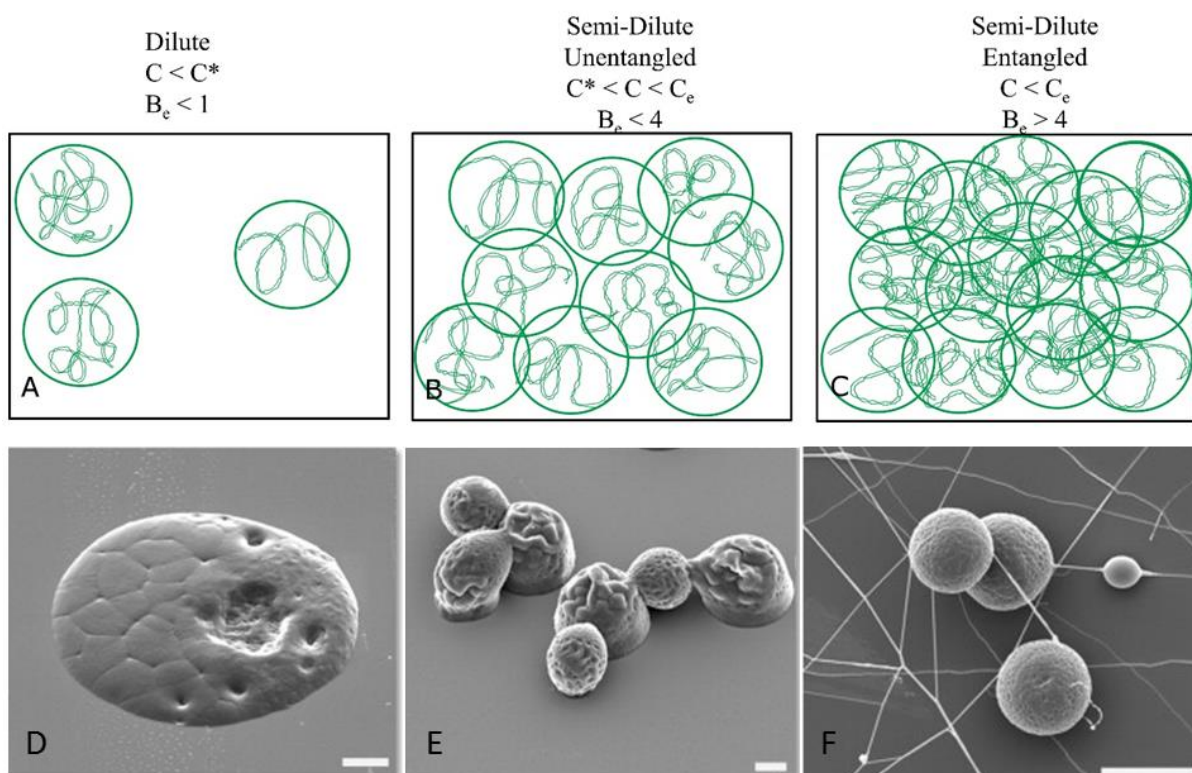


Figure 2. Physical representation of the three solutions regimes with corresponding SEM images. (A) and (D) dilute, (B) and (E) semi-dilute unentangled; (C) and (F) semi-dilute entangled. $C^* = 1 / [\eta]$. Adapted with permission from (Bock et al. 2012). Copyright (2016), Elsevier.

3. Applications.

3.1 Tissue engineering applications

Tissue engineering creates artificial materials for regeneration of tissues based on the approach from materials engineering and life science (Ma 2004; Lanza et al. 2011; Okamoto

and John 2013). Mostly, the repair or replacement of failing organs and tissues is facilitated by tissue engineering creating biological substitutes, like the growing of cells on scaffolds to support the regeneration of the desired tissue (Okamoto and John 2013). Cell interactions are extremely important for cell growth since it can change the cell functions via complex pathways (Kai et al. 2013; Temenoff and Mikos 2014).

The course after seeding of cells on scaffolds normally process with attachment followed by proliferation and differentiation. The cell ability to proliferate is one of the most important factors for tissue regeneration. With faster proliferation, the development of the injured tissue is improved and reduces scar tissue formation *in vivo* (Prakash et al. 2010; Kai et al. 2013).

Electrospinning for tissue engineering is one of the most used techniques since its fibrous structure is comparable to the tissue extracellular matrix (ECM) (Kim et al. 2005; Li et al. 2014; Guarino et al. 2015; Weng and Xie 2015). Ito et al. (2005) have shown that the attachment of fibroblast-like cell line (COS-7) was higher to nanofibers mats of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) than on regular scaffolds of the same material after a really short incubation time. The increased surface area of nanofibers helped the improvement on cell attachment due to its 3D features (Ito et al. 2005). Moreover, Chua et al. (2006) also demonstrated that nanofiber scaffolds containing amine groups had higher cell proliferation and adhesion, in comparison to films containing amine groups.

3.1.1 Choice of polymer-hydrogel for tissue engineering.

The behaviour of cells can be adjusted by focal adhesion and signalling complexes (Wozniak et al. 2004), such behaviours are intimately related to the properties of the scaffold (Kai et al. 2013). In addition, high surface area nanofibrous scaffolds are preferably used on tissue

engineering and different cells react different to the surface where it is attached (Leung and Ko 2011; Kai et al. 2015). Moreover, materials have to be biocompatible – the body must not reject the implant or scaffolds and must interact with it, so the spreading of cells is achieved faster (Yang et al. 2010; Van Vlierberghe et al. 2011; Rogina 2014). Furthermore, it must not elicit inflammatory response and cytotoxicity with a three-dimensional environment for cell proliferation and adhesion (Langer and Peppas 2003; Leung and Ko 2011; Parratt and Yao 2013). For these reasons, the choices of polymer and/or hydrogel designed for tissue engineering are important. Figure 3 illustrates the different polymers employed in electrospinning along with its properties. Currently, biodegradable scaffolds are the most investigated materials for production of nanofibers via electrospun in tissue engineering (Rogina 2014). Biodegradable nanofibers can have tailored degradation rate in the body so that it will be metabolized or excreted. The degradation mechanism normally consists of chemical degradation and the most important parameters are the polymer structure and the environment which can also influence the degradation rate such as the pH and chemical structure (Simionescu and Ivanov 2016).

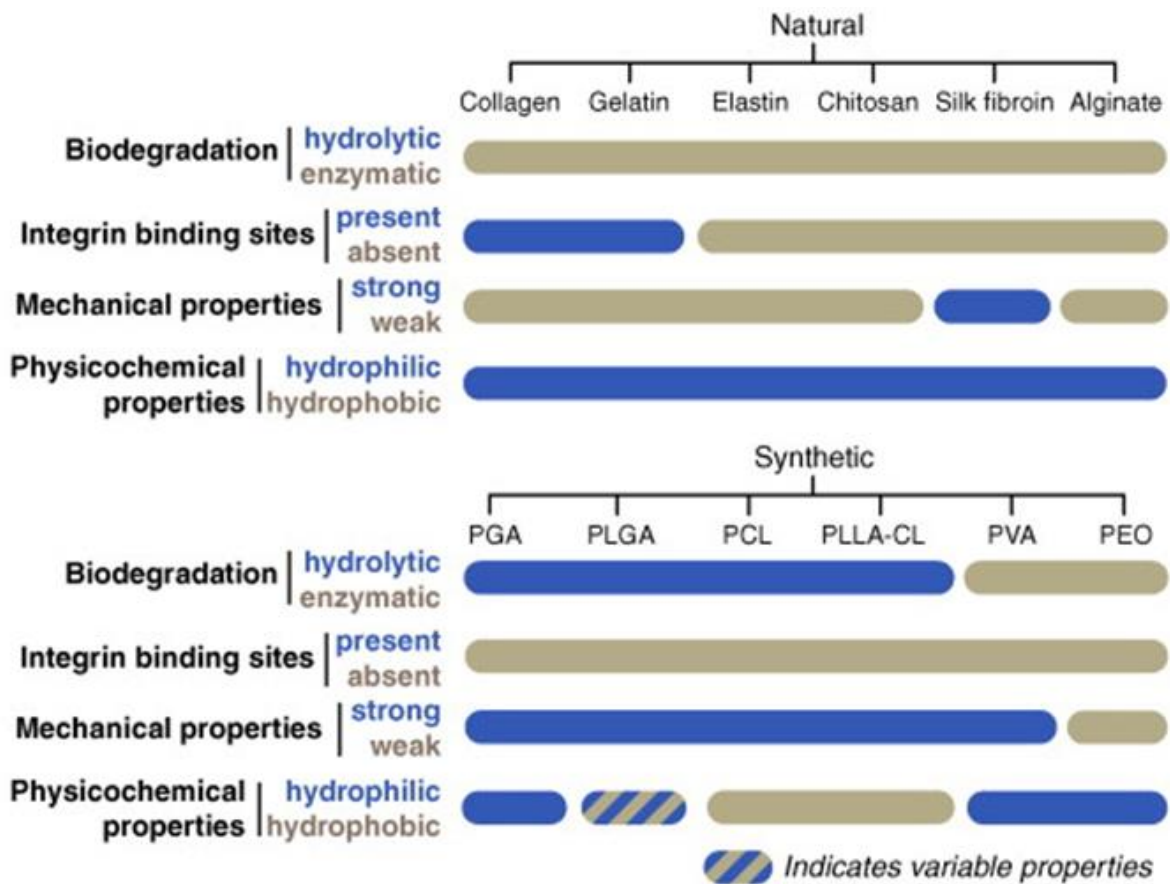


Figure 3. Most common used natural and synthetic polymers on electrospinning along with its biological, mechanical and physiochemical properties (reprinted with permission from Elsevier (Gunn and Zhang 2010). Copyright (2016), Elsevier.

3.1.2 Natural polymer-hydrogels nanofibers for tissue engineering

Natural based hydrogels/polymers are normally obtained from living plants and animals and are hydrophilic in nature; they can be extracted on polysaccharides and cellulose from plants (Simionescu and Ivanov 2016). Although most are obtained from vegetal sources, microorganisms can also synthesize biodegradable polymers. Examples are: fish – fish skin serves as a collagen; crustaceans – shells have large amounts of chitin; corals-algae - rich source of polysaccharides. These polymers are biocompatible, biodegradable and non-toxic. In addition, natural polymers have an organized structure and it helps on cell viability and tissue ingrowth (Wolf et al. 2015; Simionescu and Ivanov 2016).

The main advantages of natural polymers (collagen, chitosan, silk fibroin, gelatin, etc.) are their inherent cellular interaction and similar chemical versatility – greatly mimics the extracellular matrix structure (ECM) for bone cell support matrix; which encourages proteins adsorption and cellular adhesion, surface migration and proliferation (Lai et al. 2014). However, ECM of different tissue have unique characteristics (Li et al. 2014; Khorshidi et al. 2015).

Collagen nanofiber scaffold can be used as a matrix for osteogenic progenitor cells to adhere, proliferate and differentiate into osteoblasts (Chen and Lv 2015). Some studies performed with collagen nanofibers exhibited an adhesion of over 45% for mesenchymal stem cells population in a quickly response time of 30 min at room temperature (Chan et al. 2009).

In addition, recent studies suggest that stiffness play an important part in bone tissue engineering for nanofibers scaffolds. Collagen nanofibers when tested under MG63 osteoblast-like cells, which produces bone-ECM proteins such as osteocalcin, responsible for ECM deposition and mineralization; presents superior values of maturation of those cells and their cellular response and adhesion is increased based on the value of stiffness of the scaffold (Torres-Giner et al. 2009; Tsai et al. 2012).

Chitosan, which is biocompatible, biodegradable, presents low-toxicity and is widely investigated on electrospinning as nanofibers (Rogina 2014). However, pure electrospun chitosan fibres are difficult to obtain (Rogina 2014) and chemical modifications into derivatives that are soluble in common organic solvents are used (Neamark et al. 2006). The morphology of chitosan fibres is altered by the acetic acid concentration, whereas an increase in the solvent changes the chains entanglements and is easily to form uniform

fibres (Geng et al. 2005). The properties of chitosan nanofibers for bone regeneration have been studied (Shin et al. 2005) and cells MG63 proliferated on the nanofiber with collagen and osteocalcin induction after two weeks *in vitro*. In addition, chitosan can also maintain its integrity for as long of six weeks and enhance bone regeneration without inflammatory reaction due to its anti-fungal and anti-bacterial properties, which can be improved by UV irradiation (Shin et al. 2005). Charged reactions can occur on chitosan leading to a network between polymeric chains (Berger et al. 2004). This network acts as a hydrogel. However, one of the drawbacks is its poor mechanical strength and, therefore, it is normally used with other material or crosslinked with a copolymer.

Alginate, a naturally biodegradable polysaccharide material, is obtained from brown seaweed and is biocompatible, nontoxic and nonimmunogenic. However, is hard to obtain continuous and uniform nanofiber scaffold by electrospinning due to the rigid and extended chain conformation in aqueous solution - lack of chain entanglement (Chen and Lv 2015). Therefore, the addition of another co-polymer is usually performed on producing alginate nanofibers. The ability of alginates on absorb water and retains it is superior to of the natural gums (Berger et al. 1953).

However, nanofibers composed of natural polymers compares poorly in terms of their mechanical properties with low resistance to aqueous solutions that limits their use as tissue engineering. (Frenot and Chronakis 2003; Khadka and Haynie 2012). Many researchers reported a number of crosslinking procedures to stabilize the nanofibers synthesized using a wide range of natural polymers and their blends (Delmar and Bianco-Peled 2016; Jalaja et al. 2016). The effect of crosslinking on nanofibers create a network of 3D polymer chains which can possibly enhance fibres stability and other physical and

mechanical properties (Miraftab et al. 2015; Laha et al. 2016). Crosslinking on different natural polymers have been performed (Torres-Giner et al. 2009; Gualandi et al. 2016; Jalaja et al. 2016), such as gelatin in order to decrease its solubility for being able to use in long term (Zhang et al. 2006a). Laha et al. (2016), developed a gelatin with saturated vapor of glutaraldehyde as crosslink. The effect of crosslink induces the nanofibers to a more hydrogellic state (Fig 4).

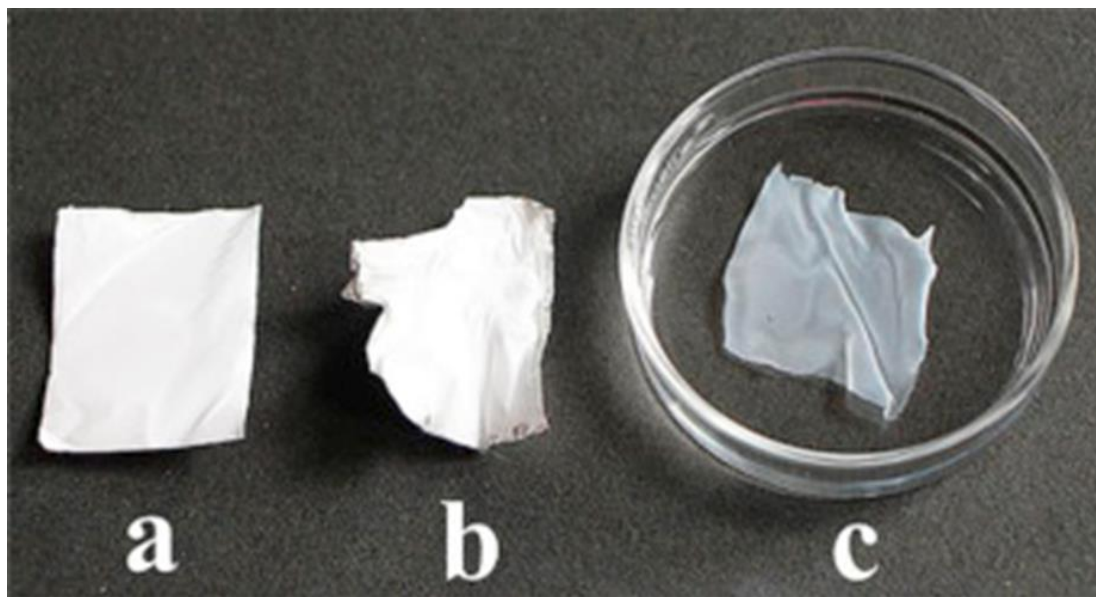


Figure 4. Digital images representing, (a) non crosslinked gelatin (b) gelatin after crosslink and (c) gelatin with crosslink immersed in water. Reprinted with permission from (Laha et al., 2016). Copyright (2016), Elsevier.

3.1.3 Synthetic hydrogels /polymer for tissue engineering

One of the drawbacks of using natural polymers is their inherent brittleness. In addition, to their restricted flexibility (Chen and Lv 2015). Additionally, synthetic polymers can be easily modified in contrast to natural polymers which have sensitivity to processing conditions such as: pH or UV radiations, water, etc (Simionescu and Ivanov 2016).

Synthetic polymers are, as the name suggests, synthesized in the lab. Between the synthetic nanofibers the most attractive ones used in tissue engineering includes Polyvinyl alcohol (PVA), poly (ethylene glycol) (PEG) and poly (ethylene oxide) (PEO), Polycaprolactone (PCL),

Poly (Lactic Acid) (PLA) and Poly-(N-vinyl-2-pyrrolidone) (PVP) (Wolf et al. 2015). With most of the biodegradable synthetic materials approved by FDA, the interest in using these materials has drawn great attention.

PVA polyvinyl alcohol is created as a result of free radical polymerisation of vinyl acetate with subsequent hydrolysis of acetate groups to hydroxyl moieties resulting in a wide molecular weight distribution (Hassan and Peppas 2000). PVA is biocompatible material and non-toxic with useful mechanical properties for tissue engineering and have the ability to swell to a large extent in solutions similar to those of human tissues. Nanofibers of PVA can be used as single scaffolds (Felice et al. 2015) or combined (Yang et al. 2008; Vashisth and Pruthi 2016) especially for drug delivery systems and tissue engineering (Felice et al. 2015). PVA can be easily dissolved in aqueous solutions and produced as nanofibers via electrospinning. Furthermore, crosslinking approaches have been performed to produce PVA hydrogels, such as chemical crosslinking, freeze/thawed, and UV-irradiation (Franco et al. 2012; Canillas et al. 2015). The crosslinking via methanol and chemical with glutaraldehyde seems to be most used method for PVA nanofibers hydrogel. However, glutaraldehyde and chemical crosslinks seems to have toxicity problems, which could impact and damage cells. Therefore, authors tried to elaborate different physical and chemical crosslinking methods (Torres-Giner et al. 2009).

PEG and PEG-containing block copolymers are used in many synthetic forms, due to its nondegradability by simple swelling and its limited metabolism in the body. In addition, PEG with molecular weight inferior of 50kDa are used in tissue engineering applications (Yamaoka et al. 1994) so that they can be totally degraded on the kidneys (<30kDa) or the liver (>30kDa) (Veronese and Pasut 2005).

PCL nanofibers, have been extensively used (Torres-Giner et al. 2009) due to their bioresorbable aliphatic polyester associated with its low cost and slow degradation. Mesenchymal stem cells (MSCs), which shows potential to treat large bone defects (Quarto et al. 2001) proliferates and grows well on PCL scaffold (Yoshimoto et al. 2003) showing bone-like appearance when implanted on rat models (Michael Shin et al. 2004). In addition, in comparison to normal PCL substrates, PCL nanofibers support higher MSCs adhesion and viability (Ruckh et al. 2010). In addition, PCL nanofiber demonstrates the deposition of HAp on simulated body fluid (SBF) (Araujo et al. 2008).

PVP Poly-(N-vinyl-2-pyrrolidone) have excellent biocompatibility with high ability to absorb water. PVP nanofibers are excellent candidates for fabrication of nanofibers with non-spinnable materials due to various inorganic ions that can be produced and its ability to disperse particles acting as covering agent, which makes it a polymer of choice for the fabrication of electrospun fibres with non-spinnable materials. However, PVP nanofibers is very soluble in water showing poor properties as hydrogel (Lubasova et al. 2015). Consequently, PVP hydrogels nanofibers with further crosslinking is required for improvements and acting as hydrogels. Recent methods have been performing through Fenton reaction and UV irradiation (Fogaça and Catalani 2013), also with Poly acrylic acid, since this reaction forms a strong hydrogen bond interaction (Lubasova et al. 2015).

3.1.4 Blend of natural and synthetic polymers nanofibers and integration of nanofibers with hydrogels for tissue engineering

The poly-blend of natural biodegradable polymers (chitosan, silk fibroin) with synthetic polymers offers a major advancement in tissue engineering by the simple and economical approach of the favourable biological properties on natural polymers and the excellent

mechanical properties of the synthetic polymers that favour cell growth and proliferation (Gunn and Zhang 2010; Abdal-Hay et al. 2016). Various poly-blend polymers have been researched in the past years (Cosme et al. 2016; Hu et al. 2016a; Mahoney et al. 2016; Zhijiang et al. 2016; Ziaee et al. 2016).

A common polyblend scaffold is made from PCL/gelatin mixture and demonstrates increase hydrophilicity, promotes bone osteogenesis and mineralization of MSCs in vitro (Alvarez Perez et al. 2012). In addition, with the support of PCL the scaffold provided improved mechanical and biochemical properties to guide bone regeneration. Currently, various types of polyblends have been researched for the application intended, such as polyester urethane urea (PEUU) with Gelatin for application in myocardial tissue engineering. Since cardiac tissue challenges are the development of scaffold with suitable Young's modulus, the polyblend PEUU/gelatin provided relatively desirable values for the application intended. In addition, cardiomyocytes were able to proliferate and grow in this nanofiber scaffold (Jamadi et al. 2016).

The combination of nanofibers and pure hydrogels may offer the advantages of incorporating both structures and minimize their problems. Although this concept is in early stages some researchers developed different designs for tissue engineering applications, for this matter see (Bosworth et al. 2013) and (Xu et al. 2015).

Laminated composites. The easiest way to incorporate both structures is to fabricate individually and mix them by the layer-by-layer method, this method is adjustable by the layers, class of fibers and hydrogels which can be tuned to the morphology and mechanical properties required. Another technique is to

crosslink the hydrogels directly onto nanofiber films, dropping the solution onto the nanofiber (Quinn et al. 2007; Manna and Patil 2009; Shi et al. 2015). In this application the voids that separate each nanofiber are filled with hydrogels. Yang et al. (2011), developed a layer by layer of oriented nanofibers of PLA with collagen type 1 hydrogels. These were separated by filter-paper with cells deposited onto the fibers and incubated before adding another layer. The authors investigated the different fiber directions on the arrangement of the layers. The results found that there was a difference in direction of cells which were dependent of the orientation of the deposited fiber. This suggested further studies were possible into developing complicated structures to simulate ECM found in some tissues, such as skin and cartilages.

Encapsulating fibres in hydrogel Controlled placement of fibers with an ordered structure can be achieved by encapsulating the fibers in hydrogel. McMahon et al, (2011) achieved a nanotubular scaffold with nanofibers of PEUU (poly(ester urethane) urea and PEG-fibrin hydrogel (Mcmahon et al. 2011) for the application of coronary artery bypass grafts. Basically, rectangular segments of aligned electrospun fibers was revolved around latex mandrels. Smooth Muscular progenitor cells were embedded onto the fibers and incubated for two

days. After incubation, the fibers were revolved again through a latex tube with the cells facing outwards. The tube was added in a hollow Teflon cylinder and a thrombin/fibrinogen-cell was added to encapsulate the fibrous layers before polymerization and support enough mechanical strength so that after the removal of the latex, the composite hydrogel would be intact. After incubation, the latex and Teflon tubes were removed and the final composite was immersed in diacrylate-derivatized polyethylene glycol (PEG) solution for the final construct of PEG on fibrin gel. Finally, polymerization was achieved by UV. This structure was hypothesized to mimic the coronary artery vessels, showing a biphasic layer of hydrogel fibers and collagen. Suture strength results showed that this construct has similar values to those of human artery. In addition, smooth muscle cells were able to proliferate and migrate on this hydrogel-nanofiber construct.

Injectable Composites

A recently technique has been developed by Brown et al. (2011) named as melt electrospinning writing which can produce complex porous fibres structure using an automated stage, this technique can create scaffolds by stacking melt electrospun fibres on top of each other which is similar to melt-extrusion based on direct writing but in a sub-micrometre magnitude. (Visser et al. 2015) use the innovation

of this technique to produce scaffolds for cartilage tissue and via an injection mould prepared a reinforced hydrogel scaffold nanofiber by adding the polymer GelMA with an etched PCL nanofiber scaffold produced by melt electrospinning. These constructs have increased stiffness compared with hydrogels or nanofibers scaffolds alone approaching that of articular cartilage tissue. The scaffolds were also embedded with human chondrocytes and show viable responses, retaining their morphology and can respond to biological regime in terms of matrix production and gene expression, making it a feasible material to culture cells in various environment mechanically diverse.

3.2 Controlling of fibres parameters for tissue engineering

With recent progress of cells interactions and tissue microenvironment, important investigations on the structural nanofibers scaffolds on different cells environment have been performed (Liu et al. 2012; Li et al. 2014). For the application intended, a control of the fibres parameters is important. Between these parameters four majors, as pointed by a great recent article Xu et al. (2013), have been deeply investigated: fibres diameter, packing, orientation and 3D shape.

Researchers have been trying to control the nanofiber diameter (Du et al. 2008) since it can regulate cell behaviours influence like adhesion, proliferation, migration, differentiation and protein adsorption (Christopherson et al. 2009). Currently, controllable diameter size of the electrospun fibres produced have a range of 150 nm – 5 um (Ishii et al. 2008; Nasouri et al.

2012). These ranges of fibre are in much smaller size than those produced by conventional electrospun methods and some cells which help facilitate contact guidance of cells (Wang et al. 2010a). Normally, the size controls are dependent on the polymer solution parameters (Zhang et al. 2005) and processing conditions (Deitzel et al. 2001).

The conventional electrospun method forms 3D tightly packed structures and studies indicate that cells can only grow and migrate on the superficial surface, resulting in a 2D membrane rather than a 3D structure. Additionally, these scaffolds restrict cell infiltration and limit nutrient exchange. This effect results in loss of cells and the unsuccessful or partial regeneration of tissues (Li et al. 2014). To overcome this issue, the control of fibre packing is important when designing the structure for tissue engineering culture. One strategy is increase the pore size and porous structure of these scaffolds (Baker et al. 2008; Kim et al. 2008). Various techniques to increase the porosity and pore sizes have been developed such as increasing the diameter of nanofibers (Rnjak-Kovacina and Weiss 2011), evaporating constituents of a mixed polymer after electrospun process (Wu et al. 2014). These allow a low packing density of nanofibers which helps the cells to infiltrate the scaffold.

Researchers are trying to obtain oriented fibres since the extracellular matrix in tissues of human body have anisotropic architectures, so the scaffold needs to display the same anisotropic behaviour for the tissue application intended (Li et al. 2014). In addition, cell migration and extension are improved when nanofibers are oriented to a single axis direction, such as neural cells, in which, oriented nanofibers help direct the growth of axons in healing process on neural tissue engineering. However, random orientation is typical in electrospun fibres which are attributed to the bending instability related to the spinning jet. To overcome this, fibre orientation can be produced on electrospun fibres by using two

grounded rods (Li et al. 2003) or a rotating drum (Kim and Reneker 1999) or. A rotating drum at high speed can be used to obtain aligned fibres but their orientation is not perfect and disk collectors have also been used (Xu et al. 2004). Usually, on this configuration the electric field is higher at the disk edge and the fibres are well aligned along the edge of the collector. Another technique is the use of two conductive strips separated by a void gap and due to this void gap, that acts as an insulated region, results in electrostatic interactions on the nanofibers and they are stretched to form a parallel array across the gap.

Since then, different designs have been developed to improve this system such as conductive coil for the collector (Lee et al. 2016) and addition of finite-length hollow cylindrical electrode along the jet trajectory, to suppress the coil formation. Parallel-plate electrodes in between the collector and a cylindrical electrode to orientate the nanofiber scaffold at the collector have also been used (Karatay et al. 2014).

Furthermore, research is ongoing to produce fibres with similar morphologies and characteristics of the native tissue ECM. As an example for muscle layer of blood vessel, generating a nanotubular scaffold that simulates the work of a smooth muscle layer – elasticity, mechanical strength, and high surface area – is a high achievement and it is possible to be obtained by electrospinning. Tubular electrospinning structures gives aligned fibrous scaffold with a high surface area that can induces the proliferation and adhesion of loads of cells for a faster and complete healing of tissues (Wang et al. 2014). In addition, recent studies have shown that patterned nanofibers in 3D tubular scaffolds can be produced easily, using designed collector templates. The collector template can be designed based on varying the nanopatterns with different shapes, producing various nanofibers

structures that can be tailored by the collector template (Daming and Jiang 2008). These specific controlling parameters tend to be important for specific tissues engineering.

3.2.1 Controlling of fibres for Neural tissue engineering

Neural cells are affected by the 3D shape of electrospun membranes (Christopherson et al. 2009; He et al. 2010; Wang et al. 2010a) and its fibre diameter affects adhesion, proliferation, migration of rat hippocampus-derived adult neural stem cells (rNSC) (Christopherson et al. 2009; He et al. 2010; Li et al. 2014). Studies performed by (Meng et al. 2015), tried to simulate the ectoplasm of nerve (polyanionic nanofibrous cortical layer) by producing a tubular-shape PAA nanofiber hydrogels. The results from Meng et al., concludes that PAA fibrillar structure might be a good candidate as a macromolecular who mimic of an axon concluding that aligned fibres might help improve the mimic of the cortical layer.

The effect of alignment was reported by several authors (He et al. 2010; Wang et al. 2010a; Hu et al. 2016b), aligned fibres appears to enhance Schwann cell maturation more than randomly oriented fibres (Wang et al. 2010a).

Finally, hydrogels seem to affect the neural tissue. In the great work of Hodde et al. (2015) layers of oriented PCL nanofibers were embedded with fibrin hydrogel. Fibrin was selected because it could act as targeting tissue healing, depositing fibrin at the site of the injury and acting as extracellular matrix for migration and proliferation of perineural fibroblasts, Schwann cells, and regenerating axons during nerve healing (Weis et al. 1994). The hydrogel-fibrin nanofiber structure was similar to the ECM of peripheral nerves. In addition, the fibre hydrogel on 2D structures retained the deficient simple flattened, unipolar morphologies of Schwann cells, whether the 3D construct had a complex, highly branched morphology similar to neuron-like morphology and the maximum outgrowth was observed

after one day of incubation. In addition, Schwann cells were oriented to the direction of PCL fibres (Hodde et al. 2015).

3.2.2 Controlling of fibres for Vascular tissue engineering

Alignment of fibres seems to improve the proliferation of vascular cells (Del Gaudio et al. 2009; Ma et al. 2012; Li et al. 2014; Shalumon et al. 2015; Ercolani et al. 2015). Shalumon et al. (2015), studied PLLA/gelatin electrospun aligned fibers in smooth muscle cells (SMCs) cells and its results indicates that aligned fibres supports SMCs cells and improve the proliferation (Shalumon et al. 2015).

3D shape of electrospun fibres also seems to strongly affect vascular tissue cells (Ma et al. 2012; Merkle et al. 2015) with a preferable of tubular shapes for the reason that it mimics the natural blood vessels (Stitzel et al. 2006).

Electrospun fibres produced in a core-shell design promotes NIH 3T3 fibroblasts (FBs) and rat smooth muscle cells (rSMC) viability (Merkle et al. 2014; Merkle et al. 2015). In addition, the packing of fibres have an effect, according to Zilla et al. (2007), tubular scaffolds must possess a porous structure with pore diameter of 10 μm in average and 20-80 μm^2 minimum area to allow penetration of cells so to obtain a fast regeneration of vascular tissues (Zilla et al. 2007). Vascular tissue grafts also needs to possess proper mechanical properties and a confluent endothelialized lumen to resist thrombosis (Lin 2011). Hydrogel-based systems such as hydrogel nanofibers scaffolds are generally more biocompatible in the peritoneum than hydrophobic polymeric devices (Yeo et al. 2007). Stefani et al., produced a novel hydrogel nanofiber with PCL with copolymer of acrylated poly((l-lactide-co-trimethylene carbonate), aPLA-co-TMC, the mixing of the two incompatible polymers as melts with UV-crosslink produced core-shell tubular fibers and the scaffolds supported the migration,

alignment and proliferation of human mesenchymal stem cells (hMSCs). In addition, cells followed the fibres alignment; random fibres gives raise to diverse migration and adhesion, more spread and disorganized cell orientation, while aligned fibres promoted cell elongation with high organized and oriented pattern (Stefani and Cooper-White 2016).

3.2.3 Controlling of fibres for bone tissue engineering

Diameter of fibres influence bone forming cell behaviour, Badami et al., investigated MC3T3-E1 cells and found that the range of fibres diameters is usually between 0.14–2.1 μm for proliferation of cells and they are likely to reproduce on larger diameter fibres; the optimal correct diameter for specific cells can help the regeneration of bone tissues (Badami et al. 2006). The fibre packing also affects bone tissue, enhanced porosities significantly improve the cell penetration and distribution (Baker et al. 2008; Vaquette and Cooper-White 2011). Since the bone ECM is oriented in the same direction of the collagen nanofibers in basic structure of bone; alignment of fibres takes an important place in bone tissue (Cai et al. 2012). The orientation and pattern of the fibrous scaffold have to resemble the fibrous structure of the natural extracellular matrix (ECM) because it can improve osteoblast cell migration, proliferation and simulate the ECM structure (Wang et al. 2009a; Wang et al. 2010b).

Hydrogel nanofibers with PVA/gelatin followed by crosslinking based on methanol, for improvement on the mechanical structure, show a well-spaced 3D structure with growing and proliferation of MG-63 (human osteosarcoma cells) (Linh and Lee 2012). Recently, Pangon et al. (2016), developed a chitosan/chitin whisker with Hap hydrogel nanofiber for the objective of promote bone cell response, the results indicate that after the simulated body fluid (SBF) the samples were randomly mineralized with Ca-deficient Hap. In addition,

the hydrogel presented nontoxicity to osteoblast cells with proliferation and viability enhanced by increasing the chitin whisker (Pangon et al. 2016).

3.2.4 Controlling of fibres for ligament and tendon

Alignment of electrospun nanofibers structure is important on ligament and tendon because it mimics the dimensionality of collagen fibrils that comprise native tendons and ligaments (Li et al. 2007; Choi et al. 2008). In this case, it is extremely important that the orientation of the nanofibres with a focus on the mechanical properties, since it has to mimic the native tissue structure. Deepthi et al., aligned PCL nanofibers and coated with a hydrogel chitosan-hyaluronic acid in a crosslink of N,N-(3-dimethylaminopropyl)-N-ethyl carbodiimide (EDC) and lyophilized by stacking multiple nanofibers mats. The cell results with rabbit ligament fibroblasts exhibited better migration, attachment and proliferation along the direction of alignment of fibres with elongation in comparison to PCL random ones where cells just spread onto the fibres (Deepthi et al. 2015). However, the results from Deepthi et al. (2015), showed that PCL fibers with random orientation infiltrated more onto the scaffolds owing to the increased porosity. However, ligament tissues require strong scaffolds and the random PCL fibres exhibits poor mechanical properties. In addition, cell arrangement along the direction of the acting force is a prerequisite for ligament tissue engineering and the mechanical strengths of most of the scaffolds presented in literature are yet inferior compared to the ligament tissue (ACL) (Liu et al. 2012; Deepthi et al. 2015).

3.2.5 Controlling of fibers for Skin tissue engineering

Skin tissue consists of multi layers, they are named as the epidermis, dermis, and hypodermis. These diverse tissue layers comprised diverse cells and diverse functions, so complex structured scaffolds are needed for this tissue engineering. The effect of

electrospun nanofibers have been extensively studied (Cui et al. 2008; Huang and Fu 2010). Studies have been showed that fibroblasts proliferated best at 350-1100 nm. Few results have been reported on the parameters for fiber orientation and fiber packing on skin regeneration (Li et al. 2014). Hydrogels nanofibers containing gellan/PVA and its effects on human dermal fibroblast (3T3L1) cells were investigated showing positive results (Vashisth and Pruthi 2016). Various similar results have been reported (Cui et al. 2008; Loh et al. 2010).

3.2.6 Controlling of fibers for cartilage tissue engineering

Cartilage wounds can advance to osteoarthritis, and are actual challenges for regenerative medicine (McCullen et al. 2012; Steele et al. 2014). One of the unique features of cartilage tissues is that they have anisotropic mechanical properties due to differences in density and structure arrangements (Becerra et al. 2010). This leads to three zones with collagen fibers varying their orientation, developing from aligned in the surface regions, to random and orientating perpendicular to deep zones (Steele et al. 2014). These profiles for cartilage zones contributes in terms of mechanical properties (Klein et al. 2009; Becerra et al. 2010).

Authors tried to mimic this cartilage unique zone behavior. Steele et al., developed a trilayered electrospun fibers (Fig. 5) with scaffolds nanofibers regions by using the same polymer with varying the alignment, packing and diameter of fibers. The combination of the two distinct zones is designed to yield an anisotropic scaffold with a smooth articulating surface and a more porous region for ECM deposition. The morphological changes were able to provide desired functionality, in terms of mechanical stability, while impacting both chondrocyte gene expression and ECM accumulation. Hydrogels with PLA and Cytosan have also been successfully reported for the regeneration of cartilage tissue (Mallick et al. 2016).

The water adsorption of hydrogel nanofiber is a significant parameter during the chondrocyte culture and cartilage regeneration (Mallick et al. 2016). Chitosan has a similar structure than of glycosaminoglycan (GAG) - a natural biopolymer found in tissues and ECM. Results from Mallick et al. (2016), using a nanofibers scaffolds for cartilage engineering shows that rabbit chondrocytes can attached well and proliferated throughout the nanofibers. In addition, the glycosaminoglycan values resulted from chondrocyte culture from a specific design scaffold exhibited a desired enhancement in the GAG release, which the authors suggest it might be due to suitable modulation and maturation of the chondrocytes (Mallick et al. 2016).

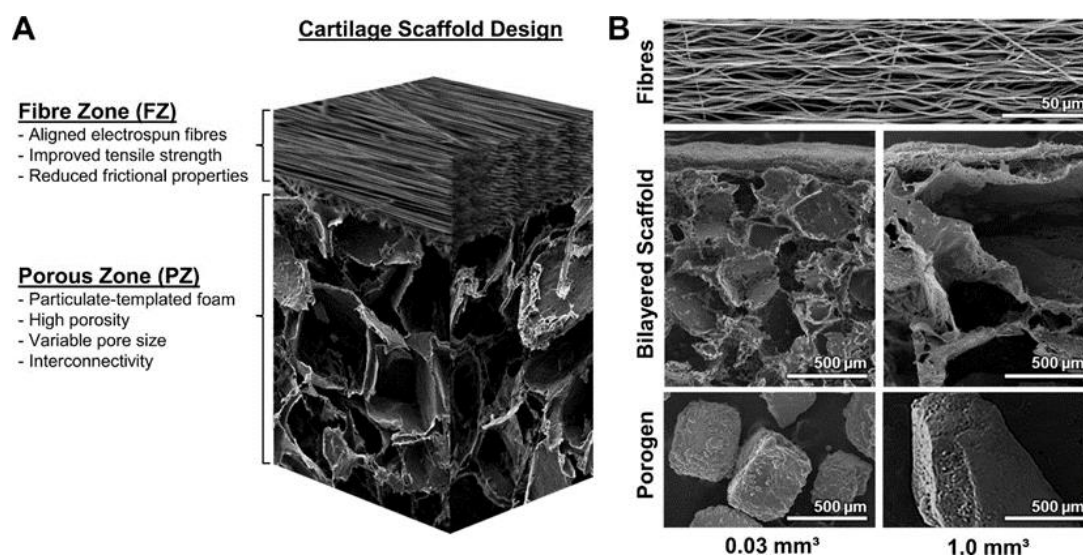


Figure 5. Bilayered cartilage scaffold schematic. (A) A diagram illustrating the electrospun fibre zone (FZ) and a porous zone (PZ). (B) SEM images of (top) the aligned fibre zone that is shared between both scaffold varieties, (middle) the complete bilayered scaffolds with 0.03 mm³ (left) and 1.0 mm³ (right) pores and (bottom) the sodium chloride porogens used to produce higher porous zones. Reprinted from (Steele et al. 2014).

3.3 Dressings for wound healing

Wounds, if exposed and untreated, are susceptible to water loss, toxins and bacterial infections, and could promote unnecessary and continued inflammatory response that

delimits the regeneration process. In this way, wound dressings materials are used for regeneration and repair of the dermal tissues, protecting it mainly against microorganism (Mogoşanu and Grumezescu 2014).

Ideal dressings must contain certain characteristics, among them are the efficiency as absorption ability of wound fluids and pus, bacterial barrier, functional adhesion - which can adhere to healthy tissue but not adhere to wound tissue, easy to remove and low cost (Thomas 1990; Bhardwaj and Kundu 2010).

Hydrogels nanofibers for wound dressing applications meet most of the requirements as dressing material due to their microfibrinous and/or nanofibrinous structure related to the electrospinning, producing a moist environment and helping the regeneration of skin with no scar (Alvarez et al. 1983). In addition, hydrogels have great ventilation ability and it can be suitable and efficient on absorption of contaminated exudates (Dumville et al. 2013). Furthermore, electrospun nanofibers can prevent and control microbial biofilms, cleanse the injured tissue and eliminate/minimalize pain (Lee et al. 2003; Mogoşanu and Grumezescu 2014).

Xu 2016, electrospun a mixture of Chitosan/PLA and PEG and the formed nanofibers were evaporated to remove the solvent at 60°C. The crosslink of nanofibers was done with Glutaraldehyde vapour at room temperature followed by 0.1M glycine aqueous solution to block unreacted aldehyde groups. These fibers presented fast kinetics and equilibrium water absorptions. These fibers can captivate excess exudates and create a wet wound healing environment for the wound. The swollen fibers reduced the size of the pores and permeability of air but the crosslinked reaction allow flux of oxygen and carbon dioxide

allowing a functional adhesion necessary in this situation. Finally, Xu et al demonstrated that these hydrogel nanofibers mats showed good antibacterial activities (Xu et al. 2016).

3.3.1 Choice of polymer for wound healing

Natural polymers are widely used for wounds and burns dressing due to their biocompatible properties and similarity to ECM. Natural polymers can stimulate the healing process and repair the damaged tissues and skin regeneration (Huang and Fu 2010). Between the natural polymers, the most common used are cellulose – used in chronic wound dressing (Hunt et al. 1984; Montesano and Orci 1988), chitin and chitosan – anti-inflammatory and wound healing properties (Anitha et al. 2014; Mogoşanu and Grumezescu 2014), alginates – hemostatic properties in exudation/bleeding wounds and burns (Wang et al. 2002; Paul and Sharma 2004). Numerous hydrophilic polymers have the ability of sharing the hydrogel properties and ligands for natural polymers, such as Poly ethylene glycol (PEG). Due to the 3D cross-linked networks, polymeric hydrogels are extensively used in pharmaceutical and biomedical area, tissue engineering, drug delivery (Peppas and Sahlin 1996; Peppas 2000; Samchenko et al. 2011). Therefore, hydrogels cross-linked natural polymers can be used for wounds and burns dressings (Mogoşanu and Grumezescu 2014; Wolf et al. 2015). Synthetic polymers on the other hand, are usually selected as carriers for drugs (Peppas 2000; Chaterji et al. 2007; Nguyen and Alsberg 2014; De Lima et al. 2015) and can be designated for the choice as hydrogel blend.

3.4 Drug delivery applications

3.4.1 Methods of drug loading unto electrospun fibres.

Several methods can be used in order to incorporate antibacterial drugs into nanofibers.

1. One of the most common methods is to mix the drug with the polymer solution directly following the electrospun fibres. This method does not require any additional steps and is one of the simplest systems to obtain effective nanofibers as long as the drug and the polymer lipophilic or hydrophilic interactions are considered (Zeng et al. 2005)
2. Mix the drug with the polymer solution, as per method 1, and add another polymer electrospun layer to encapsulate the drug to act as a shell. This method is particularly useful for sensitive drugs. In addition, shell layers improve the sustainable release of the drug and overcomes the initial rush initial release typical on drug delivery systems of nanofibers produced with electrospinning. This method is effective for the delivery of active pharmaceutical ingredients and permits a biphasic drug release (Miao and Liu 2015). The effective prolonged release of core shell structure is demonstrated by Zhang et al. (2006b), core-shell nanofibers BSA loaded onto PEG slows the release of BSA for one month. In addition, this technique shows superior link with fibres allowing uniform fibre structure and the protection of the drug in the early activity with the biological ambient (Miao and Liu 2015)
3. Nanoparticles of drug are loaded onto the polymer and finally electrospinned forming the fibre matrix. Due to the specific properties that are obtained by some materials at nanoscale, such as silver nanoparticles (Zhao et al. 2012) and bioactive europium-doped hydroxyapatite (Hap:Eu³⁺) (Hou et al. 2009)], it is necessary to process these materials to obtain nanoparticles before processing the electrospinning.
4. Adsorption of the drug by immersing the fibre mats into a required amount of drug. This method can be achieved by using nanofibers mats that have a high surface,

resulting in greater drug loading amount. This method allows immediate release of drugs from the surface of the scaffold and allow facile dosage control (Yoo et al. 2009). Chen et al. (2007), developed a PLA nanofiber and immersed in TiO₂ nanoparticles. Following, the authors immersed this nanofiber mats in daunorubicin drug, for treatment in cancer. The AFM results shows that after immersed in daunorubicin, the TiO₂ nanoparticles and drug packed together and form spherical particles on PLA nanofibers. This surface incorporated with anti-cancer drug can adhere to the surface of the targeted cancer cells and approach the damaged cell surface slightly improving the metabolic system.

5. Immobilize the drug by surface activation the polymer mats and following immersion in a required amount of drug (Method 4) (Yoo et al. 2009). Immobilization of bioactive molecules on the exterior of electrospinning nanofibers are also performed to produce reactive functional groups. Owing to benefits of polymers process, greater selection of molecules with distinct biological features can be immobilized onto the nanofibers mat without compromising the bulk properties. Chemical immobilization reduces release rate of the biomolecule but also allows a precise control via introduction of responsive materials to local external cues (Goonoo et al. 2014). The immobilization can be performed either by physical and chemical methods (Yoo et al. 2009); however, it is usually done with chemical method since they are covalently attached to nanofibers and they are not easily removed from this changed nanofiber mat when incubated over long periods of time. Zomer et al. (2012) tested chitosan nanofibers mats, surface activated and coated with heparin-containing polyelectrolyte complex nanoparticles (PCN) which presents basic

fibroblast growth factor (FGF-2). The PCN help protect FGF-S for over 30 days and it could be modulated.

6. Drug nanoparticles and the polymer solutions are electrospun by the side by side method to form a biphasic layer of fibre mats. In this method, two liquids (polymer and drug solutions) comprises two parallel metal capillaries. These form an interesting morphology behaviour (Liu et al. 2007). Such fibres are also called Janus fibres (Yu et al. 2016). However, with different nozzles, the fibre is formed with the junction of the drug and the polymer. In addition, the drug is well trapped between the vicinity of each fibre connection.

Yu et al. (2016) tested a new design of using a Teflon barrier between the nozzles in the side-by-side method and it was possible to observe for the same fibre two different morphologies with a poorly soluble drug amorphously distributed, with biphasic controlled release and achieving an initial burst and a slower sustained release phase.

All these methods are different approaches that can be used to incorporate various active pharmaceutical ingredients (API) in electrospun fibres that can control the release profile through changes in the fibres morphology, porosity and composition.

3.4.2 Choice of polymer/hydrogels for nanofibers in drug delivery systems.

Target-drug delivery nanofibers can be produced by using matrices with either biodegradable or nondegradable polymers. The drug release mechanism may vary depending of the type of polymer, diffusion for nondegradable and matrix erosion for biodegradable (Pillay et al. 2013). The attainable delivery of the drug can be achieved depending on the polymer used. However, the parameters polymer type, solvent and drug

compatibility are important process variables when designing stable nanofibers (Zeng et al. 2005; Pillay et al. 2013). However, recent studies have shown that it is possible to design hydrophobic drug in hydrogels electrospun carriers (Laha et al. 2016) without losing the properties of the polymer matrix.

The release of drug in nanofibers produced by electrospinning is controlled by diffusion of drug and/or degradation of the polymer matrix (Cui et al. 2006; Loh et al. 2010; Laha et al. 2016). However, Laha et al. (2016) tested the effect of crosslink on hydrophobic drug loaded gelatin nanofibers. Laha et al. (2016) found that nanofibers in the polymer matrix, without crosslink, usually led to deficient exchanges with the poorly water soluble drug molecules and as such, the hydrophilic nanofibers mat results in rapid release of drug within few hours. When crosslink was achieved on the nanofibers mats, their swelling nature and osmotic behaviour provided the principal mechanism for delivery of the hydrophobic drug in the medium and consequently, even at longer periods of time, there was sustained release of drug as it diffused to the release medium through the carrier gradually (Laha et al. 2016).

3.4.3 Electrospun nanofibers that contain natural products for drug delivery systems and tissue engineering applications

Recently, blended nanofibers with plant-derived natural biomaterials has gathered great interest since it is possible to incorporate the curative, regenerative, antimicrobial and anti-inflammatory properties of plants with the incorporation of good mechanical properties and slow biodegradation of synthetic polymers. One of the advantages of blending plant-derived materials into polymers is that plants have usually large molecular size and most compounds are unable to cross the lipid membranes of the cells which results in poor adsorption and loss of bioavailability and efficacy (Venugopal et al. 2014). So electrospinning

fibers deliver the active ingredients of the plants at sufficient concentration during the entire treatment period to the host site (Venugopal et al. 2014).

Many plants have been tested in electrospun nanofibers for drug delivery, as example there is *Aloe vera* – antioxidant and good medicinal properties for tissue engineering. Nanofibers with *Aloe vera* show that human dermal fibroblasts can have better and faster attachment, proliferation and guided growth (Tam et al. 2014). Moreover, it can accelerate the healing of open wounds in type 2 diabetic radiation-exposed rats (Venugopal et al. 2014).

Asian ginseng (*Panax ginseng* root) has numerous applications on central nervous systems, , cardiovascular and human skin (Lee et al. 2007). *Panax ginseng* extracts can also promote collagen in human dermal fibroblast cells (Lee et al. 2007; Pajoumshariati et al. 2015) and positive effect on osteogenesis and cell proliferation. On nanofibers scaffolds, (Pajoumshariati et al. 2015) results indicate that ginseng extracts shows an improvement in cell attachment and proliferation, it also enhanced the MSCs osteogenic differentiation with high level of calcium content deposited on the surface of fibres which shows a potential candidate for bone tissue engineering.

The range of nanofibers medicinal plant is vast and this field has been only recently studied and it opens large areas of studies with it.

3.4.4 Electrospun nanofibers with drugs for tissue engineering applications.

Besides bone regeneration skills that nanofibers can induce, drug loading contents can be added as well so that they can prevent any kind of infection, since the number of patients with infections is rising owing to the lifelong risk for bacterial seeding on the implant (Zimmerli et al. 2004). Infections associated with guided tissue regeneration (GTR) and

guided bone regeneration (GBR) implants are mainly caused by anaerobic bacterial infections (Ulubayram et al. 2015). In this way, nanofibers scaffolds have been currently used as an anti-infection barrier with loadings of various different drugs such as: metronidazole (MNA) and silver nanoparticles and since the barrier had a sustainable release of those drugs; the membranes maintained its antibacterial effect for a long term (Xue et al. 2014). In addition, recent studies have shown that it is also possible to obtain a biphasic release kinetics with good antibiotic encapsulation (~75 – 100%) on PLGA electrospun fibres; the researchers used 10% (w;w) fusidic acid and 5% (w;w) rifampicin which help reduce the number of adherent bacteria by 99.9% in an *in vivo* rodent model of implant associated infection (Gilchrist et al. 2013; Ulubayram et al. 2015).

3.4.5 Drug-encapsulated nanofibers for wound recovery

Nanofibers scaffolds and hydrogels nanofibers have been successfully employed as vehicles for anti-bacterial agents for wound recovery (Lee and Yoo 2008; Choi et al. 2015; Vashisth et al. 2016). Antimicrobial agents are preferred to tailor the role in the wound healing process and preferably such products usually must protect against, gram negative, positive and antibiotic-resistant bacteria. One natural product that have protection from gram negative and positive is propolis (De Lima et al. 2015) and from our knowledge, it has not been overlooked as nanofibers scaffolds. On the other hand, strong antibiotics have been encapsulated into the nanofibers scaffolds, such as neomycin (Nitanan et al. 2013), ampicillin (Sabitha and Rajiv 2015), ciprofloxacin (Canillas et al. 2015). Cefixime a very effective antibactericidal antibiotic for *Escherichia coli* and *Staphylococcus aureus* bacterial strains, which are very common in wound infections (Bergeron and Turcotte 1986; Arshad et al. 2012). Shahzad et al. produced nanofibers mats of chitosan, PVA and HAp with Cefixime for wound healing, the nanofibers were crosslinked via Freeze-thawed method, freezing the

mats at -80°C for 24h, freeze dried to form porous scaffolds and finally heat treated at 80°C for 10 min. Shahzad et al. demonstrated that heat treatment affects the structure of the mats, have excellent interconnected porous structure, high swelling capabilities with sustained release of cefixime, inhibition against *S. aureus* and *E. coli* and cytocompatibility with VERO cell line (Shahzad et al. 2015). Ciprofloxacin have also been studied with PVA and PVA/PAA hydrogel structures for the objective of reducing infection against osteomyelietes (Canillas et al. 2015); however, its investigation still needs to be analyzed for nanofibers.

4. Recently strategies developments in electrospun for drug delivery.

Although many works have been described in terms of drug delivery on wound healing and tissue engineering it still needs more investigation in terms of poorly water soluble drugs and localized targeting delivery. The effect of initial burst release also needs to be improved so the next session describes innovations on recent fronts of targeting and poorly water soluble drugs.

4.1 Multi-layered nanofibers

The ability to engineer drug release produced by nanofibers can also be improved or modified by the polymers matrix. If the drug is incorporated in separate nanofiber matrix, the diffusion is different and can promote a time-engineering release of the drug (Wang et al. 2010d; Huang et al. 2012). This way, complex nanofiber mats can be produced with various polymers to attain multi-phased drug delivery. In this configuration, after the first layer of electrospun deposited with the first polymer, another polymer sequentially is deposited on the same collector. This process can be repeated multiple times to produce meshes of multi-layered fibres with ordered structure and different kinds of polymer. Recently, controlled release systems for oral delivery of poorly water-soluble drug have

been studied with multi-layered nanofibers, such as ketoprofen (KET) incorporated in a trilayered electrospun with two different polymers (Huang et al. 2012).

In addition, a recently new triaxial electrospinning strategy has been adopted with a gradual layered structure mesh that are dependent of the various working fluids containing varied concentrations of the drug which was designed to incrementally increase the content of drug moving from the exterior of the fibres inwards resulting in a gradient distribution of the drug - linear release of KET. By incorporating this formulation into an enteric-coated capsule, a linear release colon-targeted oral drug delivery system can be produced as shown in fig 6.

4.2 Hollow and core-shell electrospun fibres

In contrast to solid nanofibres, the hollow cores have interesting advantages in terms of higher specific surface area, lower density and multiphase interfaces (Wu et al. 2011). Hollow spheres as compared to normal nanoscaffolds have gathered interest as drug delivery systems owing to this hollow-void that can encapsulate large amounts of drugs. In addition, it also controls the release rate, so that it prevents the initial burst and short-term release that occurs in solid nanofibers (Wang et al. 2010c). Wu et al., created a hollow hydroxyapatite fibre for protein delivery systems, the hydroxyapatite nanofibers produced an ultrafine fibre diameter and interconnected pores providing large specific surface area and demonstrated great protein adsorption ability with long-term sustained release. However, hollow fibres have poor mechanical properties (Kang et al. 2015). To overcome the deficiencies of this technique, core-shell nanofibers have been produced on top of the hollow fibres (Kang et al. 2015). The principle behind using core-shell fibres on hollow fibres was that, since large quantities of drugs can be encapsulated onto the hollow fibres it might

be useful to protect them, in which the core-shell technique preserve the biological activity of the drug (Su et al. 2012; Zhang et al. 2013).

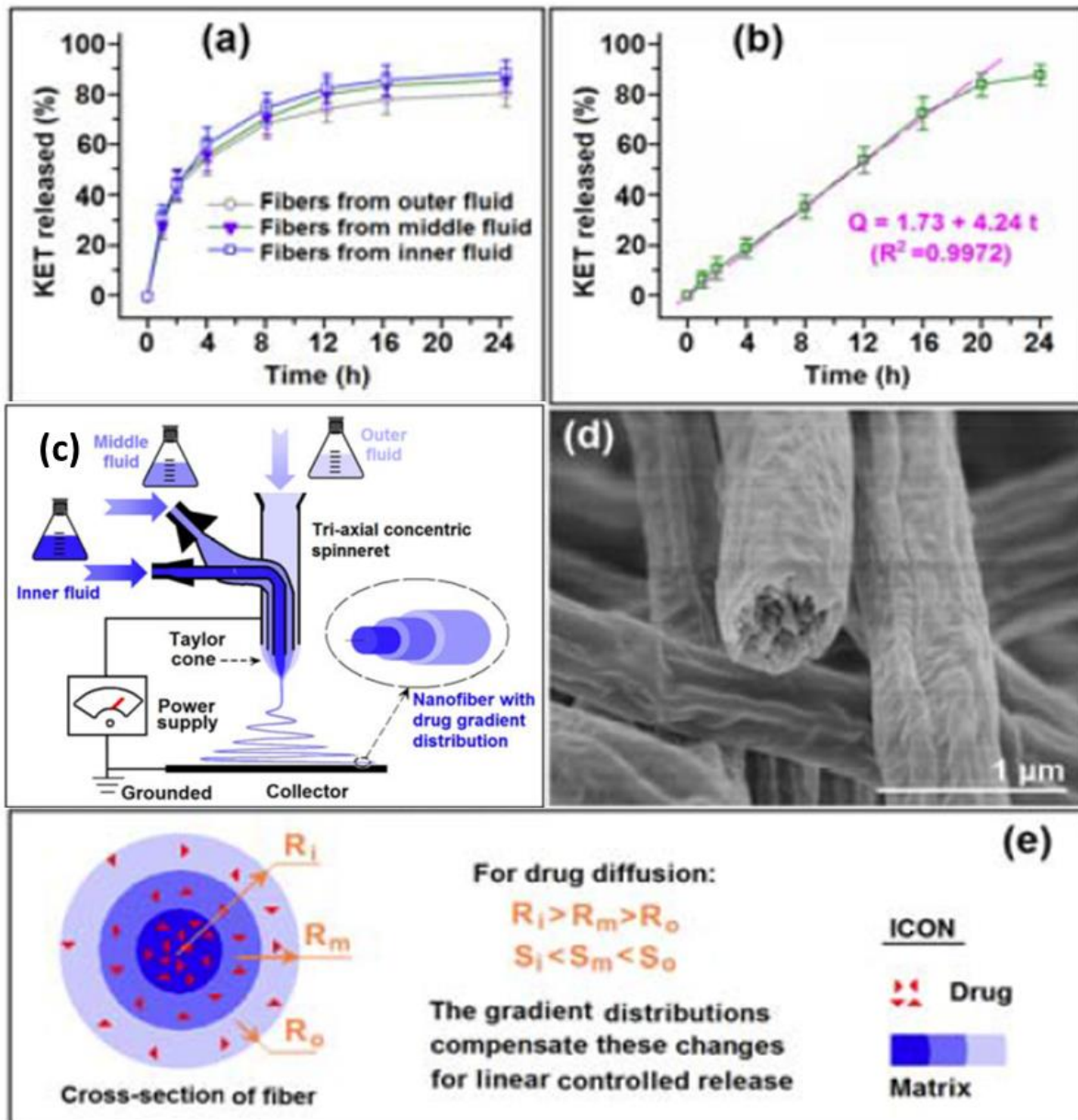


Figure 6 (a) In vitro dissolution test results for KET incorporated in each monolithic nanofibers and (b) the tri-layer nanofibers, (c) a schematic of the triaxial electrospinning process (d) FESEM images of the tri-layer nanofibers after release of all the KET loading, and (e) a diagram explaining how the gradient drug distribution can yield a linear release profile; adapted with permission from (Yu et al. 2015). Copyright (2016) American Chemical Society.

4.3 Patterned and alignment structures of nanofibers

There are several methods to produce nanofibers with controllable patterns (Zhang and Chang 2007), as a simple method it bases on the theory that the orientation of nanofibers are affected by the topography of the collector, so a patterned nanofibrous scaffold can be generated by using a patterned conductive collector (Zhang and Chang 2007; Li et al. 2014). Alternating the patterned collector and distribution, nanofibers can be assembled into well-ordered nanofiber meshes with both types of topographies, random and parallel alignment existing in the same mesh (Daming and Jiang 2008; Wang et al. 2009b).

The orientation effect and pattern on nanofiber have also been investigating for drug delivery applications. Results shows that the drug delivery compartment can be adjusted by the surface wettability of the drug carrier. Meng et al. (2011) showed that aligned scaffolds of nanofibers had a lower release rate compared to random orientation, suggesting that alignment of fibers could also influence the drug release.

5. Conclusion and future perspective

Nanofibers produced by electrospinning are versatile materials that can have many applications on biomedical field. Nevertheless, polymer jets are relatively unstable so controlling the solution and electrospinning parameters are really important to obtain uniform nanofibers.

The electrospun nanofibers scaffolds mimics the natural ECM, this feature leads these materials as ideally candidates for tissue engineering. From today, the focus and specific knowledge gathered and applied of nanofibers on different tissues of the body are helping on further progression on this field immensely. Such knowledge is helping achieve regeneration of tissues at relatively fast rate time. Nonetheless, the designed scaffold needs

appropriate properties for the application intended, so the evaluation of the nanofibrous scaffold material was necessary to be discussed in this chapter. Currently, the blend of natural with synthetic polymers offers the appropriate properties for biomedical applications. Furthermore, the incorporation of nanofibers with hydrogels seems to be a solution for the current problems and challenges in this particular field.

We evaluated the effect of controlling the nanofiber diameter, packing, orientation and 3D shape on different tissues environment for cell behaviors such as attachment, orientation, proliferation and migration. As we progressed, it was possible to conclude that parameters of fibers are really important for the tissue application intended, such as cartilage that has three different layers and requires a complex nanofibrous scaffold with varying porosities and alignment. However, this field still needs further investigation for better understanding on tissue regeneration such as physiological signals.

Nanofibers can also incorporate drugs by different methods and it can not only help on tissue engineering by creating antimicrobial barriers (Huang et al. 2012; Xue et al. 2014) but it can also help on wound healing. Hydrogels nanofibers are suitable candidates for wound healing applications, their swelling ability coupled with permeability for gaseous transfers can promote a perfect condition for healing environment. In this way, the ability of incorporating drugs and control the release rate can also impede antimicrobials to infect the wound and spread.

As technology advances and studies progress, novel techniques for incorporation of drugs in specific target delivery are now in development. The field of drug delivery on nanofibers is certainly making fast progress for the main current problems on biomedical field such as poorly water soluble drugs and biofilms of bacteria (Ahire et al. 2015; Paaver et al. 2015).

6. References

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Figure 2. Physical representation of the three solutions regimes with corresponding SEM images. (A) and (D) dilute, (B) and (E) semi-dilute unentangled; (C) and (F) semi-dilute entangled. $C^* = 1 / [\eta]$. Adapted with permission from (Bock et al. 2012). Copyright (2016), Elsevier.

Figure 3. Most common used natural and synthetic polymers on electrospinning along with its biological, mechanical and physiochemical properties (reprinted with permission from Elsevier (Gunn and Zhang 2010). Copyright (2016), Elsevier.

Figure 4. Digital images representing, (a) non crosslinked gelatin (b) gelatin after crosslink and (c) gelatin with crosslink immersed in water. Reprinted with permission from (Laha et al., 2016). Copyright (2016), Elsevier.

Figure 5. Bilayered cartilage scaffold schematic. (A) A diagram illustrating the electrospun fibre zone (FZ) and a porous zone (PZ). (B) SEM images of (top) the aligned fibre zone that is shared between both scaffold varieties, (middle) the complete bilayered scaffolds with 0.03 mm³ (left) and 1.0 mm³ (right) pores and (bottom) the sodium chloride porogens used to produce higher porous zones. Reprinted from (Steele et al. 2014).

Figure 6 (a) In vitro dissolution test results for KET incorporated in each monolithic nanofibers and (b) the tri-layer nanofibers, (c) a schematic of the triaxial electrospinning process (d) FESEM images of the tri-layer nanofibers after release of all the KET loading, and (e) a diagram explaining how the gradient drug distribution can yield a linear release profile; adapted with permission from (Yu et al. 2015). Copyright (2016) American Chemical Society.