Review Paper:

Biodegradable natural and synthetic polymers for the development of electrospun nanofibrous scaffolds for various tissue engineering applications

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Abstract

Following a tissue injury/ damage, the regeneration of cells at the affected area is very critical. Fewer cell types like dermal cells are capable of cellular division to a smaller extent. But following more significant cellular damage, there comes an urgent need to replace these defective cells or tissues, where most traditional approaches of medicinal treatments fail. Tissue engineering offers an alternative solution that is more ideal under these circumstances. It is an interdisciplinary field of science that tries to integrate life science into engineering principles with the purpose of replacing damaged cells/tissues.

In addition, the overall process of tissue growth and differentiation in vivo requires the presence of various growth factors at the nano-sized range. The current trend is to encompass nanotechnology along with tissue engineering aspects. Scaffolds being the most important component of tissue engineering application, the electrospinning process is used to generate nanofibrous scaffolds. Electrospinning makes use of different natural and synthetic polymers. These polymers can provide structural support upon which cellular growth occurs and hence these polymers are suitable for various biomedical applications. This review study attempts to cover the basics of the electrospinning process, different natural and synthetic polymers and their significant biomedical applications.

Keywords: Tissue engineering, electrospinning, nanofiber, natural polymers, synthetic polymers.

Introduction

Most often, cellular/tissue damages are associated with certain ailments, injuries and traumas. Immediate replacement of such tissues is a crucial event in survivability. Transplantation biology helped in solving the dilemma to some extent. But the constraints associated with transplantation surgery like painful and expensive removal of autografts, immunological incompatibility of allografts and even the non-availability of suitable recipient-specific cells/tissues pointed up the necessity of some new approaches. Tissue engineering (TE) is an interdisciplinary field that incorporates life science aspects into engineering which aims to improve, restore and maintain the functional aspects of damaged tissue[47].

The term tissue engineering was officially coined in 1988 at the National Science Foundation workshop to imply "the applications of life science and engineering principles and methods to develop an understanding on fundamental structure-functional relationship of both normal and pathological mammalian tissues and for the development of biological substitutes to maintain, restore or to improve tissue functions." This fascinating science thus offers an alternative choice for treating damaged tissues with one's own normal healthy cells/ tissues. Such cells are usually seeded onto certain polymeric biomaterials commonly referred to as scaffolds in TE. The important steps in tissue engineering is given in figure 1.

In vivo, extracellular matrix (ECM) provides the growing cells with a meshwork of a diverse number of biomolecules (mainly proteins and some carbohydrates) to aid in the process of cellular maintenance, growth and regeneration. These ECM components make up a large volume of tissues and the rest includes the cells. But however, in TE applications, scaffolds act as the critical component, biomimicking most of the ECM functions. Thus, scaffolds function as one of the chief components of TE application studies. Even though they provide the cells with a suitable adhesive surface, there are certain hurdles still associated with scaffold usage. Improper cellular vascularization, poor nutrient availability, the poor mechanical strength of engineered cells and immunological incompatibility with host tissues are some major obstacles[116]. Currently, a diverse range of scaffold fabrication strategies exists. But many suffer the aforesaid limitations.

Nanotechnology, the science of nano-sized particles, offered a new possibility for scaffold synthesis. It is one of the most exploited fields of science in the 21st century. The birth of nanotechnology opened the gateway to an immense number of applications, specifically in the field of biomedicine. Basic concepts of this science were laid down by Richard P. Feynman[39] who suggested, "*There is a plenty of room at the bottom*". During the recent decade, several applications of nanotechnology have gained research interest. One such major and remarkable effort was observed in association with TE. With the integrated approach of nanotechnology and tissue engineering, new means of scaffold fabrication have been made possible.

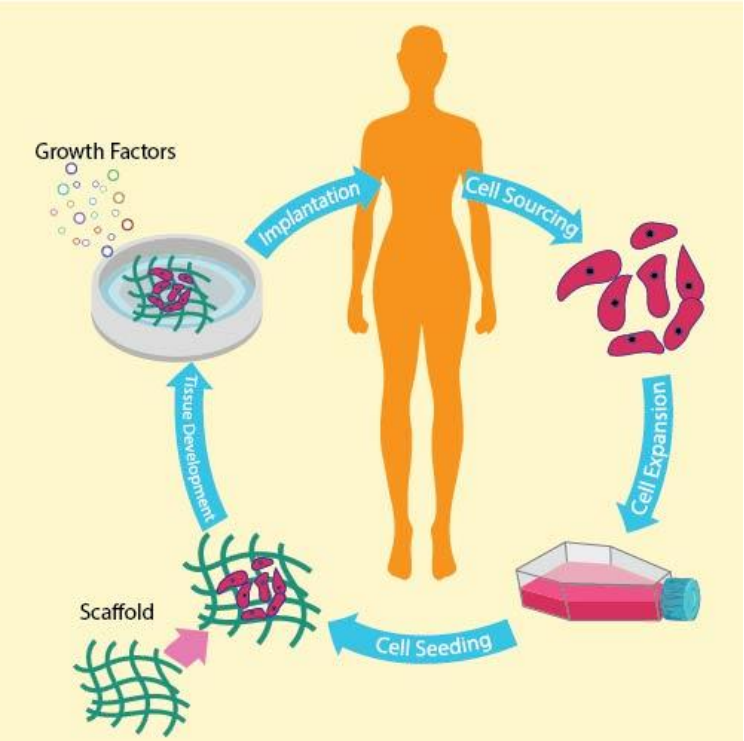


Figure 1: Graphical representation of steps involved in tissue engineering process

Electrospinning technique gained wide acceptance for nanofibrous scaffold generation.

With the electrospinning technique, different kinds of polymers in solution form can be used to generate very thin and highly delicate nanofibers. Besides the porous nature of the scaffolds, it also allows the cells to eventually gain a 3D structure. These electrospun fibers have proven their applications in cardiac cell regeneration[138,153], bone tissue regeneration[60,110] nerve regeneration[24,153] cornea regeneration[38], skin regeneration[64,143] and so on. Different biomaterials such as natural, synthetic and even ceramics, are being utilized for scaffold fabrication.

Natural polymers enjoy special recognition as many of these natural polymers serve an exclusive part of ECM. But some natural polymers in their pure state are unable to undergo electrospinning. This led to the idea of blending natural polymers with other naturally or synthetically derived polymers. This review focuses on presenting a discussion emphasizing the method of electrospinning, identifying suitable polymer in the solution for electrospinning application and their associated biomedical applications.

Electrospinning Process

The term "electrospinning" was coined in the year of 1994[30]. The electrospinning technique makes use of polymer in solution form for nano and micro-fiber construction. In 1934, Formhals[41] introduced the fundamental idea of electrospinning. It is a very simple, inexpensive process for generating nanofibrous scaffolds under the influence of applied voltage across the electrodes[3,111,130]. Like, freeze-drying and solvent casting method, the electrospinning technique can also be employed for synthesizing porous 3D scaffolds. Control over orientation and surface morphology, the high surface-to-volume ratio and the cross-sectional configuration of electrospun nanofibers are key characteristics that make electrospinning more attractive than any other method[100].

A high voltage power supply system, a syringe pump, a metallic needle (or the spinneret) and a ground collector make up the most important components of a standard electrospinning system[47] (Fig. 1). Under the influence of applied voltage, the polymer solution is injected through the metallic needle. The flow/feed rate of the polymer solution is set with the help of a syringe pump. Once the applied strength of the electric field exceeds that of the polymer solution's surface tension, a thin liquid jet produced is then collected in a collector. In order to attract the polymer solution, the collection area is supplied with a negative charge[78]. The produced jet solidifies to yield nanofibers accompanied by solvent evaporation[1].

Characteristics such as process parameters, solution parameters and ambiance/ environmental parameters are thought to collectively determine the morphology and other associated characteristics of developed nanofibers, especially the deposition manner of nanofiber[83]. Orifice size of the nozzle, feed rate, distance between nozzle and collector, applied voltage etc. are some process parameters that are pivotal. It is understood that a minimum feed rate is necessary to generate uniform fiber production because with either increase/ decrease in feed rate, an alteration in the fiber morphology and diameter is observed[93]. The critical voltage required for promoting nanofiber formation differs from

polymer to polymer. Usually, smaller diameter nanofiber production is correlated with the application of higher voltage[130]. Baumgarten[9] reported an interrelationship between the applied voltage and fibre diameter where an increased voltage causes to increase the fibre diameter[9].

The application of high voltage is very crucial in achieving successful nanofiber production. It is usually observed that the release of the polymer solution under the influence of high voltage causes the release of polymer solution as a thin jet rather than spherical droplets to change into the form of a cone. This cone is commonly known as Taylor's cone. However, the release of the solution under low or no electric field results in the formation of spherical droplets. The generation of these spherical droplets is the effect of the solution to reduce the surface tension. When the jet comes in contact with the collector, the spinneret discharges the thin fiber[146]. Fibers of different shapes and sizes can be generated through the process. Even though the process involves the application of high voltage (except for polysiphone polymer system), no electrospun fibers are found to retain the residual charge but get discharged once the process has been completed[133].

Likewise, characteristics such as conductivity, polymer concentration, viscosity and surface tension are observed as the chief solution parameters. The number of surface electric charges in polymer solution is thought to affect the ability to form nanofibers. It is due to the presence of certain additives/impurities; polymer dissolution in solvent causes an increase in the number of such ionic species. But it is also observed that the increased polymer concentration affects the process conductivity negatively[5]. Similarly, another parameter the solution viscosity is greatly thought to control the diameter of these nanofibers. The higher is the viscosity, the larger is the diameter of the nanofiber[88]. Although a higher viscosity corresponds to a larger nanofiber diameter, too high or too low viscosity can negatively influence the nanofiber formation. A very high solution viscosity makes it difficult for the release of the fibre and a very slow viscosity causes interruptions in electrospray and polymeric solutions.

In addition, the polymer's molecular weight and the solvent nature are also found to influence the minimum threshold viscosity required for electrospinning[5]. High molecular weight polymers are preferred to low molecular weight polymers as they can produce enough force of elongation to overcome the surface tension properties[146]. Surface tension is a predominant property of a liquid at its surface. This property enables the molecules in the liquid state to resist any kind of external force primarily due to cohesive interactions. The surface tension of the polymer solution is largely decided by the solvent being employed. Hence the correct solvent selection allows the smooth generation of nanofiber following electrospinning.

Pressure, humidity, atmospheric gas, temperature etc. constitute some of the important environmental parameters of the process[133]. Humidity is one such notable parameter influencing fiber production. A low humidity of less than 35% is suitable for electrospinning[30]. An increase in temperature can also produce a bleak outcome. Since a high temperature promotes increased solvent evaporation, the fiber diameter gets readily altered and is not suitable for TE studies.

Scaffold design

Scaffolds are 3D biomaterial associations that are capable of performing functions like cell-biomaterial interaction, gaseous and nutrient transport, cellular proliferation and differentiation, biodegradability and minimum cytotoxicity/inflammation[67]. Thus, scaffolds provide a meshwork for the attachment of growing cells and thereby promoting cellular regeneration. Numerous scaffold fabrication technologies direct the synthesis of the scaffold by utilizing different polymeric solutions, with the sole aim of promoting tissue regeneration. Therefore, several factors are under consideration while developing an ideal scaffold.

a. **Porous nature:** They are 3-dimensional porous structures with an interconnected porous network for cell growth to provide a way for flow transport of nutrients and metabolic waste. The porous nature of the scaffold usually tends to improve the mechanical properties of the scaffold. Besides affecting mechanical properties, it also promotes mass oxygen transfer[1], water absorption and cellular migration[152]. Furthermore, the products of scaffold degradation should also be able to migrate through these porous structures without having eliciting any immunological response.
b. **Biodegradability:** Usually, the scaffolds employed for cellular growth and differentiation are not intended to stay forever *in vivo.* With overtime, their biodegradation is essential and gives the cells an opportunity to synthesize their own ECM components[6].Controlled degradation of scaffolds also permits the easy elimination of the biomaterial without triggering any unfavorable immunological reaction. Hence, scaffold biodegradation should have to coincide with the rate of tissue formation. Most biodegradable polymers have been shown to exhibit highly unstable backbone functional groups that can be readily targeted for the process of hydrolysis, or in some instances, these polymers are targeted by certain microbial enzymes for the degradation in nature[111].
c. **Biocompatibility:** The most important criterion of any scaffold is its biocompatible nature. Biocompatibility allows the seeded cells to adhere, grow and differentiate normally. Similarly, whenever the scaffolds are placed *in vivo*, the chemical composition of the scaffold also should be biocompatible with the bodily conditions causing no/less immune response.
d. **Low immunogenicity:** Immunogenicity refers to the tendency of a material to elicit an immune response. So, for *in vivo* applications, biomaterial for scaffold preparation should evoke no/less immune response to

ensure that scaffold is being retained until an adequate amount of cell growth and differentiation occurred. Whenever these biomaterials fail to meet up the biological cellular needs, they often trigger severe immune reaction leading to graft rejection.

Polymers for scaffold fabrication

Scaffold fabrication is the main principle for TE studies. First, biological scaffolds obtained from human and animal cells have been a choice. Later by analyzing the troubles in association with biological scaffolds, the use of synthetic scaffolds expanded. Synthetic scaffolds are the fabrication products obtained from polymers. Cellular differentiation and maturation are greatly influenced by ECM components and different growth factors. Therefore, maintaining and culturing of different cell types are much easier if they are provided with a medium equivalent to ECM *in vivo*[7]. Hence, chemical characteristics of the scaffold should be similar to ECM components.

Moreover, once appropriate growth statistics have been achieved, scaffold degradation at the target site without leaving any traces of toxicity is also desirable. There are several biological, structural and chemical characteristics considered for the development of a scaffold. The main biological properties ideal for a scaffold include biocompatibility and non-cytotoxicity. Biocompatibility is the ability of scaffold material to promote cellular growth followed by degradation. Likewise, many properties of certain natural and man-made synthetic polymers are found to be beneficial in TE applications for various biomedical purposes. Different kinds of polymers, both natural and synthetic ones are being utilized for scaffold fabrication (Figure 3), of which the commonly used polymer biomaterials are outlined in this study.

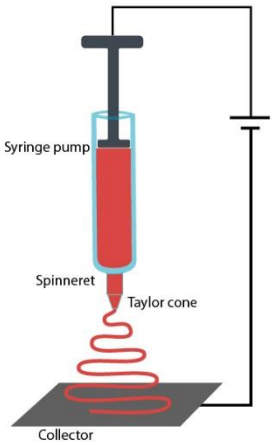


Figure 2: Simplified graphical representation of electrospinning system

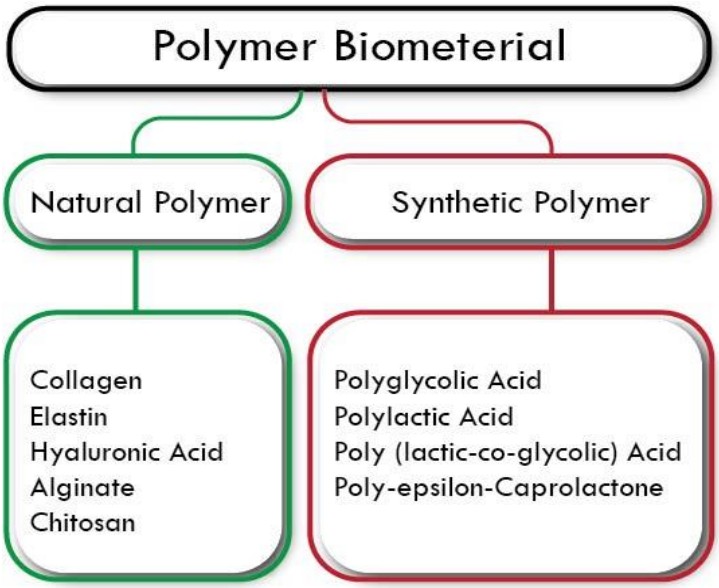


Figure 3: Commonly used polymer biomaterials for scaffold fabrication

Table 1
Distribution pattern of collagen in different tissue types

Collagen Type	Distribution in Tissues
I	Bone, tendon, cornea[19], skin, ligaments
II	Cartilage, vitreous body, corneal epithelium
III	Skin, cartilage[149], blood vessels, lungs, spleen, liver
V	Cornea[19], lung, bone
XI	Vitreous body, cartilage[43], intervertebral disc[29]
XXIV	Bone, cornea[29]
XXVII	Cartilage[29]

Natural Polymers

As the scaffold should mimic the ECM *in vivo*, the best solution is to use natural polymers to act as scaffolds. Collagen, elastin and hyaluronic acid are most commonly reported for their importance in ECM along with other natural polymers like alginate and chitosan for their ability to promote cellular growth and differentiation.

Collagen: Most abundant structural protein of ECM is widely distributed in almost all different soft and hard tissues of the body. These dynamic, triple helical proteins have four major types, I, II, III and IV proteins. Among which type I is widely used in tissue engineering applications. Moreover, type II, III, V and XI are also utilized in biomaterial construction due to their fibrillar nature[32]. There are mainly eight families of collagen protein in which type I, II, III, V, XI, XXIV, XXVII are the members of fibril forming collagen sub-family and have a great interest (Table 1).

Cells like osteoblast, chondroblast and fibroblast are involved in collagen synthesis[69]. But however, in connective tissues, fibroblast cells are duly responsible for collagen production[107]. Because of poor immunogenic properties[23], high stability, tensile strength, non-toxicity and bioresorbable nature[69], collagen gained wide interest in TE applications[109]. Collagen is widely employed in neuronal[151], vascular tissue[65], tracheobronchial[106] and bone repair and related studies[89,90].

Elastin: It is another dominant extracellular matrix protein which is composed of monomers called as tropoelastin. *In vivo*, different proteins such as fibrillins, fibulins, versican, MAGP-1 and MAGP-2 (Microfibril associated glycoprotein -1 and 2), EMILINS (Elastic microfibrils interface located protein) and EBP (Elastin binding proteins) serve to play a crucial role in elastin formation. Elastin protein in ECM provides tissue with additional elastic properties[147] and lends resilience to cells and tissues. Thus, elastin commonly occurs in elastic tissues of blood vessels, skin, lung parenchyma etc. and is capable of undergoing stretching and contraction without causing damages to these tissues.

However, in large vessels, elastin constitutes approximately about 50% of ECM, whereas in the skin, it is only merely 5% in composition[118]. This chemically inert polymer is also an attractive molecule of TE. This insoluble polymer is being naturally synthesized by cells such as fibroblasts, chondrocytes, vascular smooth muscle cells and epithelial cells. Elastin is being used in two forms-soluble and insoluble. But the use of soluble form over insoluble is advantageous as the analysis and handling are pretty much easier than insoluble form[125]. Smooth muscle cell studies[117], wound healing[145], axial vascularization studies[124], vascular cell growth studies[142] and other vascular studies[131] make use of the elastin polymer.

Hyaluronic acid (HA): This unsulfated[4] glycosaminoglycan (GAGs) molecule is also known as hyaluronan and exists as a linear polysaccharide made up of repeating d-glucuronic acid and N-acetyl-D-glucosamine. Unlike the other GAGs, HA is being synthesized in the plasma membrane with the help of HA synthase (HAS) membrane protein[68]. These carbohydrate polymers provide cells with a structural framework. In vertebrates, it is a major component of ECM of certain body fluids and some tissues. In tissues, HA forms the part of ECM of skin, umbilical cord and hyaline cartilage whereas in the case of bodily fluids, they occur as the part of synovial fluid and vitreous humor[94].

Pure forms of HA lack mechanical properties; therefore, chemical modification makes it more usable for diverse applications. So, the ability to undergo modifications along with biocompatibility and biodegradability has made HA, a potent candidate for TE purposes[28]. HA is mainly being used in cartilage tissue engineering[89], wound healing[80] and bone tissue engineering[61].

Alginate: Alginate is a polysaccharide molecule also known as algin or alginic acid. It is a linear copolymer made of D-mannuroniic acid (M) and L-guluronic acid. Brown algae such as *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum* and *Macrocystis pyrifera* are the major sources of commercially available alginate[132]. Besides, certain bacterial species like Pseudomonas and Acetobacter are also capable of producing alginate with highly specific chemical structures and physical properties[70]. As the alginate is natural in origin, it might possess different types of impurities which are needed to be removed during purification steps.

Therefore, the biocompatibility of alginate varies according to the purity of the molecules. Other important properties which put alginate as an alternative in TE applications include its comparatively low cost, no/ low toxicity and gelation properties[45]. Alginate has been widely studied in areas such as neovascularization[48], bone tissue engineering[8], cartilage repair[22], skeletal muscle regeneration[122] and nerve repair[113].

Chitosan: Chitosan is a linear polysaccharide made up of deacetylated beta-(1,4)- glucosamine residues. In nature, chitosan is formed by the degradation of chitin which is assisted by the activity of various groups of enzymes such as deacetylase and chitinase produced by the activity of a diverse group of microbes. At the commercial level, chitosan is formed as a result of the deacetylation of chitin which is exclusively derived from the shells of crustaceans such as shrimps, crabs, prawns etc. Deacetylation is achieved with the help of various alkaline methods, ultimately yielding a non-toxic and bio-degradable polymer[119]. It is used in cartilage and bone tissue engineering[86], neo-vascularization[87] and so on.

Synthetic Polymers

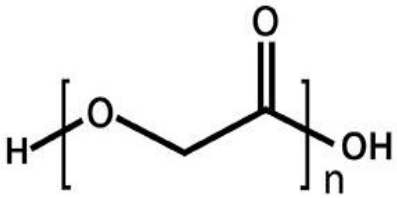
Synthetic or man-made polymers are yet another choice for tissue engineering applications. They represent a large class

of polymers that can be synthesized under *in vitro* conditions. Since several polymer characteristics such as mechanical property, biodegradability and porosity can be altered easily, they are also a good choice of interest. Unlike natural polymers, these synthetic polymers are easily available and are of low cost. Poly-lactic acid (PLA), poly-caprolactone (PCL), poly-glycolic acid (PGA) are most commonly used synthetic polylactone polymers for TE applications. Besides, Poly(lactic-*co*-glycolic) acid (PLGA) is also in use.

Poly Glycolic acid (PGA)*:* PGA is a linear aliphatic polyester known for its high biodegradable nature (figure 4). It can degrade into simple monomer units of glycolic acid at a faster rate than PLA[102]. Both low and high molecular weight PGA can be manufactured, but the process involved varies. When polycondensation of glycolic acid yields low molecular type PGA[21], the ring-opening polymerization of glycolide produces high molecular weight PGA[121]. Apart from these two processes, other methods like Bronsted polymerization (of carbon monoxide and formaldehyde) and solid-state polycondensation of halogenoacetates also result in PGA production. PGA is a highly crystalline polymer that is barely soluble in most organic solvents but is soluble in fluorinated organic solvents such as hexafluro isopropanol. PGA is recently being exploited to a great extent due to its biocompatible nature[59]. Both find applications in the field of neuronal tissue studies, cardiac tissue engineering[35] and bone tissue engineering[26].

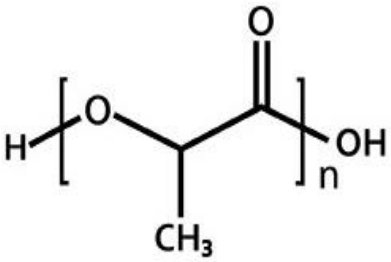
Poly-lactic acid (PLA): PLA is synthesized from the commonly occurring organic acid lactic acid[2]. This hydroxycarboxylic acid widely occurs in two enantiomeric forms (D- and L-lactic acid). The basic structure of PLA is given in figure 5. Commercially lactic acid can be produced by two methods: fermentation or chemical synthesis. Due to its eco-friendly nature, however, fermentation is more frequently used in lactic acid production rather than chemical synthesis using different petrochemicals. Different methods operate for the synthesis of PLA from lactic acid. The ring open polymerization and direct polymerization methods are most frequently employed. PLA properties vary according to the temperature and time required for processing, molecular weight and isomer component being used[99]. PLA has convincingly been used for its biodegradable, biocompatible ability for surface modification and lower cost. PLA using research activities occurs in the following areas: cutaneous tissue engineering[98], neuronal[13], bone tissue engineering[52], ligament tissue engineering[136] and vascular tissue engineering[66].

Poly(lactic-co-glycolic) acid (PLGA): PLGA is a copolymer that can be synthesized using two different monomer units: lactic acid and glycolic acid. The structure of the molecule is given in figure 6. Their ability to be processed into desired shape and size, biodegradability and biocompatibility put out this molecule as a good candidate for TE. However, the solubility of PLGA is worth mentioning as it can be easily solubilized by many more common solvents such as ethyl acetate[85]. PLGA finds applications in brain tissue engineering[157], bone tissue engineering[25,33], neural differentiation studies[76] and drug delivery[42].



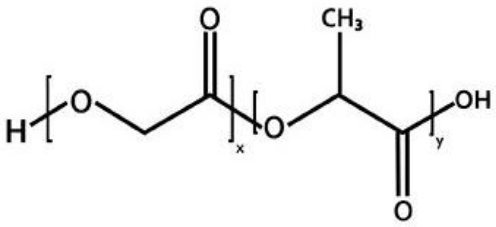
Poly(Glycolic acid)

Figure 4: Structural formula of Polyglycolic acid



Poly(Lactic acid)

Figure 5: Structural formula of Polylactic acid (PLA)



Poly(Glycolic acid co Lactic acid)

Figure 6: Structural formula of poly(glycolic acid co lactic acid) (PLGA)

Poly-epsilon-caprolactone (PCL): PCL is another biodegradable polymer of semi-crystalline nature which is synthesized using epsilon-caprolactone monomers. This aliphatic polymer exhibits non-cytotoxicity and biocompatibility. The degradation rates of PCL are reported to be lower than any other poly lactones in the order of PGA > PLGA > PLLA > PCL[36]. The solubility of the molecule significantly differs from PLG, in only slightly being soluble in ethyl acetate at room temperature but soluble in other solvents like chloroform, benzene, toluene, carbon tetrachloride etc.[148] PCL is used in conducting studies in the areas of bone tissue engineering[77] and skin regeneration[144].

Electrospinning of Natural polymers

Electrospinning of Collagen: One apparent aspect of nanofibrous collagen is that it lacks sufficient mechanical strength. The best choice to overcome this hurdle is by crosslinking the collagen with any other polymers. Like alginate, the fabrication of electrospun collagen can be done employing either pure collagen material or in combination with any other molecules of either natural or synthetic origin. Since collagen is the major component of ECM, the application of the polymer at the nanosized range has several advantages over other polymeric substances.

The biocompatibility of the polymer is also an obvious reason for the relatively very low immunogenicity exhibited by the cells *in vivo*. Successful electrospinning of collagen fibers of type I, II, III and IV is reported[69]. Type I collagen fiber was extensively used for understanding the cellular differentiation of many cells such as endothelial, osteoblast, dermal and muscle[58]. With respect to the endothelial cells, the pore diameter of collagen is very crucial in determining endothelial cell infiltration through scaffold such that increased pore diameter caused the cells to infiltrate through the scaffold material. Type I collagen could also provide several structural motifs to promote osteoblast differentiation, mineralization and formation of hydroxyapatite crystals.

Type II collagen has been studied in association with articular cartilage tissues. It comprises a greater dry weight proportion of articular cartilage. Studies on collagen type II for articular cartilage repair are also expanding. Chicken sternal cartilage collagen type II was used to produce electrospun fibers to provide a substrate to promote chondrocyte cell growth and infiltration[91]. Shields et al[129] used adult human chondrocyte seeded on to type II collagen nanofibers, cultured for a period of 7 days and found out that cells were capable of both adhering and infiltrating the scaffold material. Electrospinning of type I and type III collagen in association with elastin has been done to develop a three-layered vascular construct, thereby opening a new gateway in the field of vascular tissue engineering[14].

One significant application of collagen nanofibers is in the process of wound healing. Grafting of athymic mice with electrospun collagen skin substitute promoted a high engraftment rate of 100%, then employing freeze-dried collagen substitute for skin[112]. Cross-linking of collagen with chitosan for electrospinning purposes greatly enhanced the wound healing process[27]. Since chitosan and collagen nanofiber alone possess poor water resistance and mechanical firmness[95], the crosslinking method speeded up the wound healing process with better performance. Nanofibrous collagen-containing non-woven fabrics are generated by employing collagen type I in relation to PEO for better wound healing[54].

Electrospinning of Elastin: The availability of the pure form of tropoelastin, the precursor of elastin, always posed a challenge in the incorporation of elastin for scaffold engineering. With genetic engineering principles, recombinant tropoelastin of human origin synthesis was made possible. Such recombinant tropoelastin was electrospun and then was used to analyze the effect of this synthetically prepared scaffold material on dermal and vasculature cells. This study demonstrated that synthetic elastin fibers could support the growth of elastin-relevant primary human cells[104]. Due to the poor mechanical properties and higher water dissolution of pure electrospun elastin, crosslinking of certain chemicals with the polymer is an obvious choice. McClure et al[92] used polydioxanone to coelectrospun with elastin to establish that no or fewer changes in material properties were observed upon addition of cross-linking agents such as 1-ethyl-3-(dimethylaminopropyl)-carbodiimide (EDC) and genipin.

Currently, elastin is used in combination with both natural and different synthetic polymers for improving various functional aspects. In one such approach, elastin was used in combination with PLGA to produce electrospun fiber along with sodium chloride. This combination brought about a significant increase in scaffold elasticity. Later this scaffold preparation was then evaluated for the proliferation, apical polarization and organization studies of the salivary gland and epithelial cells [40]. Meshes of elastin and collagen were developed and were used to study the cell growth of smooth muscle cells. A 1:1 ratio of collagen: elastin along with cross-linking agents N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) helped in stabilizing the mesh. In the presence of this mesh, after 14 days of culture, smooth muscle cells could grow and establish a confluent layer[20].

Similarly, the combination of elastin with collagen was observed in association with skin tissue engineering applications. Successful electrospun fibers of less than 100nm diameter were produced which displayed satisfactory cell adhesion and proliferation under observed conditions[139]. With the aim of enhancing the venous structural integrity, electrospun collagen and elastin along with poly (ester urethane) urea have been developed. This blend was then placed onto excised fresh porcine internal jugular vein segments. This study also indicated that fibre developed through electrospinning has no deleterious effect on cell viability[34].

Electrospinning of Hyaluronic acid: Due to the polyelectrolytic nature of HA, synthesizing nanofibrous mesh using pure HA molecules is very difficult for TE application. So, in order to overcome this hurdle, different approaches were laid down. In one study, electrospinning of hyaluronic acid (HA) was made using *N*, *N*-dimethylformamide (DMF) and a water-mixed solvent system. DMF in the solvent system was capable of lowering the surface tension, thereby promoting nanofiber formation. So developed HA fibers had an average diameter of about 200 nm[72].

According to Liu et al[79], electrospinning of pure HA at ambient temperature can be greatly enhanced by employing a solvent system possessing DMF, deionized water and formic acid. With this method, nanofiber with an average diameter of 100 nm can be obtained. Even though successful nanofiber fabrication was possible with these solvent systems, there were chances of solvent-induced degradation of these fibres. In order to address this scenario, Brenner et al[18] used a solvent system containing aqueous NaOH and DMF. With this study, fibres having an average diameter of 39 ± 12 nm were produced.

Apart from synthesizing pure HA nanofibers, there are efforts to blend other polymers with HA. Electrospinning of HA-gelatin blends yielded nanofibers with an average diameter ranging between 190-500nm by employing a solvent system having DMF and water only[72]. Similarly, other solvent systems are also reported to aid in HA-gelatin nanofiber. Lian et al[74] used 2,2,2- trifluoroethanol (TFE) and water system in 1:1 ratio followed by cross-linking with 1.5M ethanol solutions of N-(3-dimethylaminopropyl)- N-ethyl carbodiimide hydrochloride. The creation of water-swellable HA-Collagen-based scaffolds was carried out by making use of a solvent mixture containing both sodium hydroxide (NaOH) and N, N-dimethyl formamide (DMF). The resultant nanofiber was then seeded with bovine chondrocytes. The study revealed the cellular compatibility to the scaffold by exhibiting an increase in cellular adhesion and proliferation[62].

Recently, many different modifications are being done to develop more efficient fibres for wide range of applications. In one such effort, reinforced PVA/HA fibres having cellulose nanocrystals and L-arginine were produced. These newly developed fibres exhibited sufficient mechanical strength owing to the nanocrystal presence and L-arginine acted as an accelerator of the wound healing process[55]. Synthesis of electrospun HA -based silk fibroin/ zinc oxide was found successful in dressings for burn wound management.

Loaded zinc oxide particles significantly improved antibacterial property against *Escherichia coli* and *Staphylococcus aureus* in a dose-dependent manner[49]. Other efforts include the incorporation of certain natural agents into the electrospun hyaluronic acid/ poly(ethylene) oxide-based nanofiber to bring about improvisation in critical parametrs like water-vapor transmission rate (WVTR). Ionescu et al[56] reported that incorporation of propolis to HA-PEO matrix displayed good WVTR value which promotes sufficient gaseous exchange for wound healing.

Electrospinning of Alginate: Electrospinning of alginate polymer can be done in different routes. The electrospinning of pure alginate in an aqueous solution is particularly troublesome due to two major reasons- chain conformation characteristics and polyelectrolytic nature. Like charged polysaccharides, the alginate electrospinning is affected due to its polyelectrolytic nature. This presence of like charges causes the molecule to repel against each other[17,57,84]. So, the use of many uncharged, hydrosoluble polymers with, can overcome the repulsive electrostatic interaction.

Bonino et al[17] used polyethylene oxide as a carrier agent to enhance the solution properties which are critical in electrospinning. The carrier molecule usage has a major drawback, that is, in one such preparation always, the alginate concentration averages to 40% with a higher carrier molecule. Significant approaches were carried out to produce nanofibers with higher alginate content. In one such attempt, Mirtic et al[96] used high molecular weight (2MDa and 4MDa) PEOs to guide the synthesis of nanofibers with alginate concentration up to 85%.

Similarly, chain conformation also possesses difficulty in causing chain entanglement. The very presence of hydrogen bonding hinders the process. So pure alginate mainly yields droplets due to poor chain entanglement. Sodium salts of alginate also result in droplet formation, which is irregular. But Fang et al[37]produced regular droplets using sodium alginate upon calcium chloride addition. Several other studies focused on nanofiber production with the addition of different solvent systems. Nanofiber synthesis using electrospinning is very advantageous. Nie et al[103] reported the formation of alginate nanofibers employing a solvent system containing water and glycerol.

The chain entanglement property was significantly improved with glycerol addition contributing to higher spinnability. Glycerol was capable of breaking already existing strong intermolecular and intramolecular hydrogen bonds holding the alginate, thereby causing them to form new hydrogen bonds. Also, it was found that carrier molecules like PEO could also establish hydrogen bonding with alginate to promote compatibility.

Apart from these, spinnability varies with the viscosity of the solution also[12]. Alteration in solution viscosity can be achieved with surfactant addition[17]. Diverse attempts are being made to direct the generation of nanofibers with enhanced properties. Significant efforts have been made to increase the mechanical properties of developed nanofibers. In one approach, Vigani et al[141] used two different poly (ethylene oxide) grades (high and low) in combination with alginate to increase the functional aspects. Without affecting the nano-scale dimensions, they were able to produce nanofibers of enhanced mechanical strength with the addition of a small amount of high-PEO to low-PEO along with alginate.

In another study hybrid yarns were fabricated with the coating of PLA nanoparticles over sodium alginate/PEO nanofibers[53]. This close mutual association of nanofibers inside the yarn is thought to increase the tensile strength with respect to uncoated nanofibers[156].

Electrospinning of chitosan: Like any other natural polymer, the electrospinning of chitosan is also done with a wide variety of solvent systems. Chitosan fibers with a diameter of about 220nm were obtained when chitosan-trifluoroacetic acid (TFA) solution was employed for the electrospinning process. The addition of dichloromethane helped in generating fibers with enhanced homogeneity[105]. Other than TFA, an aqueous solution of acetic acid is also being employed in electrospinning. Here in this study, acetic acid concentration was critical in determining the generation of uniform fibers rather than beaded ones. This approach, however, could develop a nanofibrous structure with an average fiber diameter of 130nm[44].

By employing a solvent system containing TFA/DCM (dichloromethane), Sangsanoh et al[120] were capable of synthesizing nanofibers. But with this system, the stability of the fibers was affected which can be overcome with the addition of an aqueous solution of NaOH or Na_2CO_3, thereby neutralizing the spinning solution.

Owing to the presence of high surface charges, the electrospinning of chitosan is very challenging. So, electrospinning of chitosan is made possible with the addition of their agents. In one approach, fibers with diameters ranging from 40 to 240 nm were fabricated by using a 3:1 mixture of chitosan and poly (ethylene oxide) (PEO) in acetic acid. Only with the addition of surfactant and neutral PEO, the spinnability increased dramatically, accompanying homogenous fiber production[63]. Chitosan, in association with poly(epsilon-caprolactone) (PCL) and poly (vinyl alcohol) (PVA), helped in the generation of fiber with an average diameter of 100-200nm, which fond application in bone tissue engineering[154].

Electrospinning of chitosan with PVA in the presence of acetic acid solvent produced system-generated fibers. These fibers have an average diameter of 99±21nm, which makes it suitable for wound dressing[131]. Chitosan- PVA blends find greater application in the field of bone TE. In the presence of hydroxyapatite (HA) nanoparticles in an aqueous acetic acid solvent, chitosan- PVA blend yielded nanofibers of 49 ± 10nm diameter[128].

Electrospinning of Synthetic polymers

Electrospinning of Poly Glycolic acid (PGA): Electrospinning of pure PGA is challenging because the molecule is found to be less viscous when the concentration of PGA increased from lower to higher. So, the addition of any polymer to increase the viscosity would obviously prevent the molecule from assuming a beaded structure and will end in fiber formation. The blending of PGA with polymers like gelatin helped in the development of fibers and such nanofibers have always been a choice of vascular tissue engineering. Hajiali et al[50] used 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as the solvent system for electrospinning and later cultured vascular cells like human umbilical vein endothelial cells (HUVECs) and human umbilical artery smooth muscle cells (HUASMCs) onto the electrospun fibers. Results indicated that this blending could improve cellular attachment and proliferation.

Fibrous encapsulation limits the usability of PGA from serving as a scaffold for soft tissues. Efforts have been made in this area to make use of PGA more into TE. Boland et al[15] pre-treated PGA with concentrated HCl before electrospinning to generate scaffold with more biocompatibility and was thus suitable for the culturing of cardiac fibroblasts (CFs). This acid pre-treatment, however caused the carboxylic and acid groups on the surface to get hydrolyzed[16]. Moreover, this hydrolysis facilitated the vitronectin binding so that cellular adhesion was made possible. More recently, PGA was exploited in the development of a tissue-engineered vessel from humanly derived fibroblast cells. For this purpose, the electrospun PGA scaffold was co electrosprayed with PEO and porogen microparticles. Results were obtained after 10 weeks of culturing of fibroblast cells over the scaffold a tissue-engineered vessel[115].

Combination therapy involving the incorporation of methylcobalamin and PGA-collagen tube into electrospun nanofiber sheet was found effective in rat models. This combination therapy speeded up the process of recovery in sensory functions along with accelerated recovery in characteristics like electrophysiology and histomorphometry in combating sciatic nerve defects[123]. Recent reports of PGA also suggest its ability to be utilized in tackling wound infection. In this approach, polyionic polyglutamic acid was cospinned with cationic photosensitizer 5,10,15,20-tetrakis(1-methylpyridinium-4-yl) porphyrin tetra (p-toluenesulfonate) (TMPyP) for generating nanofibrous mats for wound dressings. Using animal model studies, this mat confirmed the release of reactive oxygen species (ROS) upon visible light irradiation and cytotoxic ROS was shown to have the bactericidal property[135]. Another study reported the use of PGA in association with cellulose acetate and ZnO particles for the development of antimicrobial films for wound dressing applications[73].

Electrospinning of Poly-lactic acid (PLA): Mechanical properties of pure PLA often raise certain hindrances in the electrospinning process. So most frequently, PLA is blended along with some other polymers or in other cases where nanoparticle addition is done before electrospinning. A combination of PLA, PLGA, along with microfibre yarns produces a novel type of nanofibre. This nanofibre formed as a result of modification from the conventional spinning technique was then loaded with thymosin beta-4 (Tβ4) resulting in a sustained release of the drug molecule for a period of 28 days. The process actually involves the coating of PLGA onto (PLA) micro yarn fibers to generate PLGA-PLA hybrid yarns (HY). This HY was then used for the culturing of mesenchymal stem cells derived from human adipose tissues. Results suggested that thymosin-β4 along with this hybrid yarn was sufficient in enhancing and

accelerating the tenogenesis process for tendon tissue engineering[150].

Incorporation of cellulose nanocrystals into PLA nanofibers was done to evaluate the potential of the scaffold in bone tissue engineering. The added cellulose nanoparticle was capable of establishing strong interaction with PLA, resulting in the enhancement of mechanical aspects of the scaffold. Additionally, the scaffold performance was then monitored within a rat calvarial defect model soon after three weeks of treatment. Experiment results indicated that the developed scaffold exhibits good biocompatibility along with better osteoinductivity characters[108]. First reports on the incorporation of nanoparticles like nanosilica and nanoclay at varying concentrations into PLA nanofibers and its physical and biological study were given by Lopresti et al[82]. Cell culture assay using pre-osteoblastic cells indicated that these inorganic nanoparticles could act as a potent nanofiller for bone tissue engineering studies. Moreover, the wettability of the scaffold was also found to get increased with nanofiller addition.

The mechanical properties of polymers have always been a critical factor in determining their suitability in scaffold synthesis. More often, these mechanical properties are influenced by the molecular weight of the polymer. Promnil et al[114] reported the effect of molecular weight on the mechanical and morphological characteristics of PLA for meniscus tissue engineering studies. The study suggested that low molecular weight fibers were capable of generating nanofibers that had high tensile strength. Similarly, the work also concluded the fact that a high concentration of 20% is also necessary to attain good mechanical properties. Electrospun PLA has also been utilized for skin TE studies. Lopresti et al[81]reported that the effective method of PLA/Kefiran hybrid scaffold fabrication is through the direct coating of PLA from Kef/water solution. Such developed scaffold displayed good results in accordance with embryonic fibroblast cell culture studies.

Electrospinning of Poly(lactic-co-glycolic) acid (PLGA): Scaffold topography is a salient feature for TE applications. Sequeira et al[126] used the electrospinning technique for generating both nanosized and microsized PLGA fibers to understand the organization of salivary gland epithelial cells. The results indicated significant changes with respect to cellular morphology and clustering was observed due to changes in scaffold topography rather than any other mechanical properties.

There were reports on using PLGA in combination with polyvinyl alcohol (PVA) for dermal TE studies. Electrospinning of this blend followed by glutaraldehyde cross-linking was then used for culturing fibroblast cells. PBS was used to conduct shrinkage and absorption studies. Results indicated that the cross-linking agent not only helped in reducing shrinkage in PBS but also provided the composite structure with moderate tensile properties which are significant for fibroblast attachment and proliferation[32]. Nanofibrous scaffold using three components system, PLGA, collagen type I and elastin were synthesized to study and understand the effect on cells like SMCs and ECs. No cytotoxic or neurological event was triggered with *in vivo* implantation of the scaffold material in the mice model. This study gave a possibility of using the abovesaid developed scaffold for vascular TE[134]. Likewise, another three-component system had been used for electrospinning for evaluating the scaffold efficiency to promote the growth of human embryo skin fibroblasts (hESFs).

It was found that the scaffold not only assisted cellular attachment and growth but also aided in cellular infiltration[31]. PLGA also found application in osteochondral TE. A bilayered PLGA/PLGA-Hap composite scaffold was developed to evaluate growth studies of medicinal signaling cells or bone marrow-derived mesenchymal stem cells. Rabbit models with an artificial osteochondral defect in the joint were used for study purpose and positive response with tissue regeneration was observed[75].

Nerve regeneration is very limited at the target site. It is understood that schwann cells can play a significant role in relation to peripheral nerve regeneration. With PLGA, a modified supporting system was developed that can be used for inducing human adipose derived stem cells (h-ADSC) to differentiate with schwann cells. Furthermore, these h-ADSCs strongly attached and proliferated on the surface of the modified nanofiber due to the presence of laminins[127]. In another study, PLGA/CNT nanofibers were modified by using laminins proteins either through the process of physical adsorption or polydopamine coating (PD) coating. The study concluded that PD-modified scaffolds greatly promoted PC12 attachment and growth than scaffolds generated using physical adsorption[101].

Electrospinning of Poly-epsilon-caprolactone (PCL): Synthetic polymers or their blends have been demonstrated for more value in vascular tissue engineering studies (VTE). Under *in vivo* conditions, many of the developed vascular scaffolds fail to persist due to high pressure and blood flow for a long period of time. Lee et al[71] developed 520nm-sized diameter nanofibers from PCL-collagen mixture. In order to further study the effect of the developed scaffold on VTE, the cells were seeded upon by bovine endothelial cells (bECs) and smooth muscle cells (bSMCs). The cellular study revealed that the scaffold promoted confluent layer formation on lumen by bECs and bSMCs on an outer surface of the scaffold. Also, this scaffold was capable of resisting degradation and exhibited high resistance to shear stress.

In a similar study, the PCL-collagen scaffold was used to study the adherence of vascular cells along with the stability of the scaffold material under physiological conditions. For this purpose, Tillman et al[137] used the rabbit aortoiliac bypass model to evaluate the properties and concluded that the biomechanical strength of the scaffold is very good in

comparison to that of *in vivo* conditions. Moreover, this support system also promoted significant cellular adhesion-promoting growth.

Venugopal et al[140] synthesized a scaffold using PCL and collagen I and III and seeded coronary artery smooth muscle cells (SMCs) onto it. The fabricated nanofibers displayed an average fiber diameter of 210–225 nm with a tensile strength of 7.79 MPa, showing that these structures can provide sufficient mechanical strength required for controlling cellular functions. Other polymeric blends with PCL are also available for different applications. Gelatin-PCL composite scaffolds were synthesised by making use of 10% w/v gelatin/TFE solution with equal amount of 10% w/v poly(epsilon-caprolactone) (PCL) in TFE system. This composite structure provided an excellent scaffold system by promoting bone-marrow stromal cells (BMSC) to attach, grow and migrate within one week of culturing[155].

PCL-gelatin nanofibers have been revealed to be beneficial in association with cardiovascular tissue engineering. This blend produced fibers with high tensile strength having a diameter ranging from 640–880 nm. Upon culturing with adipose-derived stem cells, both cellular attachment and penetration were visible with the PCL-gelatin blend[51].

Blending PCL with other polymers is also a choice in addressing retinal-related disorders. In a recent study, PCL was blended with poly (glycerol sebacate) to understand retinal cell progenitor cells (RPC) attachment and proliferation. Results suggested that PCL/PGS scaffold promoted better RPC growth owing to advantageous surface and bulk properties than pure PCL fibrous scaffolds[10]. PCL is also an interesting candidate for soft tissue engineering. PCL/gelatin/acetylated cellulose bio nanocomposite scaffolds fabricated using electrospinning with the incorporation of cellulose nanofibers which were then utilized to study fibroblast proliferation.

Results indicated that both the biological and mechanical properties of the scaffold were altered by the incorporation of cellulose nanofibers and acetylated cellulose. Also, fibroblast cells were efficiently proliferated in the presence of filler with no cytotoxicity[46].

Conclusion

Although different nanofiber synthesis approaches exist, the use of electrospinning is widely employed. Almost all polymers, both natural as well as synthetic ones, can be used for nanofiber generation. The electrospun fibers are usually ultra-fine, spatially oriented structures with high surface-to-volume ratio. Such nanofibers can therefore serve as an excellent scaffold material to promote cellular growth and differentiation *in vivo*. There are different applications associated with these electrospun fibers. But the most significant is its implication in the field of fabrication of scaffolds in tissue engineering. Scaffolds provide a structural framework upon which tissue reconstruction takes place. Scaffold fabrication involves the utilization of different polymers, both synthetic and natural ones, for the purpose. In this review study, almost all commonly used polymers, along with their biomedical applications, are presented.

Till today, there are many obstacles in association with the construction of structures analogous to ECM to promote cellular growth and differentiation. The electrospinning process could generate a nanofibrous support system that could resemble the native architecture of human ECM. ECM has many significant molecules that are crucial in promoting the attachment of cells. So, the use of these molecules to construct a nanofibrous scaffold system is more promising. The inherent activity of these molecules in cellular interaction along with their ability to incorporate different bioactive molecules is however helping to solve the dilemma to some extent. With the diversity of ECM molecules, a single polymer molecule cannot serve as the complex *in vivo* environment. With electrospinning, synthetic polymers alone or in combination with natural polymers can also be used to generate nanofibers. Such nanoscale fibres hold tremendous potential in tissue engineering addressing different conditions.

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