Chapter 6

Electrospinning of Functional Nanofibers for Regenerative Medicine: From Bench to Commercial Scale

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Additional information is available at the end of the chapter

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Abstract

Nanofibers are an important material for regenerative medicine as they have a commensurate morphology to that of the macromolecular matrix that supports and houses the growth of cells and tissues within the body. Electrospinning is widely used to fabricate non-woven structures on the nanoscale and the versatility of the technique has widened the application of nanofibers. This is due to ease of extending nanofiber functionality through the incorporation of active materials both during and after electrospinning. Recent developments in electrospinning devices, such as needle-free systems, have reinvigorated research as these advances now allow fabrication of nanofibers at commercial scales. The process of electrospinning has a number of operating parameters that are adjusted in optimisation to achieve ideal fibres and a multitude of instrument configurations can be adopted to achieve the required manufacture. The innate properties of nanofibers, such as high surface area to volume ratio, have many proven benefits for regenerative medicine and the chapter examines these before discussing how functionality can be further improved. Numerous materials can be incorporated in the manufacture of electrospun mats, however when choosing materials for regenerative medicine, biocompatibility and biodegradability are the dominant functionalities that are required.

Keywords: electrospinning, tissue engineering, wound healing nanofibers, biomaterials, antimicrobials

1. Introduction

Electrospinning is an extremely versatile technique for the production of nanofibers. As a consequence, electrospun fibres have been fabricated for a wide range of applications from separation processes to tissue engineering. The versatility of the electrospinning process has allowed the functionality of the nanofibers to be extended beyond the innate improvement of properties enabled by the fabrication of materials with nanoscale dimensions. Further functionality has been achieved by the incorporation of nanoparticles and other bioactive compounds, this has been particularly important for the application of nanofibers for tissue engineering, wound healing and drug delivery; the three themes of regenerative medicine. The developments of regenerative medicine that we seem to be witnessing every day is just one example of the increasing demand not just for novel nanofiber constructs but manufacture of functional nanofibers at economic scales, whether that is at high value and low volume, as in tissue engineering scaffolds or high volume manufacture as in wound dressings. Indeed, scale of manufacture is another advantage for the application of electrospinning, as relatively recent instrument developments have reinvigorated the research area through the increase in volume of manufacture that they now allow. Thus, this chapter will examine the state of the art technology for electrospinning in the context of improved functionality and scale of manufacture, which is essential for the modern healthcare system and the realisation of the potential that regenerative medicine promises.

2. Electrospinning

2.1. Electrospinning process

Electrospinning uses a high voltage power supply to create a large potential difference between a grounded "collector" structure and a polymer solution or melt being delivered at a constant rate through an aperture, such as a blunt end needle. As the voltage is increased the like charges within the polymer fluid directly oppose surface tension, resulting in the normally spherical droplet at the aperture distending into a conical shape. This cone is referred to as the "Taylor" cone, after Sir Geoffrey Taylor who first mathematically modelled the phenomenon [1–3]. At a critical voltage the electrostatic attractive force between the solution and the collector causes a jet of polymer solution to be expelled from the cone tip towards the grounded collector surface. This jet then undergoes a whipping instability and dries in flight, depositing the nanofibers on the collector [4] (**Figure 1**).

It is the interaction of the applied electrical field and the electrical charge which is being carried by the jet which generates the tensile force required for electrospinning [5]. A stable electrospinning jet can be described as having four distinct regions [5]. These four regions are the base, the jet, the splaying and the collection regions. The base region can be referred to as the "Taylor Cone", where the jet is expelled from the polymer solution [1, 2]. This is where the polymer becomes electrically charged. When the voltage is increased to a critical voltage the

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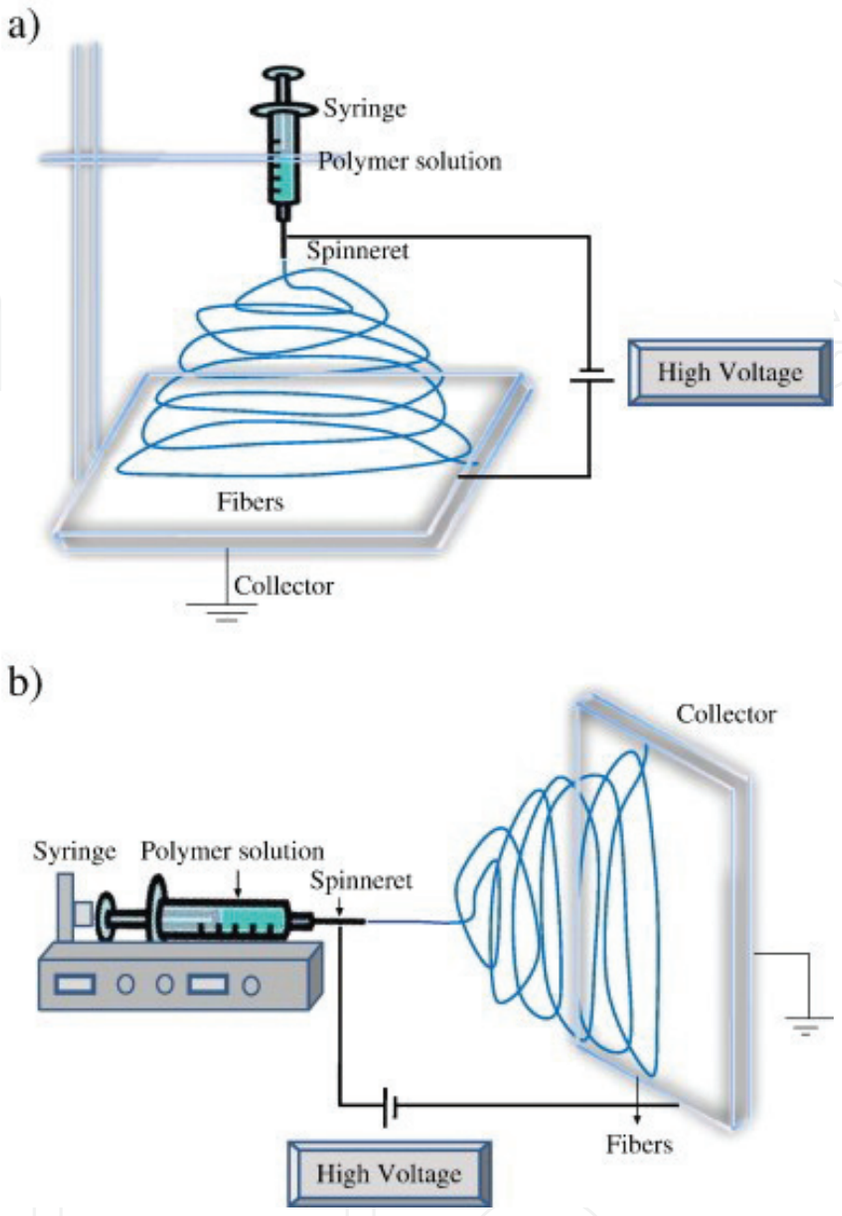

Figure 1. A schematic diagram of electrospinning apparatus in (a) a vertical setup and (b) a horizontal setup. Reprinted from Copyright (2010), with permission from Elsevier [4].

electrostatic attractive force between the solution and the collector cause a jet of polymer solution to be expelled from the cone as the repulsive forces generated by the applied electric field overcome the surface tension of the polymer. Once the jet is expelled from the Taylor cone it continues to accelerate towards the grounded collector. This region can be described as the jet region. The splaying region is where the jet undergoes a whipping instability. At the tip of the spinneret where the Taylor cone forms the jet is very stable but as it accelerates towards the collector and the solvent evaporates, the jet then undergoes what can be described as a whipping instability. This whipping instability is caused by an electrostatic repulsion within the polymer solution which is initiated as small bends in the fibres. It is the combination of acceleration of the jet and also evaporation of the solution which causes the jet to stretch

decreasing its diameter. As this occurs the electric charge on the jet causes it to stretch in the radial direction, which as the jet becomes smaller allows the jet to split into two or more fibres as the radial forces manage to overcome the forces that are holding the jet together. This continues to occur as the jet travels towards the collector. Multiple fibres are formed all with like charges which then repel each other resulting in the 'splaying' effect causing a number of fibres to be deposited on the collector.

Despite the relative simplicity of the equipment involved, by carefully controlling processing parameters the fibre's diameters, orientation, total mat porosity and other properties can be controlled, allowing optimisation of the mat for a given application. In addition, the technique's ability to work with a wide variety of materials allows a range of specific biological, mechanical or chemical properties to be achieved [6]. Therefore by controlling solution properties such as the viscosity, conductivity, molecular weight and surface tension along with processing parameters such as the applied electrical field, distance from the syringe tip to the collector and flow rate of the polymer solution, a range of desirable characteristics can be attributed to the nanofibers [4].

2.2. Controllable variables and their effects

2.2.1. Solution properties

An increase in polymer concentration in a given solution will result in an increase in the solutions viscosity. It has been shown by a number of research groups that a decrease in polymer concentration results in a decreased fibre diameter [7–10]. Although this offers a certain level of control as the polymer concentration decreases it also leads to beaded fibres and a wider size distribution. Mit-uppatham et al. demonstrated this with Polyamide-6 showing at low concentrations a large number of droplets. As solution concentration increased the number of beads decreased and fibre morphology improved [8]. Katti et al. also demonstrated that at lower concentrations when beading is present fibres deposited are wet and therefore tip to collector distance must be increased [7]. Electrospinning will only occur when the polymer concentration is high enough to allow adequate chain entanglement resulting in continuous formation of uniform nanofibers when a high enough voltage is supplied [11]. Rayleigh instability occurs when electrostatic repulsion of charges in the electrospinning jet tends to increase its surface area while the surface tension opposes this force reducing the surface area of the jet [12]. At polymer concentrations too low to initiate electrospinning no polymer chain entanglements can occur and as such the polymer solution cannot resist the Rayleigh instability sufficiently, which results in the break-up of the jet into droplets. Increasing charge density on the surface of the droplet leads to the formation of smaller droplets and results in electrospraying. At higher concentrations the high viscosity hinders jet stretching in the whipping region. The high viscosity can also lead to practical difficulties when pumping the solution through an aperture [11]. The viscosity is the governing parameter when changing the polymer concentration but changing the solution composition also has an effect on fibre diameter. As the polymer concentration increases, the proportion of polymer to solvent in the jet increases and as such fibre diameter increases. This is due to the higher volume of polymer remaining once the solvent has evaporated off.

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The conductivity of the solution is a crucial parameter. When the conductivity is low this results in the production of fibres with a greater diameter. This is most likely due to poor jet elongation [10]. As the conductivity increases the fibre diameter tends to decrease. This shows a clear relationship between the conductivity of a solution and the level of jet elongation. As the conductivity of the solution is increased the jet undergoes a higher degree of elongation along its axis due to the repulsion of charges under the electric field [13]. Tan et al. reported fibres with beads as a result of a low conductivity which is again most likely due to insufficient jet elongation resulting in the solution 'spitting' [10]. Increasing the conductivity of a polymer solution can also initiate the electrospinning of smooth fibres at lower polymer concentrations [14]. This is because the increase in conductivity results in an increased charge density at the surface of the jets which decreases the likelihood of bead formation and improves fibre extension in the whipping region [11]. The conductivity of a polymer solution can be adjusted by the addition of an inorganic salt or ionic organic compound [11]. This addition can also affect the surface tension and dielectric constant of the solution which can make assessing the effect of conductivity difficult [11].

Viscosity of a polymer solution will increase with increased molecular weight, meaning a lower concentration of polymer is required to form non beaded fibres with tight size distributions. At higher molecular weights there are a greater number of chain entanglements and therefore a higher viscosity at equivalent concentration compared to a lower molecular weight. As a result even at a low polymer concentration a high molecular weight polymer can provide sufficient chain entanglements to overcome the effects of surface tension and result in a uniform jet [10].

There is no evidence to show that the surface tension of a solution affects the morphology of fibres although this does not mean the surface tension of the solution is irrelevant as if it becomes too high it can result in jet instability which can have a drastic effect on the electrospinning process. Surface tension can also be adjusted to induce beads formation [11]. Generally, it is the surface tension and solution viscosity that are used to determine the range of polymer-solvent combinations that electrospinning is possible. The spinning voltage at which electrospinning is initiated tends to increase with surface tension. Fridrikh et al. suggest that when all other parameters are kept constant a lower surface tension is desirable [15]. Unfortunately, this is not simple as surface tension varies both with varying concentrations and due to the chemical nature of the polymer [16]. Surface tension can be adjusted through the selection of different solvents or through the addition of a surfactant. A surfactant is a substance containing hydrophobic groups (head) and hydrophilic groups (tails) and they tend to reduce the surface tension between liquids and solids.

A solvent with a high vapour pressure at normal temperatures can be referred to as being volatile. During the electrospinning process this results in the jet spending less time in the elongation stage undergoing stretching as the solvent evaporates off. Therefore, a more volatile solvent (higher vapour pressure) will result in fibres of a larger average diameter. This allows the researcher to control the fibre diameter to a certain degree through the choice of solvent based on its vapour pressure, although a solvent with a particularly low vapour pressure may be unsuitable and result in the deposition of wet fibres, with coalescence at fibre junctions.

2.2.2. Processing parameters

It has been shown by a number of research groups that the applied voltage does not have a great effect on the fibre diameter [7, 10, 17]. Electrospinning will only occur at a certain range of voltage although if the voltage is too high this can result in multiple jets forming which can decrease diameter of fibres but widen the size distribution [10]. Generally, fibre diameter decreases as applied voltage increases due to the greater columbic forces causing greater stretching of the solution and resulting in a smaller fibre diameter with fibres drying more quickly [4, 9]. Baumgarten showed that as voltage increases jet diameter initially decreases and then increases as the voltage continues to increase [18]. As a result of this flow rate must increase as the voltages increases. It has also been reported that deposition rates increase with increased voltage [17]. This could explain the inconsistent reports of the effect that voltage has on fibre diameter and other parameters such as the feed rate and tip to collector distance must also be considered when observing the effects of applied voltage [11]. By measuring the current flow Deitzel et al. showed that for a PEO/water system the increase in mass flow rate is almost linear with applied voltage [16]. In increase in voltage which results in an increased mass flow rate can also result in spinning from within the needle as the Taylor cone recedes which can result in uneven and beaded fibres [19].

The flow rate is the rate at which the solution is pumped through the needle to maintain a droplet of solution available for Taylor cone formation. The flow rate of the solution has not been shown to have a significant effect on the fibre diameter or morphology [10] although some studies have shown an increase in flow rate can result in an increase in fibre diameter [20]. The ideal flow rate should match the rate at which the solution is being ejected from the tip [11]. At lower flow rates electrospinning tends to be intermittent, with the Taylor cone often receding into the needle as previously mentioned.

If the distance between the spinneret and the collector is either too small or too large, the result is beaded fibres. The ideal distance is the minimum distance required to allow the fibres enough time to dry between the spinneret and the collector [17, 18]. Tip to collector distance is usually selected according to the vapour pressure of the solvent. When increasing the distance, the voltage applied to the spinneret needs to be increased to maintain the electric field. Further to these controllable variables it has been shown that by modifying the collector architecture, for example by using two parallel conductive substrates of varying gap size, fibres can be aligned uniaxially into arrays [21]. This alignment of fibres has led to anisotropic mat properties in terms of tensile strength as well as directional cell growth, as previously discussed. Further modifications to the collector have been illustrated to expand the possible fibre orientations including rotating drum electrode [22] and knife-edge collectors [23]. Furthermore, "coaxial" electrospinning allows for a more complex fibre architecture, forming a fibre comprised of two non-mixed materials in a core-sheath arrangement [24].

2.2.3. Collector modifications

To produce aligned fibres, one of the techniques used employs a rotating drum electrode as a collector. In this setup the standard collector (usually flat, aluminium foil or similar) is replaced

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by a rotating mandrel [22]. When the rotational speed of the mandrel is sufficiently high it results in electrospun fibres which are aligned along the rotational axis of the mandrel.

Teo et al. described a knife-edged collector system [25] adapted from a similar setup described by Bornat [26]. The setup consisted of a parallel grid with a negative charge applied to a knife-edged aluminium bar. A Teflon tub was allowed to rotate between the electrospinning jet and knife-edged aluminium bar while fibres were electrospun on to the Teflon tube [25]. This technique was also used to electrospin tubular structures aligned in a diagonal direction.

A similar setup to the rotating drum electrode is the rotating disk electrode. In this setup instead of electrospinning onto a rotating mandrel a rotating disk is used as a collector [27]. This results in fibres which are aligned relatively to the disk.

Li et al. demonstrated the ability of uniaxially aligned arrays of nanofibers formed using parallel electrodes as collectors [21]. Their setup consisted of a standard system where a needle was aligned vertically electrospinning downwards where the collector consisted of two strips of conductive silicon separated by a gap. As the fibres are formed the ends of the fibres (those which are closest to the collector strips) will generate a stronger electrical force than anywhere else in the fibre. This results in the fibre being stretched across the gap and alignment along a single axis (**Figure 2**).

2.2.3.1. Control of the electric field

The path of an electrospinning jet can be altered by an electric field using its charge [28]. This can be achieved through the addition of auxiliary electrodes which can be used to align nanofibers (using two collectors separated by a gap), form simple patterns and control the deposition of nanofibers [21, 22, 29]. The auxiliary electrodes could include a base electrode, steering electrodes, focussing electrodes, guiding electrodes and the collector itself [28]. Yang et al. showed the use of a base electrode results in a more uniform electric field meaning a larger voltage can be applied to the nozzle, increasing the average field strength and resulting in fibres of a smaller diameter [30]. This could also be seen as a disadvantage as the use of a base electron means a higher voltage is required. A focusing electrode can be used to 'dampen' the chaotic motion of the electrospinning jet so the fibres are deposited in a more localised area. These are usually shaped as ring surrounding the jet but can also be a cylinder or cone. Using multiple pairs of steering electrodes can allow complex patterns to be fabricated.

2.2.3.2. Coaxial electrospinning

One method, coaxial electrospinning allows the encapsulation of materials which cannot usually be electrospun in to a core-shell fibre. A basic coaxial electrospinning setup will consist of a coaxial needle with 2 separate syringe pumps, one pumping a solution (conductive or non-conductive) through the inner lumen of the needle and another pumping a conductive electrospinnable solution through the outer needle. By adjusting the respective flow rates and applying a suitable electric current the solution being pumped through the outer lumen can be electrospun forming fibres encapsulating the solution being pumped through the inner lumen [31, 32]. This technique makes electrospinning a suitable fabrication method for a number of applications including for the controlled release of drugs and proteins [33], for nanowires

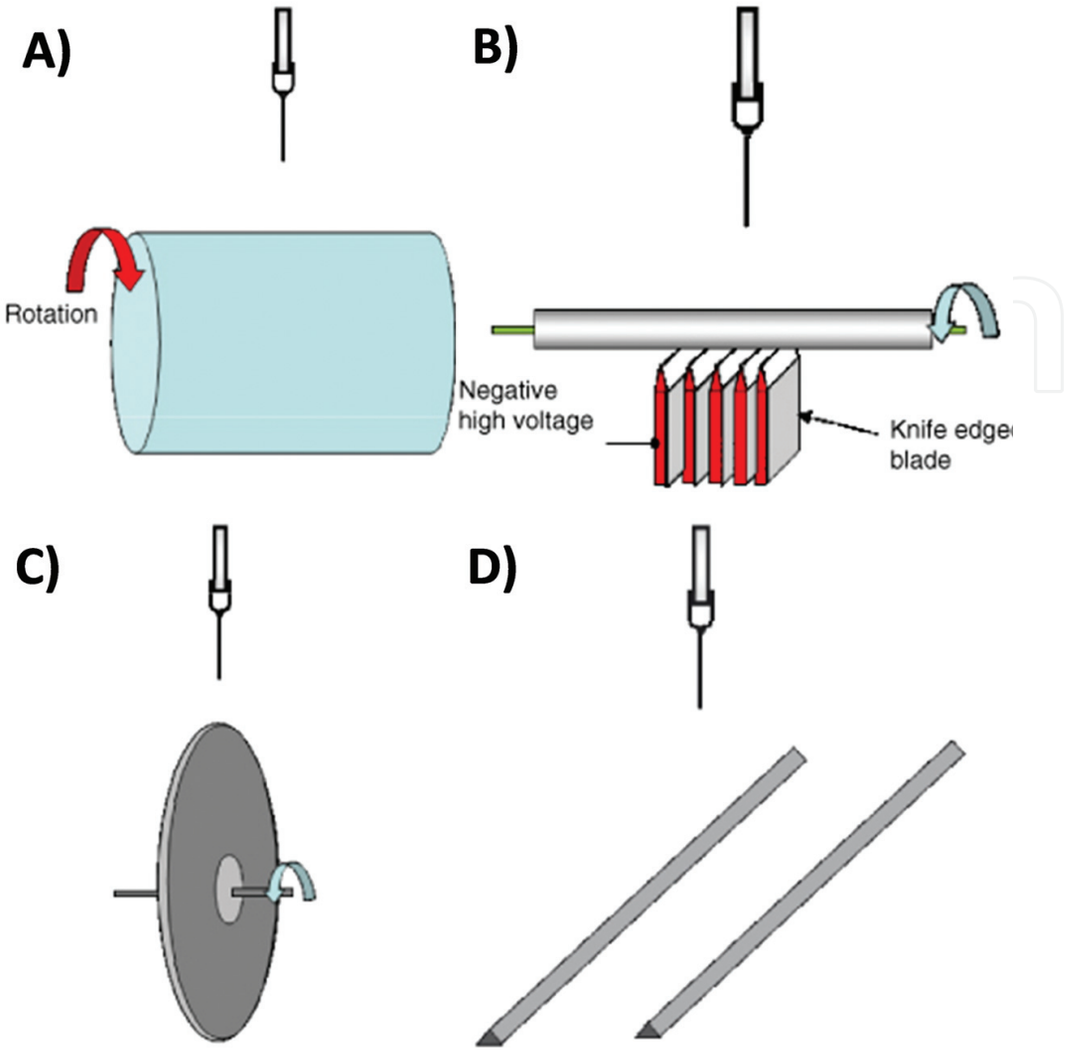

Figure 2. A schematic diagram of electrospinning apparatus in (a) a vertical setup and (b) a horizontal setup. Reprinted from Copyright (2010), with permission from Elsevier [4].

offering a number of potential applications in microelectronics and optical electronics [34] and also for the incorporation of stem cells in to tissue engineering scaffolds through a technique such as cell electrospinning [35, 36]. The latter of these applications is discussed below.

Once a polymer scaffold has been developed it can then be manually seeded with cells, frequently the cells employed for this are stem cells. The scaffolds are usually seeded with cells by adding a concentrated cell suspension to a suitable medium containing the scaffold material, and incubated for a period of time allowing them to proliferate and penetrate the scaffold. In a study by Vunjak-Novakovic et al. it was demonstrated that the requirements for cell proliferation can be easily satisfied by the use of well-mixed spinner flasks during the incubation. In their experiments all cells attached to the scaffolds and no cells were damaged in the process [37]. However, manufacturing polymer scaffolds and manually seeding them with cells has some key limitations. Seeding is not always uniform so the scaffolds need to incubate for up to 72 hours in a bioreactor. Also, cells do not always fully penetrate the entire depth of

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the scaffold resulting in a non-homogenous distribution of cells. This is not usually an issue in simple, thin constructs but when thick more complex scaffolds are used, such as when attempting to grow a whole organ, this becomes an issue [36].

To overcome the issue of full-depth cell penetration 'cell electrospinning' can be employed. This technique encapsulates living cells into the electrospun fine composite threads. Jayasinghe et al. demonstrated this method, successfully electrospinning living organisms into Poly (dimethylsiloxane) (PDMS) scaffolds [35]. In the study, a coaxial electrospinning setup was used with an inner lumen delivering a suspension of living cells and an outer lumen delivering a PDMS solution. This work suggests the possibility of incorporating living cells into polymer scaffolds with full-depth penetration using electrospinning. This is a significant achievement which has yet to be replicated using any other jet-based techniques [38]. This work from Jayasinghe's group clearly demonstrates the ability to functionalise scaffolds with living cells.

2.2.3.3. Free surface

Although needle nanometre diameter fibres of polymer, produced by electrospinning allows excellent control over both fibre diameter and their composition it has an extremely low throughput where basic systems are often limited to flow rates of less than 0.5 mL per hour. A free-surface electrospinning setup such as the El Marco NanoSpider™ Lab 200 system is capable of forming fibres with throughput many thousands of times greater than the conventional needle-based electrospinning setup [39]. In the bowl-based electrospinning approach the spinning solution is simply held in a bath, rather than being delivered through an aperture, with the whole bath then being connected to a high voltage power supply. In the specific case of the NanoSpider™ a rotating metal mandrel is half-submerged in the bath to concentrate the electric field on the thin layer of polymer which coats the mandrel. In this process, many Taylor cones are formed on the surface of the polymer solution, and electrospinning upwards onto a collector above the bath. This increases the throughput of the process many thousands of times above the conventional needle-based system, however much higher voltages, up to 82 kV in the case of the NanoSpider™ are required and solution properties such as viscosity, conductivity and surface tension must be more tightly controlled [40, 41] (**Figure 3**).

2.2.3.4. Multiple spinnerets

An electrospinnning setup consisting of multiple spinnerets can allow scale-up from a single needle system. This is a relatively simple setup and can also allow different materials to be mixed during the electrospinnning process. The main disadvantage of the technique is the complicated interactions between the jets. However, it can provide scale-up from a single needle system with similarly tight size distributions. The scale-up is generally linear with the limit being set by the number of needles used.

2.3. The commercial state of electrospinning

The emergence of more scalable electrospinning techniques in recent years has allowed for electrospinning to be used as a commercial fabrication method for non-wovens. For example,

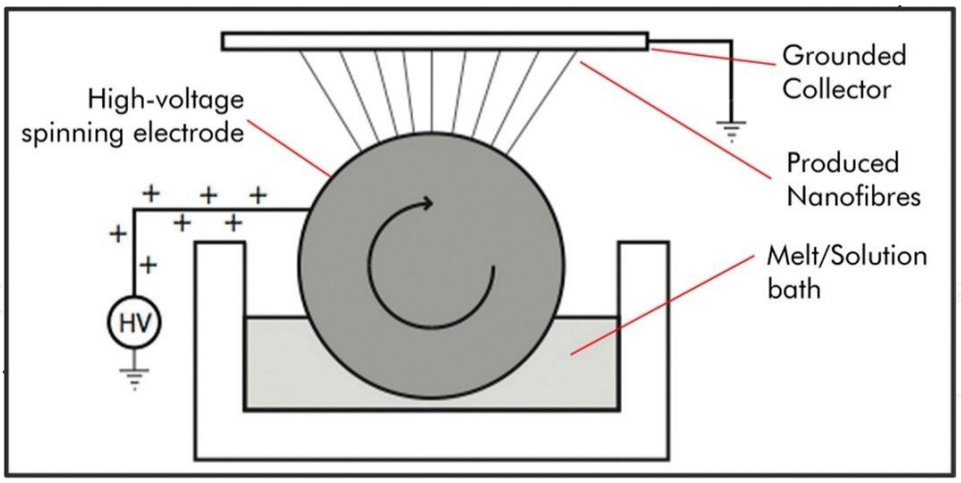

Figure 3. A schematic diagram showing a free-surface electrospinning setup. A polymer solution/melt is held in a bath and a spinning electrode connected to a high voltage power supply is utilised to form multiple jets. Nanofibers are electrospun upwards and collected on a grounded collector plate.

El Marco offer a number of devices, from a lab scale system, NS Lab 200, with a production rate of up to 5.5 g/hour to the NS8S1600U, with a production rate of up to 352 g/hour. These production rates are all significantly higher than for needle electrospinning, and the design of the nanospider devices allows for their incorporation into a production line. This opens up applications with high volume but low value which previously were not feasible using needle electrospinning. These include filtration materials, textiles and dressing materials.

Despite the efforts of researchers to commercialise electrospinning, the upscale is still a significant barrier for many applications. Companies such as Espin technologies (USA) and Fintex Inc. (Korea) are producing nanofibres commercially for specific applications, including air filtrations. Companies such as Nanofibre solutions (USA) and The Electrospinning Company (UK) have been using electrospinning technology to produce nanofibres for niche biomedical applications. There are also companies seeking to take advantage of the properties of nanofibers for a wide range of versatile applications. These companies include Revolution Fibres Ltd. (New Zealand) and Fibre Rio Technology Corp (USA) [42].

There are commercially available nanofibre products in a number of different industries. These include filtration (companies include AMSOIL, Carcor and Donaldson in the air filtration market and Donaldson, DuPont and Finetex in the liquid filter market), acoustic technologies, skin care and biomedical products (companies include Arsenal Medical, The Electrospinning Company and Nanofibre Solutions), Composite materials and sensing and electronics. A more detailed discussion on the companies providing these products and their applications can be found with their commercial applications, challenges and opportunities examined [42].

3. Functional morphology

The morphology of nanofiber mats has many proven benefits for processes including the control of bio interface systems within regenerative medicine. These benefits are innate to

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nanoscale materials and the high surface to area ratio impacts regenerative medicine in many way including the increased porosity and absorbance of wound dressings of nanofiber mats compared to those formed by micron fibres [43, 44], the biomimicry of the extracellular matrix (ECM) of nanofiber tissue engineering scaffolds [45, 46] and improved control of drug release rates when moving from micro to nanoscale fibres [7, 47]. The following section considers the improved functionality of nanofiber mats compared with materials of more traditional morphology and discusses some recent observations on the impact of application of nanofibers on cellular integration and control.

3.1. Control of mammalian cells

Nanofiber-based scaffolds are of great interest in tissue engineering applications. Their usefulness has been extensively assessed both through *in vitro* and *in vivo* settings. The selection of biocompatible polymers, the modifications to fibre production and processing has been examined in great detail. Of particular interest are the changes to the use of crosslinking agents from cytotoxic chemicals to novel methods which avoid their use. We also assess how structural and orientation changes to the fibres allow the scaffold to fit into a specific niche in the body while the polymer of choice also compliments these changes. Here we will briefly review the recent findings in relation to fibre interactions with mammalian cell systems and the modifications which have been made in order to improve their usefulness in tissue engineering.

One of the largest challenges with electrospun scaffolds is not simply the degree of porosity, which is usually in the region of 91.6% [45], but how accessible the pores are for cell infiltration. Wang et al. propose the use of the Darcy permeability coefficients to determine how cells will respond to surfaces and the architecture of tissue engineering scaffolds through their porosity [48]. They also describe 3 types of pore shown in **Figure 4**; a blind end pore leads to a section of scaffold where material cannot infiltrate further. A closed pore is isolated inside the scaffold and is therefore inaccessible to cells. The desirable architecture is an open pore network where the pores branch between each other and allow cell migration to occur.

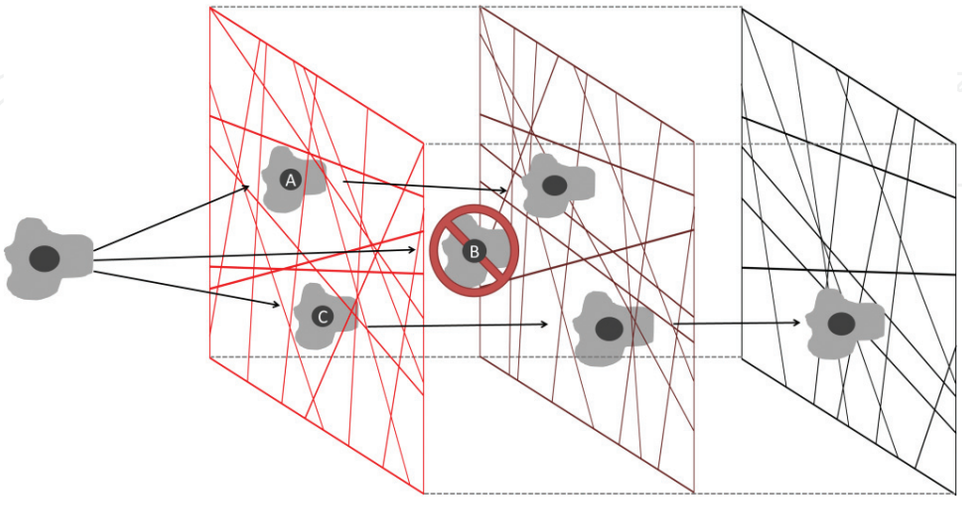

Figure 4. Cell migration through an electrospun scaffold can be difficult due to 3 types of pore: A: Blind end pore where cells cannot completely infiltrate. B: A closed pore inside the scaffold cannot be accessed by cells. C: Open pore network allowing complete cellular infiltration throughout the scaffold.

Li et al. assessed the state of nanofibrous scaffolds for tissue engineering, with great focus on the technology of electrospinning [49]. This review looked into the ideal characteristics for a tissue engineered scaffold, and the questions which should be answered for a particular niche. These begin with architecture; are the fibres of aligned or random orientation? The porosity of a scaffold which must allow for cell migration and nutrient diffusion, and if a scaffold is to recreate blood vessels, are red blood cells contained? This is one of the potential downfalls for an electrospun scaffold, as pore size cannot be easily controlled during production. To avoid this, various methods such as salt leaching have been employed to increase and control pore size [50]. The mechanical properties of a scaffold must also be assessed to match the niche it will be integrated into as these can have drastic effects on cell morphology, cell proliferation and differentiation [51].

Polymer choice must be considered when trying to create a nanofiber scaffold for tissue engineering, especially if the construct is to become a medical device for use in the body. This polymer will need to comply with rigorous testing, and its production as well as the device fabrication will have to follow ISO 13485 standards ("ISO 13485:2016 – Medical devices – Quality management systems – Requirements for regulatory purposes," 2016), followed by European Union Directives 93/42/EEC, 90/385/EEC and 98/79/EEC conformity. As well as considering the polymers safety aspect, it must also be useful to the environment in which it will occupy. Tissue engineering biomaterials can either be synthetic polymers such as poly (lactic-co-glycolic) acid, or naturally occurring polymers; collagen, complex sugars such as hyaluronan or chitosan, or inorganics like hydroxyapatite all fit within this classification [53]. These can be further adapted with surface modifications [54, 55], drug loading [54, 56] and other techniques to increase scaffold efficiency.

Naturally occurring polymers are often favoured, in particular extracellular matrix derived scaffolds such as collagen, which provides support for cell attachment *in vivo* and has been of interest in tissue engineering for over 20 years. The issue with naturally derived polymers tends to be in their processing, many of these cannot be electrospun without the use of harsh solvents such as 1,1,1,3,3,3 Hexafluoro-2-Propanol (HFP) and 2,2,2-Trifluoroethanol (TFE) which have been shown to denature the proteins [57], removing many of the beneficial aspects of their use.

For physiologically soluble polymers it is usually required that the scaffold is crosslinked. Glutaraldehyde has for many years been regarded as the standard method of crosslinking [58], particularly with proteins due to its efficiency and high degree of crosslinking across different polymers [59]. This chemical has many setbacks with regard to tissue engineering scaffolds, foremost the calcification of scaffolds and surrounding tissues which are exposed to residual crosslinking agent which may lead to device failure [60]. In recent years, alternatives to glutaraldehyde have arisen with the desire to increase cytocompatibility *in vivo*, examples of these can be seen in **Table 1**.

Niu et al. compared PCL/Collagen scaffolds which were crosslinked with either glutaraldehyde vapour or genipin and examined their efficacy with cell infiltration, survival and proliferation (Niu et al., [58]). They found that nanofiber-based scaffolds increased cell proliferation while microfibre scaffolds showed better infiltration of cells. They also showed that genipin

Crosslinking agent	Example polymers for use	Type
1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC)	Any protein	Chemical
Genipin	Any protein	Chemical
Rose Bengal and 532 nm excitation	Collagen	Photochemical
Citric Acid	Zein, Collagen	Chemical
Thermal cycling induced crystalisation	PVA	Thermal
Pentaerythritol triacrylate (PETA) and UV irradiation	PEO	Photochemical
Lysyl oxidase	Collagen	Native Enzymatic

Table 1. Examples of crosslinking agents used for physiologically soluble polymers in electrospinning applications to increase cytocompatability in comparison with glutaraldehyde and their corresponding polymers.

showed higher levels of cytocompatibility than scaffolds crosslinked with glutaraldehyde. The increased use of less cytotoxic crosslinking agents shows a shift in the field away from the use of compounds that undo the beneficial effects of electrospun scaffolds.

Finally, we will examine the methods used to modify the architecture of the scaffold to best suit its desired niche. The work in this area mainly focuses on the creation of aligned fibres. Electrospinning typically produces a mat which contains randomly orientated 'non-woven' fibres. This type of mat is useful for creating barrier systems such as skin, however many niches are composed of a highly ordered structure in order to direct cell growth and mechanical strength. A prime example of this requirement is muscle tissue regeneration. Avis et al. produced aligned fibres of PLGA by electrospinning, where no further modification was needed [61]. The C2C12 murine myoblasts which were used in this study showed good adhesion and proliferation to the fibres and the RPM 1500 group showed good alignment along the fibre direction where nonaligned RPM 300 group showed a swirling pattern. This helps to demonstrate the importance the control over the scaffold architecture has on the course of scaffold efficacy. It should be noted however that this study demonstrates again that scaffold infiltration is hindered by poor internal access to pores in electrospun samples.

To conclude, it is important that when planning the use of electrospun fibres in applications utilising mammalian cells, there should be consideration of the type of niche the scaffold will be emulating or integrating with. The control over architecture, porosity and mechanical strength must be considered since each component characteristic can drastically affect the efficacy of the scaffold. We must also consider the post-processing that we carry out to improve the scaffold, and whether the current methods are sufficient to emulate a given niche. The move away from glutaraldehyde seeks to demonstrate that there are many minor changes to our processing which may improve the scaffolds use in tissue engineering.

3.2. Control of microbial cells

The growing use of electrospinning to fabricate nanofibrous structures for use in wound dressings, tissue engineering and filtration processes has increased the need for an understanding of the interactions between bacteria and nanostructures. The adhesion characteristics and

Polymer	Solvent	Fibre diameter	Ref
Drug delivery system			
(a) Poly(ε-caprolactone) (shell) + poly(ethylene glycol) (core)	2-2-2-Trifluoroethanol (b) water	200–350 nm	[68]
(a) Poly(ε-caprolactone) and poly(ethylene glycol) (shell)-dextran (core)	Chloroform and DMF-water	1–5 μm	[69]
Poly(ε-caprolactone) (shell)- poly(ethylene glycol) (core)	Chloroform and DMF-water	500–700 nm	[33]
Poly(ε-caprolactone-co-ethyl ethylene phosphate)	DCM and PBS	~4 μm	[70]
Poly(d-l-lactic-co-glycolic acid)- PEG-b-PLA and PLA	DMF	260–250 nm	[71]
Poly(d-l-lactic-co-glycolic acid)	DCM	1–10 μm	[72]
Poly(d-l-lactic-co-glycolic acid)	THF:DMF	400–600 nm	[73–75]
Poly(l-lactide-co-glycolide) and PEG-PLLA	Chloroform	690–1350 nm	[76]
General tissue engineering			
Poly(ε-caprolactone)	Chloroform and methanol	2–10 nm	[77]
Poly(ε-caprolactone) (core) + zein (shell)	Chloroform and DMF	500–900 nm	[78]
Poly(ε-caprolactone) (core) + collagen (shell)	2–2-2-Trifluoroethanol	500 nm	[79]
Poly(d-l-lactic-co-glycolic acid) and PLGA-b-PEG-NH2	DMF and THF	400–1000 nm	[80]
Poly(d-l-lactide-co-glycolide)	DMF and THF	500–800 nm	[81]
Poly(ethylene glycol-co-lactide)	DMF and acetone	1–4 mm	[82]
Poly(ethylene-co-vinyl alcohol)	2-Propanol and water	0.2–8.0 mm	[83]
Collagen	HFP	180–250 nm	[84]
Gelatin	2-2-2-Trifluoroethanol	0.29–9.10 mm	[85]
Fibrinogen	HFP	120–610 μm	[86]
Poly(glycolic acid) and chitin	HFP	130–380 nm	[87]
Vascular tissue engineering			
Poly(ε-caprolactone)	Chloroform and DMF	0.2–1 nm	[88]
Poly(l-lactide-co-ε-caprolactone)	Acetone	200–800 nm	[89, 90]
Poly(propylene carbonate)	Chloroform	5 μm	[91]
Poly(l-lactic acid) and hydroxyapatite	DCM and 1-4-dioxane	300 nm	[92]
Chitin	HFP	0.16–8.77 nm	[93]

Table 2. A table showing polymers used for electrospinning and their targeted applications. Reproduced with permission from [84].

colonisation of bacteria on these materials is still not completely understood but is essential to aid their future development [62].

Although there has been a vast amount of research on the interactions between nanofibers and eukaryotic cells [63, 64] there has been very little research focussed on the interaction of micro-organisms with nanofibers [45]. An improved understanding of the fundamental processes

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involved in such interactions is essential to aid the further development of nanofiber mats for application in environments such as wound dressings, filtration and tissue engineering that can all be compromised by microbial colonisation.

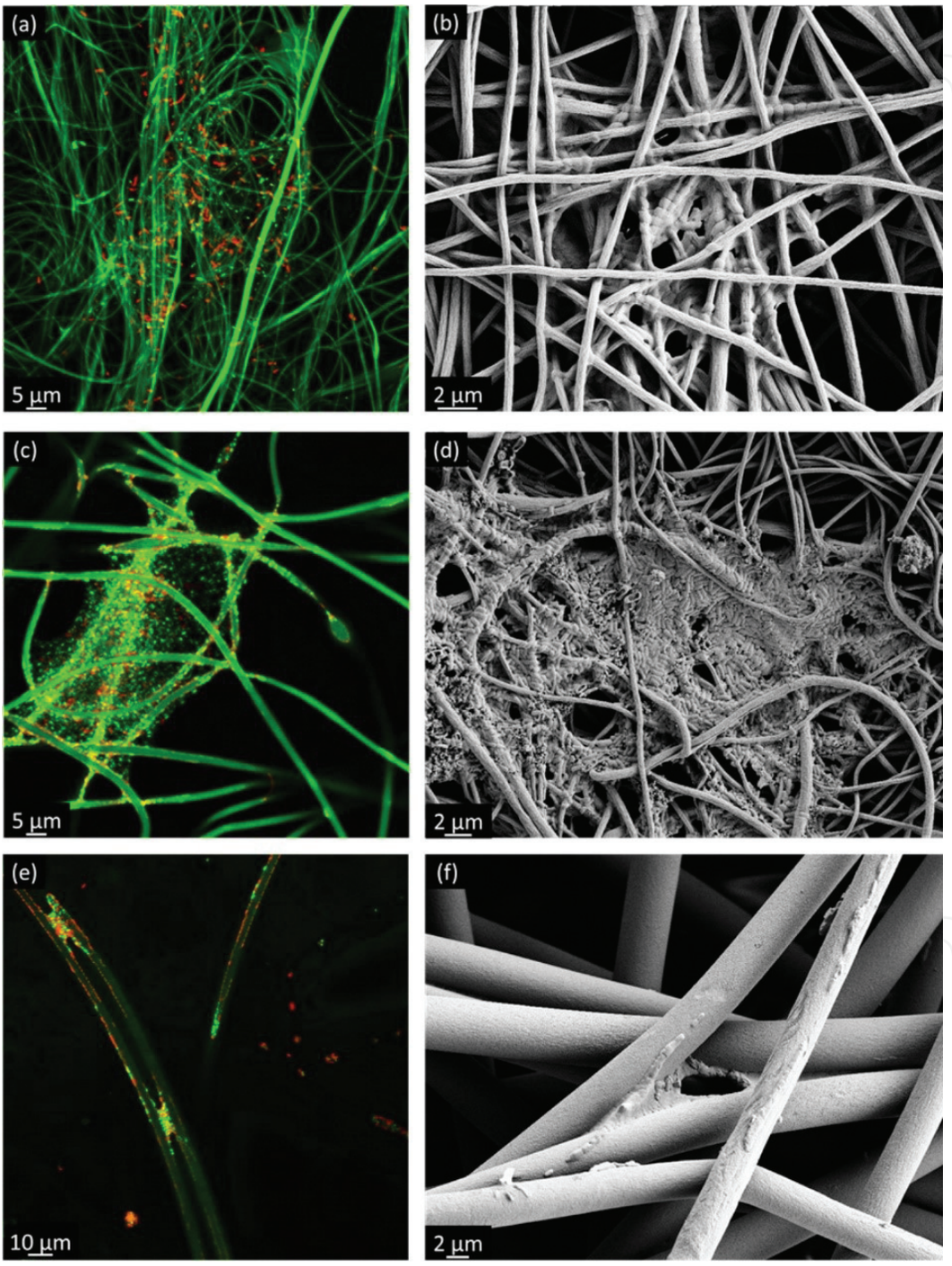

Figure 5. Confocal (a, b and c) and SEM (d, e and f) images of *E. coli* cells interacting with electrospun polystyrene meshes with varying fibre diameters. (a,b) Average diameter = 500 ± 200 nm; (c, d) average diameter = 1000 ± 100 nm; (e, f) average diameter = 3000 ± 1000 nm. Reprinted with permission from [65]. Copyright 2015 American Chemical Society.

Abriago *et al.* investigated the effect of fibre diameter on bacterial attachment, proliferation and growth at electrospun fibre constructs (**Figure 4**) [65]. In their study varying concentrations of polystyrene (PS) in DMF were used to electrospin fibres of different diameter from 300 nm to 3000 nm. Electrospun meshes were then tested against *E. coli*, *P. aeruginosa* and *S. aureus* both in solution and on agar plates. Their work demonstrated that the fibre diameter influences bacterial proliferation. An average fibre diameter close to that of the bacteria offered the best support for bacterial adhesion and proliferation. Rod shaped cells tended to wrap themselves around fibres with a smaller diameter than their length limiting the ability of the cells to bridge gaps between fibres and form colonies (**Figure 5**). Round cells tended to proliferate through nanofibrous substrates yet when the diameter was larger they were found to have adhered to the surface. Again, these findings were limited by the absence of a smooth control samples. In a further study Abriago et al. investigated the bacterial response to different surface chemistries of electrospun nanofibers [66]. Polystyrene (PS) nanofibers were electrospun and plasma coated with a number of different monomers including allylamine (ppAAm), acrylic acid (ppAAc), 1,7-octadiene (ppOct) and 1,8-cineole (ppCo). The same techniques as the previous paper were used to characterise bacterial interactions with the fibres [65]. The plasma coating did not induce a significant change in fibre morphology. The surface chemistry was found to have a significant effect on bacterial adhesion and proliferation. A ppAAm coating (hydrophilic and rich in amine positively charged groups) resulted in the highest attraction of viable *E. coli* cells forming colonies and clusters across the interstices of the mesh. There was a significantly lower number of *E. coli* cells found on fibres with a hydrophilic, negatively charged ppAAc coating. The cells spread throughout the fibrous network. Fibres with a hydrophobic ppOct coating were found to have a higher proportion of live cells when compared to untreated PS fibres forming clusters at fibre crossovers. The ppCo coating had no inhibitory effect although a high proportion of dead isolated bacterial cells were found to have adhered to the fibres. Cells were wrapped around fibres with no clusters at fibre crossovers or across the interstices of the mesh. The results demonstrate the effect of surface chemistry on the interaction between bacteria and nanofibers offering a further parameter to be altered during electrospinning fibre fabrication to meet a specific antimicrobial attachment application (**Figure 6**).

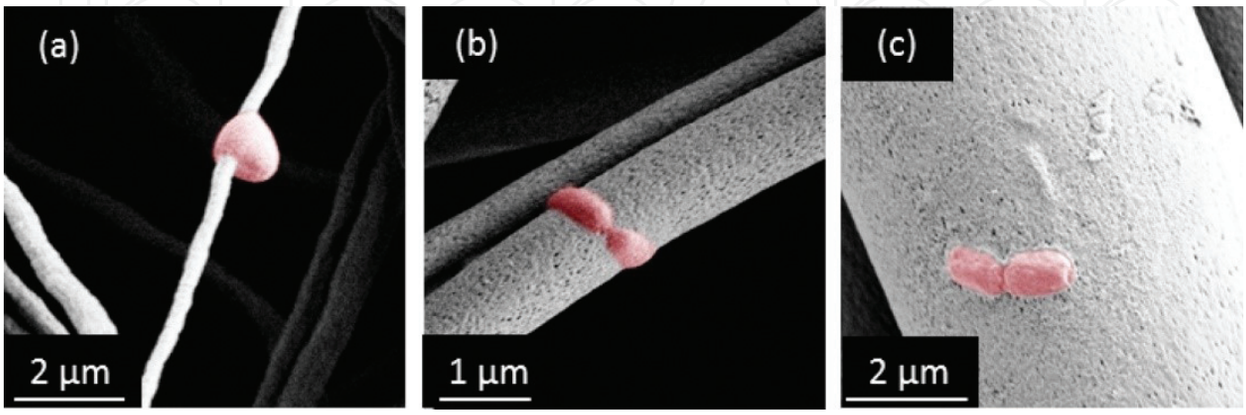

Figure 6. SEM images of *E. coli* cells (red) adhered to polystyrene fibres of average diameter (a) 0.3 µm (b) 1 µm (c) 5 µm. Reprinted with permission from [65]. Copyright 2015 American Chemical Society.

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4. Functional materials

There are a wealth of materials that have been electrospun for regenerative medicine and the choice of material is governed by the application. When choosing materials for tissue engineering, biocompatibility and biodegradability are the dominant functionalities that are required, so this has led to the electrospinning of natural materials such as collagen, gelatin and fibrinogen. However, concerns over contamination and interspecies transfer of disease agents has meant that other biodegradable materials have been considered and these include the aliphatic polyesters such as polylactic acid (PLA), polyglycolic acid (PGA), their copolymers (e.g. PLGA) and polycaprolactone (PCL). Electrospinning has an advantage of relatively easy incorporation of biologically active materials into the nanofiber construct. Obviously, this can be immobilisation after fibre fabrication, however there is scope to incorporate the material during electrospinning to create a one-step process, with economic benefit when considering scale-up, and possible mechanisms for controlled release, if required. Incorporation during the fibre fabrication stage may mean a homogeneous distribution of the material throughout the fibre construct as opposed to heterogeneous distribution at interfaces when the fibres are functionalised after fabrication. A caveat for the advantages bestowed by adding functionality through the addition of bioactive materials during the electrospinning process is the potential loss of activity due to location of the additives within the core of the fibre or compromise of the material because of the presence of aggressive solvents used in the electrospinning process. We now discuss the development and application of different materials that confer increased functionality onto the electrospun scaffold.

Material selection is critical in the design of nanofibrous scaffolds for biomedical engineering. The materials suitable for these applications are classified as "biomaterials". A common class of materials used for scaffold fabrication is that of polymers, defined as a large molecular chain composed of multiple repeated subunits. These can be both naturally occurring, "biopolymers", as well as man-made, "synthetic" polymers. The polymer size can be expressed in terms of its molecular weight which is directly related to its average chain length.

The human body's extracellular matrix is composed of natural polymers, primarily polysaccharides and glycosaminoglycan's [46]. Examples of biopolymers commonly used in artificial ECM production are collagen, gelatin, elastin, fibrinogen and chitosan. Examples of commonly used synthetic polymers are Poly (vinyl alcohol) (PVA), Poly (lactic acid) (PLA), Poly (vinylpyrrolidone) (PVP), Poly (lactic-co-glycolic) acid (PLGA) and Poly (ethylene oxide) (PEO). Biopolymers tend to be more biocompatible but have a lower tensile strength compared to synthetic polymers which are generally less biocompatible.

Polymers are generally processed in liquid form as either solutions or melts, with the choice of solvent (or lack thereof) depending on the polymer being used, its solubility and its intended application. The general chemistry rule is 'Like dissolves like', which means that only 'non-polar' solvents will dissolve 'non-polar' solutes and 'polar' solvents dissolve 'polar' solutes. As already mentioned, natural polymers show better biocompatibility than synthetic polymers. These include chitosan, gelatin, collagen, fibrinogen and many others. Synthetic polymers offer the advantage of being able to tailor the properties of the polymer for the desired

applications. A number of synthetic polymers are currently used in applications such as wound healing and tissue engineering.

Solubility is another challenge faced by engineers when selecting an appropriate polymer. Water solubility is often desirable in applications such as tissue engineering and drug delivery but for application such as filtration water insolubility and chemical stability are crucial parameters. Biodegradable polymers such as PLGA and PCL are often used for tissue engineering applications although these require a harmful chemical to be used as a solvent, which means that any residual needs to be removed before application to ensure it is not toxic. Another alternative is using a water soluble polymer such as PVA or PEO and using a suitable technique to crosslink it to reduce its water solubility. Poly (vinyl alcohol) (PVA) has been used for a number of applications in wound healing, tissue engineering and filtration. PVA is a biocompatible and biodegradable polymer which has a high hydrophilicity, good chemical resistance and is easily processed using a number of different techniques.

The water solubility of PVA presents a problem when required for most applications. This can be addressed by using a suitable method to crosslink the PVA increasing its crystallinity and thus reducing its solubility. This has been demonstrated using a number of methods such as chemical modification with functional groups including dialdehydes [67] and dicarboxylic acids, physical and chemical treatment with heat, irradiation and acid-catalized dehydration.

4.1. Natural materials

4.1.1. Collagen

Collagens are the main group of structural proteins to be found in the extracellular matrix (ECM), and are the most abundant protein found in animals, providing between 25 and 35% of the whole body protein count. Collagens share the characteristic triple helix structure of Gly-X-Y repeats, where X can be any amino acid, and Y is often proline or hydroxyproline. The individual chains form a left handed helix, and the three chains wind around one another in a right handed super helix [68, 69]. This structure makes the collagen fibres insoluble with high tensile strength [70]. Collagen is a highly versatile biomaterial, which has a wide range of applications, and is particularly suited to a wide range of *in vivo* applications due to its low immunogenicity [71–73]. These *in vivo* applications include wound dressings [74], artificial tissue and organ production [75], cartilage tissue regeneration [76, 77], drug delivery systems [78] and biomedical engineering [79].

In order for collagen to be utilised, it must be obtained in a usable form. It must first undergo extraction from an animal source. The standard procedure for obtaining a usable collagen mixture utilises the technique of solubilisation in acetic acid (AcOH), which can be accompanied by pepsin treatment where necessary to aid in both the speed of extraction and yield of the resulting product [80]. However, the use of pepsin in this process results in collagen which is lacking a significant amount of its telomeric region, and is named 'atelo collagen' as such. These extraction processes have been adapted and modified in many ways, though yield has never been shown to rise above 2% from wet weight or 20% dry weight, and some groups have modified the technique used to determine yield, based on hydroxyproline content to demonstrate small increases in yield more dramatically [81]. The extraction process does not lend

itself to be scaled to large production levels, and is usually held back by larger variants of benchtop laboratory scale equipment such as centrifuges and dialysis tubing. The difficulty in extracting collagen from source is why the current market cost per gram of collagen rests around one thousand eight hundred pounds (GBP) per gram of soluble material.

Collagen has been extracted from a range of sources, but is commonly obtained from bovine, porcine and equine sources for *in vivo* use [82]. However, these sources have problems with Bovine Spongiform Encephalopathy (BSE), other Transmittable Spongiform Encephalopathies (TSEs) and potential viral vectors that could be transmissible to humans [83, 84]. More recently, jellyfish have emerged as a source of collagen that is an attractive alternative to existing sources due to a plentiful supply Williams [85] and a safer source through lack of BSE risk and potential viral vectors [86] while exhibiting good performance *in vivo* when compared to bovine sources [87].

Once extracted, collagen can be used to form tissue scaffolds based upon various methods. Often a solution of collagen is lyophilised using freeze drying to form an open architecture based scaffold which is ideal for cell migration. This collagen can also be integrated into a solution of HFP or TFE on its own or as a copolymer and electrospun to form nanofiber scaffolds [88]. In both instances the resulting scaffold must be crosslinked using various chemical [89], enzymatic [90] or photoreactive methods [91] due to the extraction processing of the collagen which renders the material soluble to physiological and acidic conditions.

Electrospinning of soluble collagens provides a suitable way of producing scaffolds which closely mimic the high porosity and surface area often seen in small diameter blood vessels, and has the potential as a good tissue engineering scaffold for the production of three dimensional cell cultures, leading to potential applications such as skin grafts. Research into collagen electrospinning has been under pressure in recent years due to the findings that the primary solvents used to electrospin collagen, namely 1,1,1,3,3,3 Hexafluoro-2-Propanol (HFP) and 2,2,2-Trifluoroethanol (TFE) have been shown to denature collagen when dissolved into either of these solvents [57]. There have been efforts since to try and electrospin collagen using benign solvent mixtures [92] however many of these are unable to replicate the successes in fibre morphology and homogeneity that collagen electrospun out of HFP and TSE produced. As well as the use of solvent being of concern, the quality of the extract of collagen being used also affects how well the solution can be electrospun [93]. This relates to many factors from extraction conditions with critical points which must be addressed. **Figure 7** shows how collagen production can be influenced by a number of extraction conditions.

It is because of these issues that collagen has remained an undesirable polymer for electrospinning and some research has moved onto gelatin electrospinning [94]. This denatured form of collagen is a much more affordable alternative to collagen which is much more easily handled using benign solvents such as acetic acid and phosphate buffered saline (**Figure 8**).

4.2. Nanoparticle-nanofiber composites

The facile nature of the electrospinning process allows for the incorporation of additives such as nanoparticles and antimicrobial agents to form composite fibres. It is a simple process, with dispersions of polymers containing other materials being suitable for electrospinning. We now

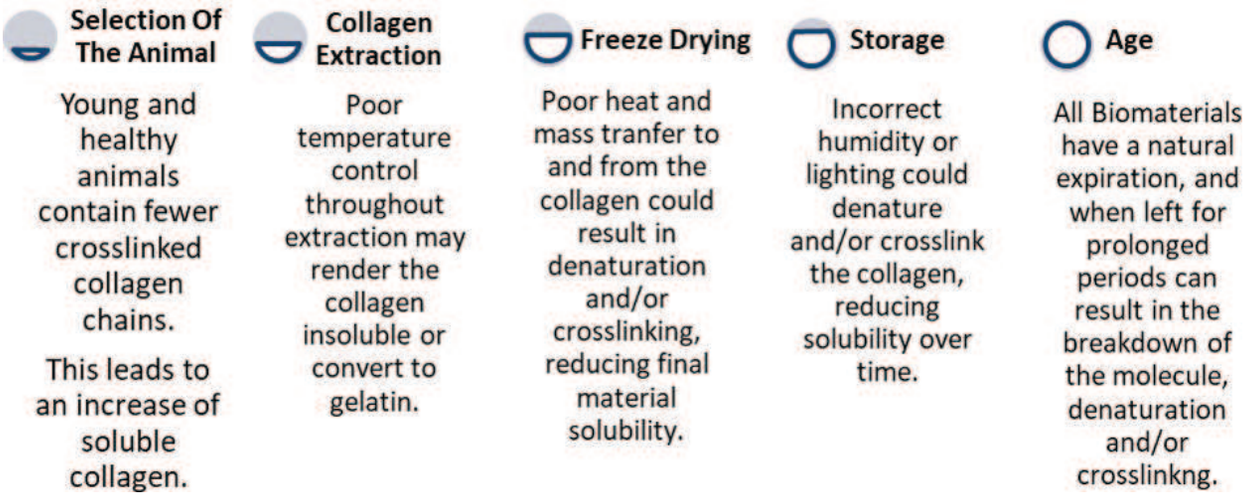

Figure 7. Chart of extraction stages which contain critical points necessary to avoid solubility issues with collagen extracts (Adapted from [93]).

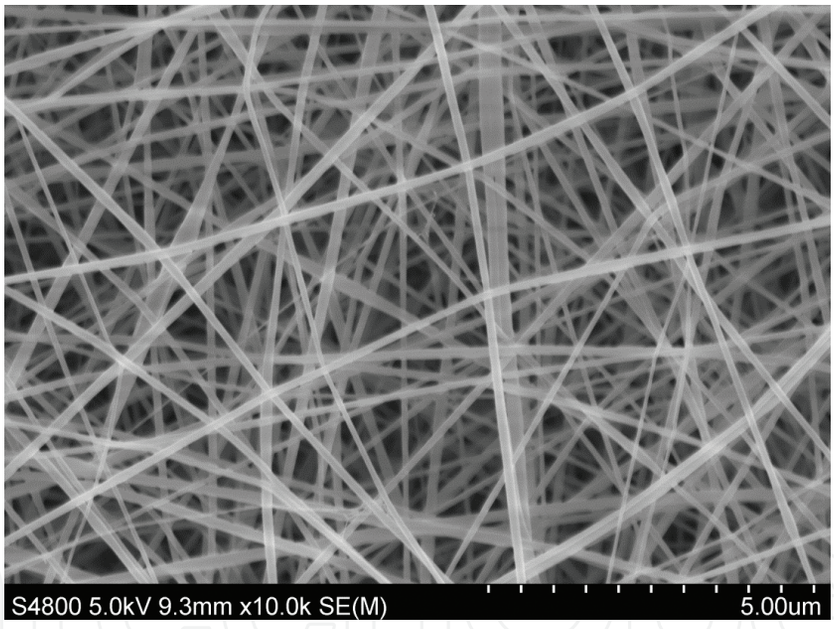

Figure 8. Scanning electron microscopy image of collagen electrospun from HFP. Fibre diameter 107 nm ± 37 nm.

examine the different modes of electrospinning including needle, free-surface and coaxial modes and how these have been used to create composite materials for biomedical engineering.

Electrospun nanoparticle-nanofiber composites have been subject to vast amounts of research in recent times, due to their functionality and unique chemical and physical properties. For example, the incorporation of silver nanoparticles into nanofibers formed from a biocompatible polymer have applications as chronic wound dressings. In another field, tissue engineering, the incorporation of iron oxide nanoparticles into tissue engineering scaffolds has been

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shown not only to improve the mechanical properties of the polymer nanofibers but also to add further functionality that improves osteogenesis in rabbit models.

There are generally 3 methods commonly used for the fabrication of nanoparticle-nanofiber composites using electrospinning; pre-synthesis, post-synthesis and *in-situ* synthesis. Each of the processes has its advantages, but the most promising technique is *in-situ* synthesis due to its ease of use, simple methodology and scalability.

Nanoparticles are more commonly synthesised before electrospinning or precursors are electrospun and the consequent nanofibers are treated to synthesise the nanoparticles within the nanofibers. More recently, *in-situ* synthesis techniques have emerged which allow for nanoparticles to be synthesised during the electrospinning process or in the solution to be electrospun with no pre-processing of nanoparticles.

The electrospinning process is generally unchanged with all particle synthesis techniques. If particles are pre-synthesised a co-electrospinning technique is generally employed where the nanoparticles are dispersed in a polymer solution before electrospinning. The other technique which can be used is coaxial electrospinning.

Electrospinning nanoparticles which have already been synthesised is the most basic and therefore most commonly used technique for the fabrication of nanoparticle-nanofiber composites. However, this process can often be multi-stage and time consuming requiring particles to be pre-synthesised and subsequently functionalised to reduce the effects of particle agglomeration and allow homogenous distribution throughout the nanofibers. A number of different nanoparticles have been incorporated into nanofibers using this technique including silica [95], titanium oxide (TiO_2) [96], Al_2O_3, Fe_2O_3, SiO_2 and ZnO [97]. Although this technique can be limiting it can also be seen as advantageous for some applications with pre-synthesis of nanoparticles is generally the only suitable method for preparing functionalised nanoparticle-nanofiber composites. Although the author would argue that *in-situ* synthesis techniques show more promise due to their simplicity and scalability, in some fields, including drug delivery pre-synthesis may be the preferred technique. This is due to the requirement of functionalised nanoparticles which is not achievable using emerging techniques.

Post treatment techniques involve the inclusion of a precursor in the electrospun nanofibers which undergo post-electrospinning processing technique to form nanoparticles within the nanofibers. This can also be achieved by immersing nanofibers in a nanoparticle solution, as presented by Razzaz *et al.* with TiO_2 nanoparticles [96], but this requires pre-synthesis of nanoparticles. Other researchers have included a precursor in the electrospun material and synthesised the particles within the fibres. These have included nanoparticles of iron oxide [98] and silver [99]. It could be argued that post-treatment techniques offer no more simplicity that pre-synthesis techniques, although both have their advantages and disadvantages. Generally, pre-synthesis techniques offer no control of the distribution of nanoparticles, although if the nanoparticles are well dispersed the distribution will be reasonably homogenous, throughout the fibres. Post-treatment does not generally allow for this, with encapsulation of nanoparticles difficult. Instead, with post-treatment techniques nanoparticles generally coat the fibres. This tends to result in nanoparticles being released or 'used up' quicker, making nanoparticle-

nanocomposites fabricated using a post-treatment technique unsuitable for long term applications but more suitable for applications where a short release cycle is desirable (**Table 3**).

In recent year's research has focussed on the development of *in-situ* synthesis techniques combined with electrospinning with less steps, more simplicity and lower production costs. This is an area that has been investigated in more depth for metal nanoparticles. For example, Jin et al. presented a one-step technique to prepare silver nanoparticles in Poly(vinylpyrrolidone) PVP nanoparticles [100]. In their study silver nitrate ($AgNO_3$) was reduced in a PVP/DMF solution with DMF as the reducing agent. Solutions were then electrospin resulting in PVP nanofibers containing silver nanoparticles. Saquing et al. presented a facile one-step technique to synthesise and incorporate silver nanoparticles into electrospun nanofibers [101]. They chose PEO as the electrospinning polymer which is also used as a reducing again for the metal salt precursor and protects the formed nanoparticles from agglomeration. The fibre quality was improved with the addition of the silver nanoparticles and fibre diameter was reduced due to an increase in the electrical conductivity of the solution. Other researchers have presented similar methods for silver nanoparticles [102] and iron oxide [103] and titanium dioxide [104].

In a recent study we have developed a novel one-stage *in-situ* synthesis technique to fabricate PEO and PVP nanofibers containing magnetite MNPs [105]. We have also demonstrated an ability to scale-up the process from laboratory to industrial scale using a commercially available free-surface electrospinning setup. In our technique, a 2:1 molar ratio of ferric and ferrous chloride is added to a PEO solution in deionised water containing sodium borohydride, used to reduce the ions to nanoparticles. The reaction is allowed to progress before being electrospun (**Figure 4**). Nanofiber mats were crosslinked using UV irradiation, EDX was used to confirm the presence of iron, DLS showed the average nanoparticle diameter to range from 8 nm (PVP) to 26 nm (PEO), XRD confirmed the phase of the nanoparticles to be magnetite and NMR showed a shortening in both T1 and T2 relaxation times confirming the nanoparticles could provide a suitable relaxation channel (**Figure 9**).

Nanoparticle material	Nanofiber material	Application
Zinc oxide	Poly(vinyl alcohol)/sodium alginate	Wound dressing
Silver	Poly(vinyl alcohol)	Wound dressing
Polyethyleneimine-capped silver	Polysulfone	Wound dressing
Silver	Polyurethane	Wound dressing
Titanium dioxide	Poly(vinyl pyrrolidone)	Wound dressing
Titania	Polyurethane	Wound dressing
Titania doped with zinc	Poly(vinyl alcohol)	Wound dressing
Silver	Polylactic-co-glycolic acid	Wound dressing

Table 3. A table showing different nanoparticle-nanocomposite materials with applications in wound dressings.

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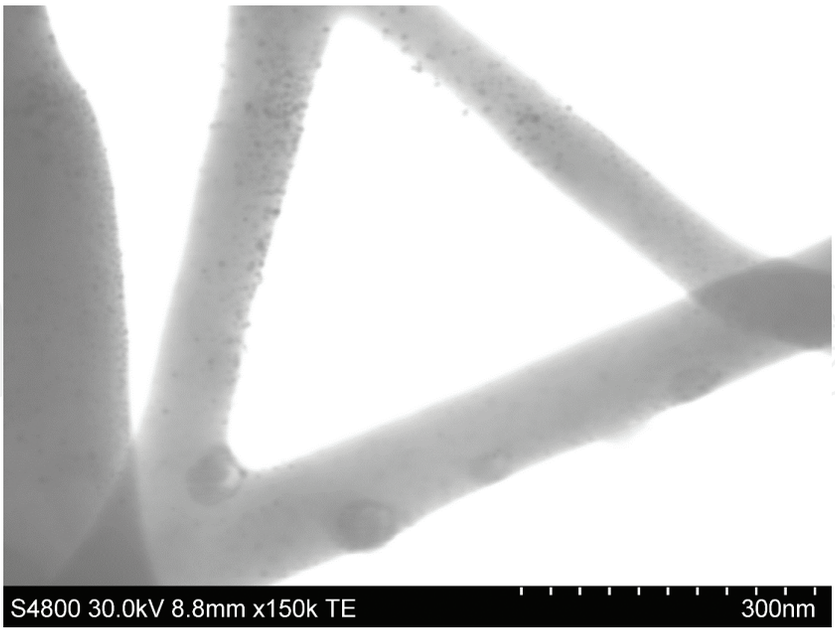

Figure 9. High magnification scanning transmission electron microscopy image of PEO nanofiber containing MNPs.

4.2.1. Applications of nanoparticle-nanofiber composites

Nanoparticle-nanofiber composites have been investigated for a number of different applications in a number of different fields, including antimicrobial applications [106].

Silver nanoparticles are the most commonly used for antimicrobial applications with various silver loaded wound dressings commercially available. Other nanoparticles with antimicrobial properties include copper [107], titanium dioxide (titania) and zinc oxide.

Nanoparticle-nanofiber composites are commonly used as tissue engineering scaffolds to improve mechanical strength, improve hydrophilicity, improve cell migration and proliferation and also for antimicrobial purposes to reduce the competition for colonising stem cells with bacteria. Mehrasa et al. electrospun PLGA/Gelatin scaffolds embedded with mesoporous silica nanoparticles [95]. The incorporation of the nanoparticles was shown to improve both the hydrophilicity and mechanical strength. They also showed improved cell proliferation when compared with pure PLGA scaffolds.

The incorporation of soluble factors and control of surface chemistry of tissue engineering scaffolds to provide biochemical cues have been well documented [108–110]. Magnetic scaffolds have been investigated for the regeneration and repair of tissues in damage and disease [111]. The incorporation of MNPs into scaffolds is also believed to increase *the rate* of both bone cell growth and differentiation. This is due to the tissues ability to recognise the mechano-electrical conversion that can lead to an increased cellular proliferation and expression levels of a number of genes related with bone differentiation [112, 113]. Magnetic scaffolds have also been shown to have applications in tissue engineering [114, 115].

4.3. Wound healing

Electrospinning is becoming a commonly used process in the development of wound dressings with the capability of spinning fibres from a range of both synthetic and natural polymers [7, 44, 116, 117]. They are suitable due to their porosity which allows them to be permeable to water. They are also very absorbent due to their high surface area to volume ratio. The small pore size can be controlled and modified offering semi permeability allowing the wound to stay moist while offering protection from bacteria.

Although they can offer protection from bacteria migrating to the wound they do not protect them from bacteria already present in the wound. Bacteria can still colonise the external surface of the dressing and this can reduce its permeability. For this reason nanofibers are often functionalised with antimicrobial agents [102, 117, 118]. There are a number of dressings on the market which contain antimicrobial agents, with ionic silver being the most commonly used. These offer a number of benefits; not only do they keep the wound aseptic, they can also stop the dressing becoming populated by bacteria.

Silver nanoparticles have been incorporated into nanofibers by a vast amount of researchers. Nguyen *et al.* prepared PVA nanofibers containing silver nanoparticles with applications in wound healing using a combination of microwave irradiation and electrospinning [119]. These were shown to have antimicrobial efficacy against *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative), 2 wound relevant organisms. Lakshman et al. also incorporated silver nanoparticles into nanofibers for a wound healing application, using Polyurethane (PU) as the electrospinning polymer due to its current use as an exudate absorptive wound dressing material [120]. The fibres showed a zone of inhibition in a Kirby Bauer disc diffusion assay against *Klebsiella* and was also capable of absorbing 75% of water compared to the control PU sponge.

5. Conclusions

Regenerative medicine is a major focus for 21st century health care and the fabrication of functional nanofibers through electrospinning is an important underpinning technology that is essential if the exciting advances in medicine are to fulfil their potential. As further understanding is achieved for the treatment of disease states by biologically active materials this must be matched by optimisation of electrospinning processes that are able to deliver the functionality to the wound bed or the bodies tissues. Research continues to focus on electrospinning control parameters, which are improving the fundamental understanding of the process with benefit to optimisation strategies and the efficient incorporation of functional materials. This fundamental and optimisation research is also necessary with the recent developments in instrument configurations as the technology of electrospinning emerges from the laboratory bench scale into the realms of economic feasibility and volume manufacture.

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