Electro spun scaffold for tissue engineering –A review article

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Abstract

In this review paper attention is given to scaffold preparation through electro spinning technique as a biomaterial for tissue engineering applications. An extensive literature review was performed on the various natural, synthetic and semi synthetic polymers for scaffold making as well as the role of process parameter on electro spinning such as concentration of the polymer solution, viscosity and delivery time etc. and the characterization related reviews were reviewed .In addition to that the recent development in scaffolds such as composite scaffolds and bi- component material based scaffolds were also described .

1. Introduction

Scaffolds are three dimensional structures with the mimicking the microenvironment of tissues [Guoping C et al,2002]. Cells will seed into this artificial structure capable of supporting three-dimensional tissue formation. Frequently it involves a biodegradable matrix containing biologically active materials which is combined with living cells to develop a construct or scaffold which can be used for the purpose of replacing damaged or lost tissue. These scaffolds must be designed to promote cell colonization, migration, growth and differentiation and should also direct the tissue development process to create the necessary healthy tissue in a topologically required form. A scaffold should have the following important properties such as it allows the attachment and proliferation of seeded cells , it allows the diffusion of cell nutrients , it exerts biological and mechanical influences to the cells.

According to Wan-Ju Li[2007] Tissue engineering has emerged as a promising alternative for the reconstitution of lost or damaged organs and tissues, circumventing the secondary complications associated with autografts, allografts, and alloplasts with polymeric scaffolds.The scaffold or three-dimensional (3-D) construct provides the necessary support for cells to proliferate and maintain their differentiated function, and its architecture defines the ultimate shape of the new bone and cartilage[Wan-Ju Li et al,2007].According to Lissa E et al[1994]Ideally cell scaffolds for tissue engineering should meet certain criteria .The scaffold surface should permit the cell adhesion .The Scaffold polymer should not be toxic when implanted in vitro culture. The scaffold should be capable of forming three dimensional structures. The scaffold porosity should be at least 90% .Scaffold regeneration rate should be adjustable to match the rate of tissue regeneration by the cell type of interest.

Electro-spinning has recently emerged technique for generating biomimetic scaffolds made of natural and synthetic polymers for tissue engineering applications. The process of electro- spinning, well known for manyyears in the textile industry in synthetic fibre production arena has recently re-emerged as a novel tool for generating biopolymer scaffolding for tissue engineering (Buchko CJ,1999). In general electro spinning produces the nanofibrous scaffolds from a variety of polymer

materials, including synthetic polymers and natural proteins. The topology of these electrospun scaffolds closely mimics that of native ECM. According to Wan-JuLi et al [2007] Scaffolds fabricated by electro spinning have a totally different appearance and structure to those made by self-assembly and phase separation. Furthermore, electro spinning technology is exceptionally useful for the fabrication of tissue engineered scaffolds, for not only is the electro spinning equipment economical, but the preparation and fabrication phases are relatively quick compared with phase separation and self-assembly. In addition, the electro spinning system is easy to set up and can be modified to fabricate nanofibrous scaffolds that meet specific requirements for structural or mechanical needs. The advantages of electro spinning technology make it suitable for both small quantity production for laboratory research use and mass production for industrial use

biomaterial scaffold serves as a 3D matrix for in vitro cultureas a template to recruit surrounding hostcells to conduct the repair process, a principal objective of scaffold design for tissue engineering is to create a structure that can simulate the native extracellular matrix (ECM) until cells seeded within the scaffold and/or derived from the host tissue can synthesize a new, natural matrix(Wan-Ju Li(2007). Among nanostructures, nanofibers are more suitable for use as the basic component of a scaffold compared with nanoparticles, due to their continuous structure.The advantage of a scaffold composed of ultrafine, continuous fibers are high porosity, variable pore-size distribution, high surface-to-volume ratio, and, most importantly, morphological similarity to natural ECM [Li WJ,2002]. The combination of these features makes nanofibrous structures a favorable scaffold for tissue engineering in recent tissue engineering applications, as shown by several studies described below.

The ability of cells to build tissues and maintain tissue-specific functions critically depends on epigenetic factors, such as the unique cell/tissue-specific microenvironment.Some of the major factors contributing to this unique microenvironment are cell–cell interactions and the organotypic extracellular matrix (ECM). Interactions between cells and ECM are crucial to cellular differentiation and in modulating or redirecting cell function [Nishimura,2003]. Fibers with diameters in the range from several micrometers down to less than 100 nm have a very highsurface area to mass ratio, and can be electro spun into3-D scaffolds with very high porosity. In this way bio- mimetic matrices can be fabricated by electro spinning technique[Min BM ,2004].

2. Polymers for biomimetic scaffolds

Recent studies, suggesting that cells are able to recognize and distinguish geometric properties of substrates ,such as shape and/or roughness. Sinha RK et al [1994] reported that surface roughness had an effect on osteoblast, endothelial cell, and fibroblast morphology, cytoskeletal properties, and proliferation. Other studies have reported that adhesion, proliferation, synthesis of alkaline phosphatase, and deposition of a calcium-containing mineral were all enhanced when osteoblasts were cultured in a nanophase ceramic, compared with micro-grain size ceramics [Webster TJ et al,1994]. Increased functions of osteoblasts have also been correlated with a decrease in the diameter of carbon nanofibers [Elias KL et al,2002]. These observations suggest that a scaffold composed of nanometer-scale components is biologically preferred. As a result, nanometer structural components should be preferred for the fabrication of functional tissue-engineered scaffolds.

The internal porous structure of void spaces, or porosity, is a physical component of a biomaterial scaffold that is also dependent on the architectural scale. These pores serve as pathways for mass transport (convection and diffusion), while also providing void space for cells to form new tissues [Muschler GF ,2004].According to Li WJ[2002] polymers such as poly(_-caprolactone)(PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and the copolymer poly(lactide-co-glycolide) (PLGA have been used for nano fibrous scaffolds.

As reported by Ramakrishna et al [2006]. A porous structure made through nanofibers is a dynamic system where the pore size and shape can change, unlike to conventional rigid porous structures. If required Nanofibers can also be formed as rigid structure.Electro spinning is the one the high productivity method to produce nanofibres.Scaffolds produced with synthetic and natural polymers through electro spinning can be produced with diameters ranging from tens to hundreds of nanometers with controlled morphology and function. The potential of these electrospunnanofibers in human healthcare applications is promising, for example in tissue/organ repair and regeneration, as vectors to deliver drugs and therapeutics, as biocompatible and biodegradable medical implant devices, in medical diagnostics and instrumentation, as protective fabrics against environmental and infectious agents in hospitals and general surroundings, and in cosmetic and dental applications etc.. [Ramakrishna et al 2006].

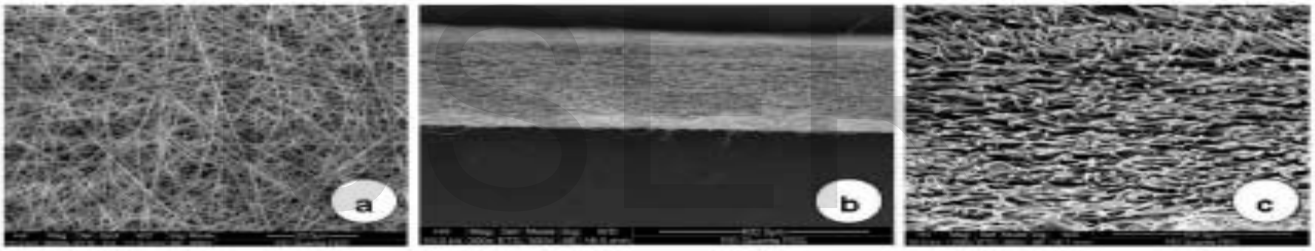

Figure.1 Ramakrishna et al [2006] Anelectrospunpolysulphone membrane: (a) surface; (b) cross-section; and (c) magnified cross-section images.

PGA has been extensively used in various biomaterial applications. Due to its high crystallinity, it is insoluble in general organic solvents, with highly fluorinated solvents. The PGA fiber diameter, ranging from 110 nmto 1.19 mm, increases with polymer concentrations from 0.05 to 14.3 wt.%. PGA polymer in a higher concentration solution encounters stronger molecular entanglement, resulting in thicker fibers during the electro spinning [Boland ED et al 2001 &2004].

PLGA has been extensively used in medical products and was one of the first biodegradable polymers electrospun for tissue engineering applications[Ko FK etal 2000]. The PLGA copolymer has an amorphous structure, because the constituent PGA and PLA molecules are unable to pack tightly to one another. Katti et al. and Berkland et al. have both used PLGA for electro spinning to investigate the parameters affecting fiber morphology [Katti DS et al 2004 &Berkland C et al,2004]. Their results showed that, in addition to polymer concentration and charge density, orifice diameter also has an effect on the morphology and diameter of electrospun fibers. poly(a-hydroxy ester) family is poly(e-caprolactone) (PCL) is also a semi crystalline biodegradable polymer. Compared to other polyester family members such as PLA, PGA, and PLGA, PCL has been used less frequently as a material for

fabricating biomaterial scaffolds, mainly because of concern over its slower degradation kinetics (Pitt CG et al ,1990).

Poly(ethylene-co-vinyl alcohol) (PEVA) PEVA is a semi-crystalline, biocompatible but not biodegradable polymer. Recently, it has been electrospun and evaluated for its potential in tissue engineering scaffolds. PEVA is a hydrophilic polymer that is insoluble in aqueous solution due to the presence of both vinyl alcohol andethylene groups. Most hydrophobic polymers have the property of slow degradation, whereas most hydrophilic polymers exhibit a rapid degradation rate. Kenawy et al. have prepared the PEVA solution by dissolving PEVA in a 70% alcohol solution (rubbing alcohol) at 65 _C for electro spinning [Kenawy EL R et al,2003]. Interestingly, the polymer tends to precipitate when sitting in room temperature for several hours.

Poly(ethylene terephthalate) (PET) PET has largely been used in biomaterial applications, especially in blood vessel prostheses, since PET is inert and does not interact with blood cells. Ma et al. investigated howelectro spinning time affects the thickness and porosity of electrospun PET scaffolds [Ma Z, Kotaki M et al,2005]. The electrospun PET nanofibers fabricated using optimal parameters ranged from 200 to 600 nm. The thickness, mass per area, and porosity of the PET fiber mats all increase with increasing electro spinning time. Polyurethane (PU) PU is a non-biodegradable biomaterial with good blood and tissue compatibility, which is primarily used in vascular implants or wound dressing. PU is ideal for applications for tissue engineering products that require stable mechanical properties or structural integrity.Poly(ethylene oxide) (PEO) PEO is a commonly used biomaterial for tissue engineering because of its capability to gel in situ. A PEO gel can be directly injected into an irregularly shaped defect site and photopolymerized to help tissue repair(Elisseeff J et al,1994).In addition to that ,poly(L-lactide-co-_-caprolactone) [P(LLA-CL)] has beenelectrospun into Zanofibrous scaffolds for engineeringblood vessel substitutes [Xu CY(2004)]. Polyurethane (PU)nanofibrous membranes have been utilized as wounddressings [Khil MS ,2003].Collagen and elastin are used as extracellular matrices of manytissues (Toshima M ,2004).

Chitin and Chitosan Chitin and chitosan are biocompatible and biodegradable natural polymers, used in biomedical applications and cosmetics. Chitosan (polyd- glucosamine) is derived from chitin (poly-N-acetyl-d-glucosamine), a polysaccharide formed in shellfish exoskeleton, which has received more attention in biomaterial development than chitin due to its solubility. Therefore, chitosan has been critically considered as a candidate biomaterial for tissue engineering scaffolds [SechriestVF et al,2000). Chitosan carries a high cationic charge density and can interact with various anionic polymers, such as chondroitin sulfate, to form a hydrogel scaffold

silk fibroin is another protein-based, natural polymer most commonly used for electro spinningfor the fabrication of tissue engineering scaffolds [Jin HJ etal,2002]. One of the most significant propertiesof silk is its excellent mechanical properties. Natural silk is produced by spiders or silkworms, with different composition and properties among species. A study by Ohgo et al. comparing three electrospun silk fibroin nanofibers suggested that silk fibroins from two different silkworms and genetically engineered silk-likeprotein each required individual optimal polymer concentration for electro spinning[Ohgo K et al,2003]. In addition, the engineered silk-like protein nanofibers were reported to be smaller than the natural silk fibroin nanofibers, and the mechanical strengthof silk fibroin nanofibrous mats was also dependent on silk fibroin type.

Fibrinogen Purified from blood plasma, is protein that plays a critical role in wound healing. In the presence of thrombin, fibrinogen gives rise to fibrin which forms fibrous clots that have found use as a clinical fixative, due to its natural role in wound healing[Silverman RP et al ,1999].electrospunnano fibrous fibrinogen mats are highly suitable for wound dressing and hemostatic products. Wnek et al. have fabricated and characterized electrospun fibrinogen scaffolds [Wnek GE et al,2003], and found the fibrinogen mats to be composed of uniform, randomly orientated fibrinogen nanofibers. Notably, the electrospun fibrinogen fiber typically exhibited a granular appearance with 22.5 nm banding, characteristic of native fibrinogen.

3. Nano Composite Polymeric Scaffolds

Current scaffold development aims to incorporate many polymer types for the fabrication of biomaterial scaffolds that are able to respond to the biological activities of cells while meeting specific host tissue site requirements. Regardless of whether a double, triple, even quadruple polymer blend/mix is used, a tissue engineering scaffold made of a polymer blend should still retain the properties of each polymer type. Therefore, it is expected that electro spinning of a polymer blend/mix will create novel composite scaffolds with enhanced performance for tissue engineering. Another practical reason for electro spinning a blend of polymers is that often the polymer of interest cannot be electrospunas uniformfibers. Three general composite nanofibrous scaffolds, natural–natural, synthetic–synthetic, and natural– synthetic, have been developed and characterized for their properties and potential applications.

3.1 Natural fibre composites scaffolds

Electro spinning of multiple natural polymer blends can yield a mixture of natural nanofibers that closely mimic the native ECM. A large percentage of native tissues contains both collagen and elastin fibers that are frequently subjected to tensile and elastic loading, respectively. Electrospun fibrous scaffolds composed of collagen types I and III, and elastin have been fabricated to replicate the native ECM of blood vessels (Boland ED ,2004). The fiber diameter found within the native ECM of blood vessels ranges from 270 to 710 nm, which is slightly larger than the 100 to 680 nm diameter range of nanofiberselectrospun from blends of collagen types I and III. Aside from protein–protein mixtures, the protein-based silk and carbohydrate-based chitosan blend has been electrospun into nanofibers as well. In the silk fibroin/chitosan blend for electro spinning, the addition of chitosan increases the viscosity and conductivity of the blend solution, thus enhancing the formation of smaller, uniform nanofibers[Park WH ,2004].

3.2 Synthetic fibre Composites scaffolds

The synthetic blending technique is followed to produce the combined properties of two or more polymers,such as PLA and PCL, are biodegradable, biocompatible, and hydrophobic whereas poly(ethylene glycol) (PEG) is hydrophilic, non-immunogenic, and nonbiodegradable. In mixing PLA and PEG, the resultant PLA/PEG blend is more hydrophilic than PLA and also more biodegradable than PEG.

In addition to improvements in biodegradation, the polymer blend has been shown to exhibit flexible mechanical properties that can be altered by the ratio of composed polymers. For example, the elastic PEVA nanofibrous mat becomes stiffer after PLA is added for blend electro spinning[Kenawy el R etal,2002].

3.3 Mixure Composites scaffolds

The mixing of natural and synthetic polymers canbe a major challenge for electro spinning since solvents functions are limited to one category of fibre. Therefore, the choice of solvent becomes a primary consideration afterdetermining the blend components. Many natural polymers are difficult to electrospininto nanofibers, especially when dissolved in water, since their polyelectrolytecharacteristic interferes with fiber formation. One alternative is to add syntheticpolymers such as PEO to facilitate nanofiber formation. The natural polymer/
PEO blend in aqueous solution can be electrospun, and the use of organic solventsmay be avoided. Duan et al. have systematically characterized the properties ofchitosan/PEO blend solutions and their electrospunfibers[Duan B etal,2004]. They concludedthat the chitosan/PEO blend retains conductivity, surface tension, and viscosity,andfavors the formation of smaller, uniform nanofibers, compared with pure chitosanor PEO solutions. Surface modification of scaffolds, with the intent to improve biocompatibility, has been extensively studied and many common techniques such as plasma treatment can also be considered for polymer nanofiber modification. Sanders et al(2005) .introduced different surface charges on electrospun PU fiber surfaces through plasma-induced surface polymerization of negatively or positively charged monomers.

4. Characterization of electro spinning processing parameters

4.1 Concentration of polymer solution

Concentration of the electrospun polymer solution has a direct relation on fiber diameter. Higher polymer concentration yield larger diameter nanofiberswhere as lower polymer concentrations results in small diameter fibers [Ryu YJ et al,2003]. If the polymer concentration is not adequate for chain entanglement, will results in fibre beads. These fibers obtained will have an odd appearance and will be depositing on a small area of the target.The concentration of the polymer solution, applied voltage, air gap distance, and delivery rate are critical experimental processing variables which determine the shape and size of electrospunfibers[BuchkoCJ ,1999 &Katti DS 2004].Mengyan Li [2005] and co-workers investigated the polymer the concentration effect on scaffold dimension .For that the solution delivery rate was kept constant at 5 ml/h and the appliedvoltage and air gap distance were set as 10–15 kV and 10–20 cm, respectively. They found that by decreasing the concentration of gelatin in the solvent HFP from 8.3% to 2%, the average size of gelatinfibers was reduced from 485±187nm to 77±41nm . Further reduction in gelatin concentration reduced thefiber size to below 100nm. However, this reduction of fiber size was accompanied by a significant formation of beads.

4.2 Spinning Voltage

Another important parameter in electro spinning is the applied voltage. Voltage has an inverse effect on the fiber diameter. As reported by Liu HQ et al,[2002]When voltage increases, fiber diameter decreases . When voltage is applying, charges will generate on the surface of the droplet and the corresponding whipping instability and stretching of the jet also increases, followed by the faster evaporation of the solvent from the polymer solution to form dry nanofibers. At very low voltage, due

to low electrostatic pulling force towards the metallic target, Taylor cone formation will be absent and thus prevents the fiber formation. If the voltage increases beyond the threshold level, the pulling force crosses the surface tension of the droplet and forms taylor cone.

4.3 Conductivity

The basic principle of electro spinning is the transfer of electric current from power source to the terminal of the needle tip through polymer solution. Therefore the polymer solution with no electrical conductivity will acts as insulators and such solutions cannot be electrospun. According to Kim B et al (2002)a minimum electrical conductivity is required for polymer solutions to spin as nano fibre. Highly conductive solutions will results in low diameter fibers compared to low conducting solutions. Generally, most of the organic solvents using for electro spinning have lower conductivities because of their dielectric nature, but when it dissolve increases the conductivity. According to Zuo WW et al,[2005] the presence of ionic functionalities increases the polymer solution conductivity.

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4.4 Surface tension

Surface tension is a property of the surface of a liquid that allows it to resist an external force. It is believed that in electro spinning, electrostatic force is countered by surface tension forces in the fiber extension region. When the surface tension of the solution is less, the force required to form the Taylor cone will be less and the reverse is vice versa[Khalid Z et al,2011]. The surface tension of a polymer solution is also be controlled by the selection of solvent.

4.5 Flow rate

The speed or rate at which a polymer solution is pumping to the tip to produce nanofibers is known as flow rate. During pumping, the solution initially becomes a drop on the tip of the syringe and when the voltage overcomes the surface tension, tayler cone will be formed and results into fibers. If the pumping rate and pulling force are equal, there will be a continuous and uniform production of nanofibers from the solution. At lower flow rate, the Taylor cone formation will start from needle interior to form low diameter fibers whereas at higher flow rates, larger diameter fibers will be formed[Ji HH et al,2005].As reported byMengyan Li et al [2005] Increasing the delivery rate from 1 to 3 ml/h yielded a significant change in fiber diameter from 431±105nm to 533±119 nm, from 349±97nm to 460±148nm for collagen and gelatin, respectively. A further increase in the rate of delivery from 3 to 8 ml/h did not significantly change the mean size (diameter) of collagen and gelatine fibers.

4.6 Polymer molecular weight

Molecular weight of a polymer is a measure of chain length of the polymer.When molecular weight increases, in general behavior, the increment in average chain length affects the whipping instability region polymer droplet and leads to slight increase in fiber diameter [Jung YH ,2005].

4.7 Capillary tip

Both conducting and non-conducting materials are used for the needles. Metallic needles are more preferable to make nanofibers because of the high conductivity. As the gauge of the needle increases, the fiber diameter decreases. This is directly related to the amount of polymer solution coming out of the tip in unit time [Doshi J etal,1995].

4.8 Dielectric constant

Dielectric constant is a measure of intensity of electrostatic lines of flux on the polymer solution. In other words, it is the quantification of the amount of electrical charge, a solution can hold. With solution of high dielectric constant, the surface charge density on the jet will be more and thus results in low diameter fibers [Ramakrishna S etal,2006].

4.9 Boiling point of the solvent

In electro spinning, the formation of nanofibers really depends on the rate of evaporation of solvent from polymer solution. Theoretically, all traces of liquids should vanish from the solution when fibers get deposited. If the solvent is not evaporating in time, fibers will be in wet condition and forms ribbon like or fluid filled shape. Use of volatile solvents will reduce this tendency and makes the fiber diameter lesser. But very high volatility of the solvents may results in the evaporation of solvent in the interior of the needle and subsequently changes into a block in the solution flow [Supaphol P etal ,2005].As reported by Mengyan Li et al [2005] For tissue engineering applications, bioartificial scaffolds are preferably porous, pliable, and elastic, so that cells will be able to integrate and/or push the ECM-likefiber aside as they grow into the scaffolds.

It is important to measure the mechanical properties of scaffolds to be utilized for Tissue engineering. Mengyan Li et al [2005] has examined the tensile properties through micro tensile test . For that for these electro spun fibers are fed in to the system in sheet form. Fig. 2shows the strain–stress curves of 08% gelatin, 10% collagen, 20% tropoelastin, and 20% elastin.

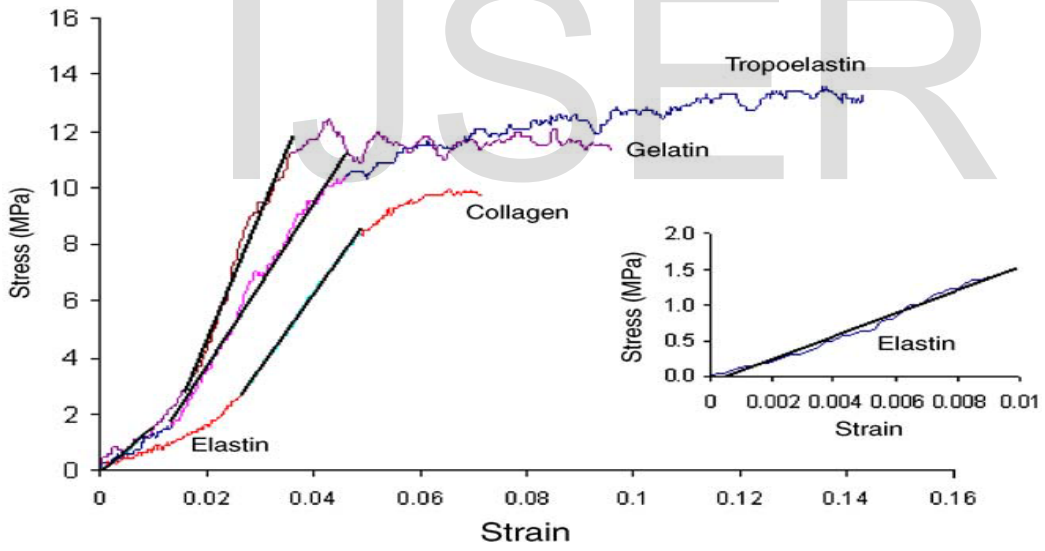

Figure-2 As reported by Mengyan Li et al [2005] Microtensile test of electrospun natural polymeric fibers. Compared are tensile strengths of 8% Gelatin, 10% Collagen, 20% Tropoelastin, and 20% Elastin fibers

From the above graph it is obvious that electro spun collagen fibers have a lower tensile modulus than gelatine fibers, but have similar tensile strength (8–12MPa) and ultimate elongation (0.08–0.1), respectively.

5. Summary

Recent days the high attention towards the electro spinning due to the interest of nano scale properties ,more specifically due to the high demand in tissue engineering towards scaffold making. The tissue engineering community has begun to capitalize on the inherent nanoscale nature of electrospun polymeric fibers to produce scaffolds which mimic native ECM. Electrospun fibres can be formed as fibre matrices, which are able to support the attachment and proliferation of a wide variety of cell types; moreover, the cells are able to maintain their phenotypes on these nanofiber scaffolds.

This electro spinning can have facility to produce the scaffolds with aligned fibers, different compositions, improved mechanical properties, varying degradation rates, or functional moieties can be produced. Nevertheless, despite the comprehensive experimental and theoretical studies illustrating the ability to control fiber formation by changing the various process parameters of elcetro spinning machine. But still precise control of fiber morphology will be necessary for improved scaffold designs that better recreate the functions of native extracellular matrix.

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