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# One Dimensional Nanostructure Scaffolds for Biomedical Tissue Engineering

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## ABSTRACT

*Biomaterial-based scaffolds have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues for medical purposes. Scaffolds serve as a critical platform both to support cell adhesion and to deliver growth factors. Various methods of fabricating scaffolds have been investigated. Developing scaffolds that mimic the architecture of tissue at the nanoscale is one of the major challenges in the field of tissue engineering. The development of one dimensional nanomaterials has greatly enhanced the scope for fabricating scaffolds. The availability of a wide range of natural and synthetic biomaterials has broadened the scope for development of one dimensional scaffolds. Nanofibers have emerged as exciting one-dimensional nanomaterials for a broad spectrum of research and commercial applications owing to their unique physicochemical properties and characteristics due to their high surface-to-volume ratio, porosity, and ease of surface functionalization. Therefore, they can provide new opportunities for cell seeding, proliferation, and new tissue formation. This review focuses on current strategies for nanofibers fabrication and their emerging applications in biomedical tissue engineering.*

**Keywords:** *Biomedical, tissue engineering, scaffolds, nanofibers, electrospinning.*

## 1. INTRODUCTION

Tissue engineering is an efficient method for the repair and regeneration of defected tissues. It is an emerging field of research that applies engineering technology and principles of biology to the development of functional tissue substitutes to expedite the regeneration of damaged tissues [1]. The key issue for tissue repair and regeneration is to provide a temporary scaffold for the defected tissue, which can induce the infiltration of cells and form new tissue with its gradually degradation. The existing procedures for constructing tissue engineering scaffolds are suitable to bulky tissue such as bone and cartilage. For some fine tissues and functional tissues, like vessel and nerve, there are no ideal regeneration scaffolds. Biomimetic functional nanofibers offer us new ideas and methods for those fine tissue engineering scaffolds and bring tissue engineering to a new stage, namely nano-biomimetic tissue Extracellular Matrix (ECM). Actually, the ECM in a body is essentially constructed by nanofibrous network containing protein with a diameter ranging from 50 to 300 nm [2]. Biomaterials play a crucial role in tissue engineering by serving as synthetic scaffolds for cellular attachment, proliferation, and in growth ultimately leading to new tissue formation. A number of novel approaches have been developed for the fabrication of biomaterial-based scaffolds. More recently, nanofibers based scaffolding systems are being explored as scaffolds for tissue engineering [3-5]. The development of nanofibers has enhanced the scope for fabricating scaffolds that can potentially mimic the architecture of natural human tissue at the nanometer scale. Nanofibers have emerged as exciting one-dimensional nanomaterials for a broad spectrum of research and commercial applications owing to their unique physicochemical properties and characteristics. As a class of nanomaterials with cross-sectional diameters ranging from tens to hundreds of nanometers, nanofibers possess extremely high specific surface area and surface area-to-volume ratio. They are capable of forming networks of highly porous mesh with remarkable interconnectivity between their pores, making them an attractive choice for a host of advanced applications. In fact, the significant impact of nanofiber technology can be traced from the wide range of

fundamental materials that can be used for the synthesis of nanofibers. These include natural polymers, synthetic polymers, carbon-based materials, semiconducting materials, and composite materials. The high surface area to volume ratio of the nanofibers combined with their microporous structure favours cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications [6, 7]. Therefore, current research in this area is driven towards the fabrication, characterization, and applications of nanofibrous systems as scaffolds for tissue engineering. Due to their potential, the nanofiber-based systems are also being pursued for a variety of other biological and non-biological applications [8]. This article aims to provide a broad overview of the various emerging nanofiber synthesis techniques coupled with their applications especially in the field of healthcare and biomedical engineering as shown in figure 1.

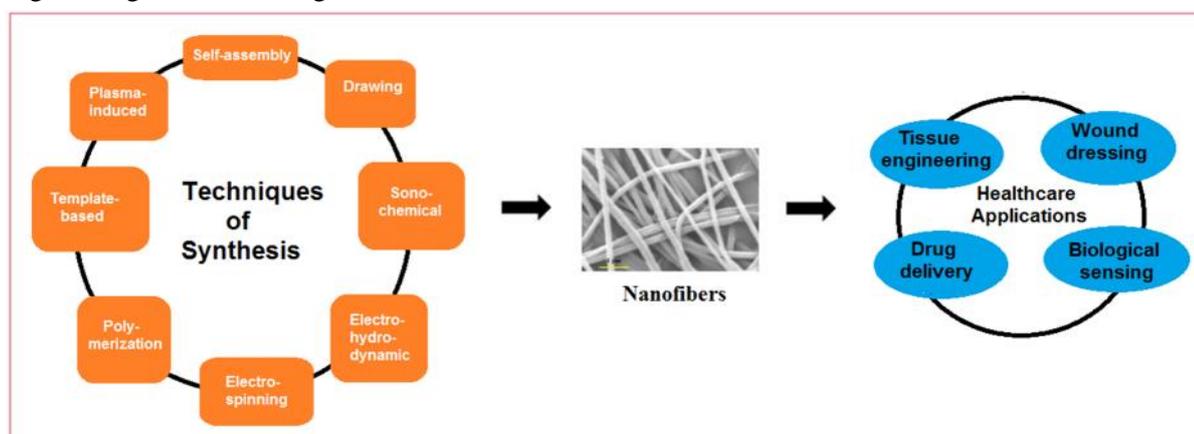


Figure 1: Techniques of synthesis of nanofibers and healthcare applications

## 2. NANOFIBERS SYNTHESIS

Currently, there are many techniques available for the fabrication of nanofibers: drawing, electrospinning, self-assembly, polymerization, plasma induced, sonochemical, electrohydrodynamic and template based synthesis. Although there are a number of techniques for the synthesis of nanofibers, electrospinning technique is the most widely studied and exhibit the most promising results for tissue engineering applications.

### 2.1 Drawing

In the drawing process, the fibers are fabricated by contacting a previously deposited polymer solution droplet with a sharp tip and drawing it as a liquid fiber which is then solidified by rapid evaporation of the solvent due to the high surface area. The drawn fiber can be connected to another previously deposited polymer solution droplet thus forming a suspended fiber. Here, the predeposition of droplets significantly limits the ability to extend this technique, especially in free dimensional configurations and hard to access spatial geometries. Furthermore, there is a specific time in which the fibers can be pulled. Viscosity of the droplet continuously increases with time due to solvent evaporation from the deposited droplet. The continual shrinkage in the volume of the polymer solution droplet affects the diameter of the fiber drawn and limits the continuous drawing of fibers [9]. To overcome the above mentioned limitation is appropriate to use hollow glass micropipettes with a continuous polymer dosage. It provides greater flexibility in drawing continuous fibers in any configuration. Moreover, this method offers increased flexibility in the control of key parameters of drawing such as waiting time before drawing (due to the required viscosity of the polymer edge drops), the drawing speed or viscosity, thus enabling repeatability and control on the dimensions of the fabricated fibers. This method is mostly suitable for the preparation of single nanofiber. Figure 2 illustrate the basic production process of nanofibers by drawing from droplet of the polymer [10]. On the substrate material is applied a millimeter drop of polymer solution (a). Micropipette then moves down toward the edge of the drop (b), there

is contact and by back motion of micropipette the fiber is to pull out of polymer droplet at a certain rate, depending on the type of polymer (c). The liquid polymer is formed in the fiber. The resultant cross section depends very much on the exact material composition, drawing velocity and speed evaporation of solvent. Although this process for the production nanofibers is very easy, but it requires a viscoelastic material that can hold on a strong deformations and tensions during pulling.

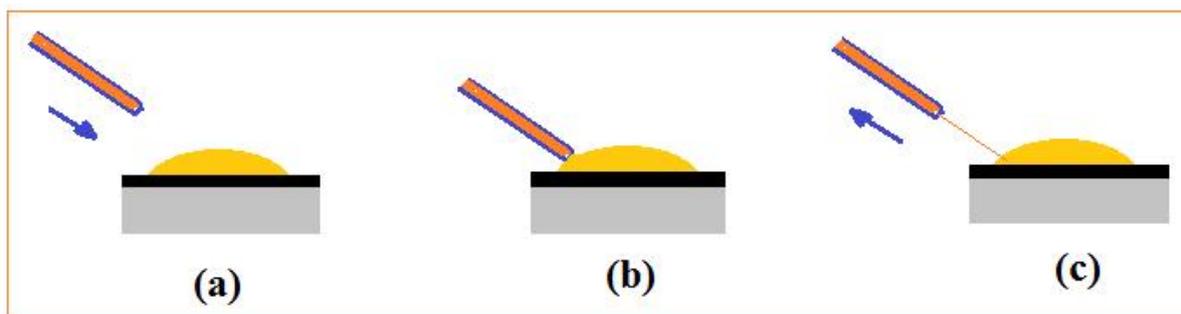


Figure 2: Schematic of single nanofiber preparation by drawing

## 2.2 Template based

Template synthesis is characterized by using a specific template in order to obtain nanofibers with a desired shape and size. Nanoporous membrane template is made of alumina. The polymer is the pressure exerted pressures to which the material is squeezed through the pores of the template into a chamber containing a solution solidifying in contact with the nanofibers solidify. The resulting nanofibers are dimensioned by the membrane pore size. The schematic of template based synthesis of nanofibers from polymer solution is shown in Figure 3. This method uses templates or membranes made from special materials such as metal oxides (alumina) to obtain the nanofibers only for laboratory production. Fiber diameters can be managed.

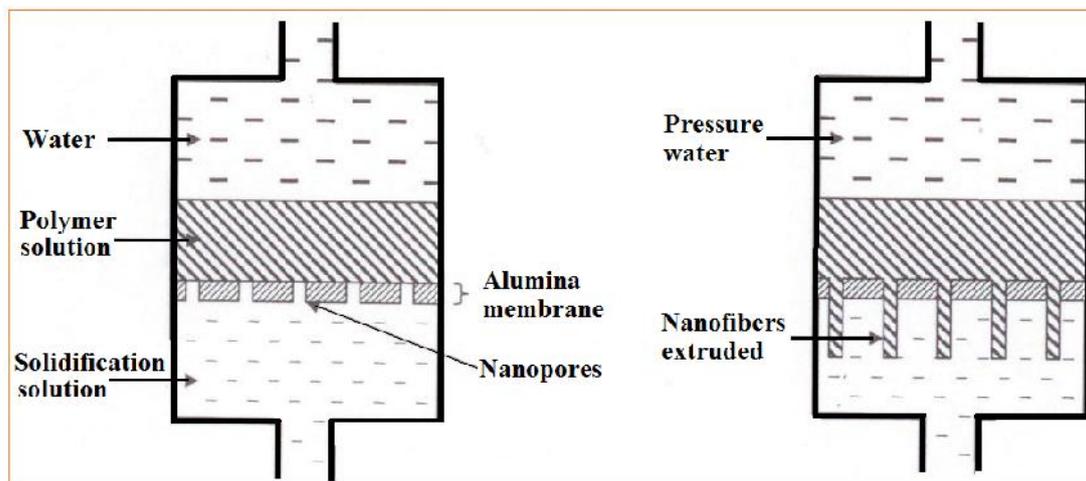


Figure 3: Schematic of template synthesis of nanofibers

## 2.3 Self-assembly

Self-assembly is based on selective control of noncovalent interactions, such as hydrogen bonds, Van der Waals forces,  $\pi$ - $\pi$  stacking interaction, metal coordination, and dispersive forces as the driving forces of self-assembly. To date, surfactants, colloidal particles, structure-directing molecules, oligomers, soap bubbles and colloids as soft-templates, as well as interfacial polymerization, have been employed to synthesize polymer nanofibers [11]. Surfactant is a class of molecules that form thermodynamically stable aggregates of inherently nanoscale dimensions both in solution and at interfaces. Surfactant self-assembly in a solution has

been investigated both theoretically and experimentally owing to its importance in synthesis of micro or nanoscale structures with controlled dimensions. The equilibrium size and shape of surfactant aggregates are controlled by the volume and length of the surfactant tail within the hydrophobic core of the aggregate and the effective area occupied by each surfactant head group at the surface of the aggregate. The favoured aggregate morphology of a surfactant in a solution is spherical, cylindrical, or a flat bi-layer, depending on these parameters. The self-assembly ability of surfactants in a bulk solution therefore creates the possibility of surfactant micelles serving as soft-templates to form polymer nanostructures. Schematic of the mechanism of the self-assembly synthesis of one dimensional nanostructures is shown in Figure 4. Micelles were formed by the self-assembly of dopants, and the polymerization was carried out on the surface of the micelles which acted as soft-templates in the formation of (a) nanotubes and (b) nanofibers can be formed by the protection of dopants. When the polymerization is carried out on the substrate producing (c) aligned nanofiber arrays. Based on the characteristic of surfactants, the micelle formed by dopants, a dopant/monomer salt, or even the monomer itself, is proposed as a soft-template to interpret the formation mechanism of template-free nanostructure synthesis via a self-assembly process [12].

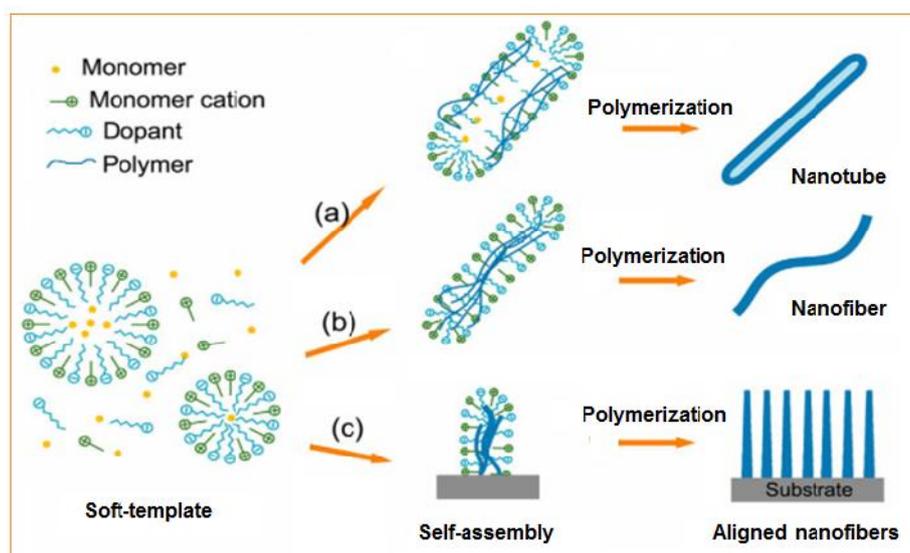


Figure 4: Mechanism of self-assembly synthesis of polymer nanofibers

## 2.4 Plasma-induced

Using the plasma-induced synthesis method, nanofibers are prepared based on five distinct steps: (1) rapid and energetic bombardment of radicals onto the electrode surface, (2) atomic vapour deposition, (3) expansion of plasma, (4) condensation of solution medium, and (5) in situ reaction of oxygen and growth of nanofibers. Plasma is typically generated from the discharge between a pair of metal electrodes in solution by a pulse direct current. The schematic diagram of experimental setup for a plasma-induced technique is shown in Figure 5. The copper wire electrodes are kept in water. The gap between the electrodes maintained at 0.3–0.5 mm by a screw micrometer during discharge period. Plasma is generated using a high voltage pulse DC power supply. When the pulse voltage is supplied, gas phase begin to form due to the Joule heating. Once increasing up to the breakdown voltage, discharge becomes visible. Water is decomposed to bubbles of hydrogen and oxygen, as well as other energetic radical particles. [13, 14]. The plasma is located at the centre and surrounded by a gas phase, which is surrounded by a liquid phase. So the energetic radical particles in the plasma region mean the kinetic energy. Once energetic radical particle reached to the interface between gas region and water medium, they converted back into water. Once plasma occurred, colourless of water changed to yellow and up to dark along with the discharge time. The oxidization of metal nanoparticles will be occurred time depending on the discharge time. If it is a short discharge time, the oxidization of nanoclusters just occurs during plasma discharge. If it is a long discharge time, the oxidization of nanoclusters occurred

both during plasma discharge and after plasma discharge, because oxygen comes from air is not enough to oxidize a mass of nanoclusters formed by discharge. Thus the nanofibers can be prepared by plasma-induced method. This method has been used to prepare CuO nanofibers in water [15].

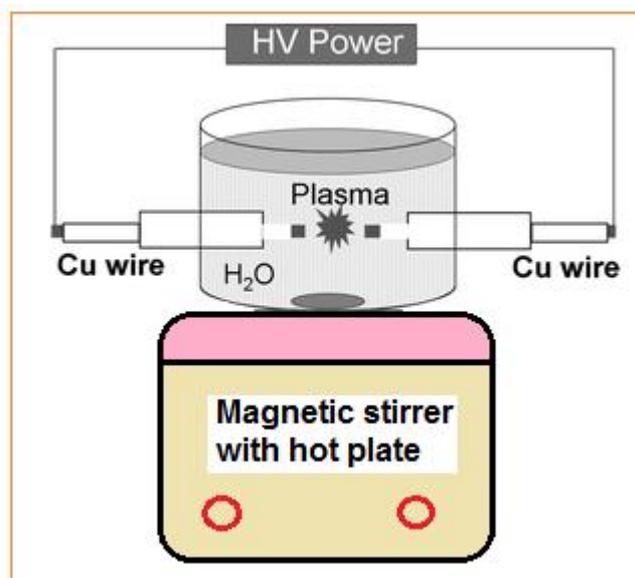


Figure 5: Schematic of plasma-induced synthesis of nanofibers in water

## 2.5 Electrohydrodynamic

Electrohydrodynamic direct writing of nanofibers is a popular method of nanofiber synthesis due to its simplicity and high throughput. A kinetically controlled mechano-electrospinning (MES) process for the continuous and programmable direct writing of hierarchical nanofibers in high resolution has been demonstrated [16]. This highly versatile and cost-effective MES technique combines both electrical and mechanical forces to drive the viscous ink for the large scale direct writing of solution-based materials. Schematic of electrohydrodynamic jet printing system is shown in Figure 6.

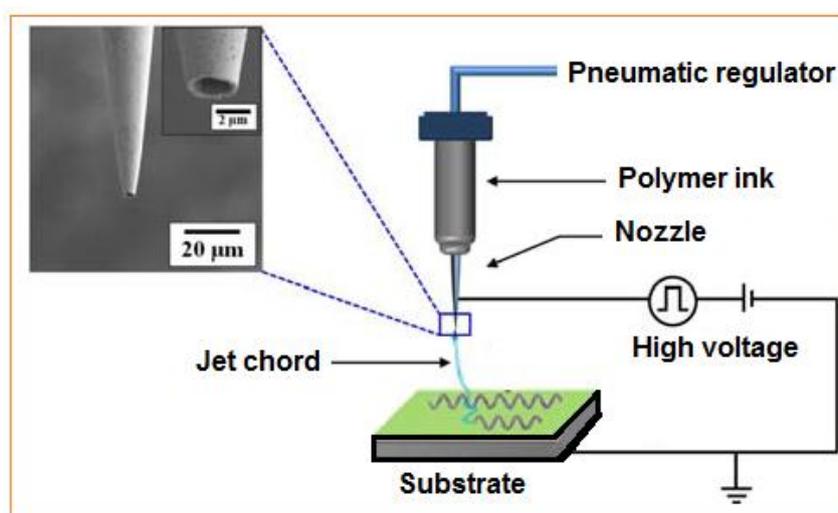


Figure 6: Schematic of electrohydrodynamic jet printing system

The operational process of the MES system can be divided into three steps (1) filling of functional ink in the syringe nozzle, (2) application and increase of an external voltage followed by the stretching of jetting fiber from the nozzle, and (3) formation of a fine “jet chord” between the substrate surface and meniscus. By

controlling the key processing parameters, several modes of the MES direct-writing process may be developed to generate distinct fiber structures [17]. MES nanofiber synthesis technique presents several advantages, notably tunable printing resolution, simultaneous control over the position and morphology of the deposited nanofibers, and direct deposition of smooth hierarchical and complex nanofiber structures. The electrohydrodynamic direct writing may also be integrated with other synthesis techniques to form novel functional structures. For example, based on the combination of electro-hydrodynamic jet printing and self-assembly, three-dimensional block-copolymer films with hierarchical configurations may be generated [18].

## 2.6 Electrospinning

Electrospinning is a cost-effective method to fabricate fibrous structures of micro to nanometer scale diameter, with very high specific surface areas. Of all the current strategies available for synthesizing one-dimensional nanofibers, electrospinning is one of the most established and widely adopted techniques. In general, the electrospinning set-up consists of a syringe with a nozzle, an electric field source, a counter electrode or grounded target (metal foil wrapped on stationary plate or rotating drum, and a pump. The schematic of electrospinning set up is shown in Figure 7, including (a) block diagram, (b) photograph of nozzle ejecting fiber, (c) fiber ejecting at a critical voltage and (d) formation of Taylor cone and jet stream of the polymer solution drop into nanofiber collecting on collector.

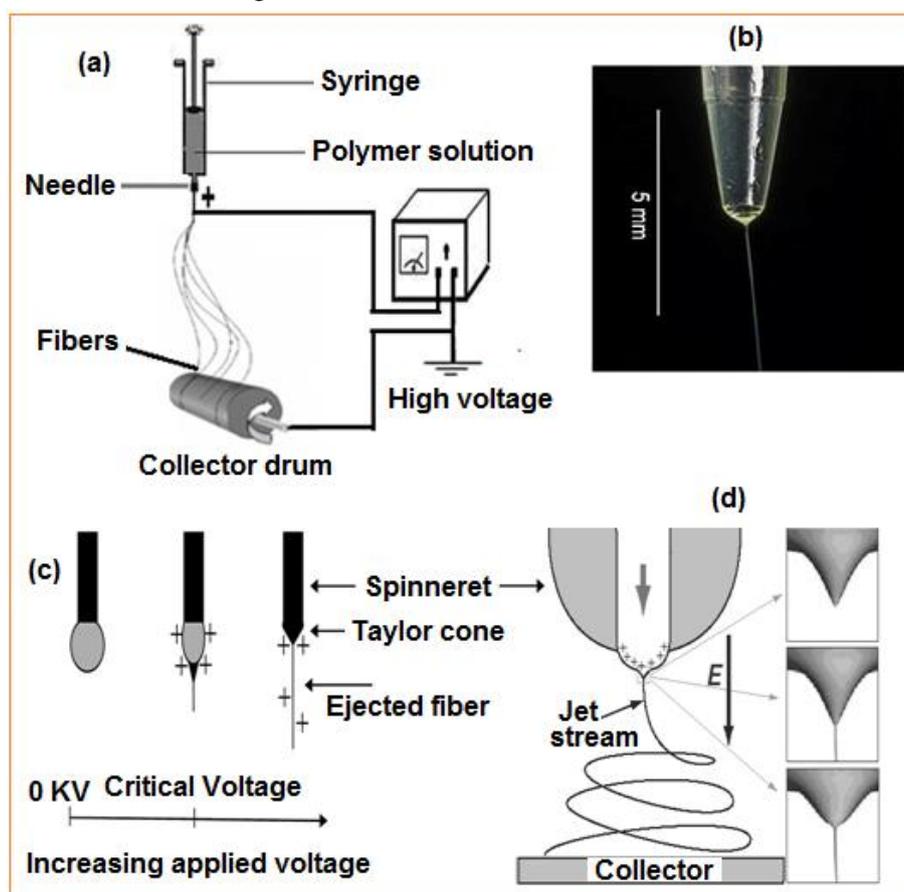


Figure 7: Schematic of electrospinning for polymer nanofibers preparation

The electrospinning process is based on the principle of electrostatics in which the electrostatic repulsion forces in a high electrical field are used for nanofiber synthesis. The solution to be electrospun is held in a syringe nozzle and a large electrical field is generated between the nozzle and counter electrode. As the solution is ejected, the solution droplet at the nozzle adopts a cone-shaped deformation due to the potential

difference between the nozzle and the grounded target. As the charged jet accelerates towards the counter electrode, the solvent in the solution evaporates, leading to the formation of solid continuous nanofibers on the grounded target. The physical properties of the electrospun nanofibers are heavily dependent on a multitude of parameters, such as solution properties (e.g., conductivity, viscoelasticity, and surface tension), environmental factors (e.g., processing temperature and humidity), and technical variables (e.g., tip-counter electrode distance, applied electrical potential, and flow rate). In fact, a wide range of fibrous nanostructures have been successfully prepared using electro-spinning. Besides the conventional electrospinning technique, several variations of this method have been developed lately. These include the multi-needle, needleless, and co-electrospinning or co-axial electrospinning [19-22]. The multi-needle and needleless electrospinning techniques are utilized to enhance the productivity of the conventional electrospinning. The co-axial electrospinning, on the other hand, is developed to synthesize core-shell and multi layer composite nanofibrous structures with additional functionalities and improved quality. In co-axial electrospinning, two distinct nanofiber building blocks are supplied through different coaxial capillary channels and then integrated into core-shell composite nanofibers. In fact, the emergence of co-axial electrospinning has significantly contributed to the development of numerous novel functional nanomaterials in a large scale manner. There is an extremely broad range of potential applications in which electrospun nanofiber nonwovens can make major contributions. These include textiles, filters, composites, tissue engineering, drug delivery, wound healing, sensor, optoelectronics, catalysis, and many more. Electrospun nanofibers could be randomly oriented but by rotating a cylindrical collector at a very high speed up to thousands of rpm (revolution per minute), electrospun nanofibers could be oriented in proper alignment in one direction for the application in tissue engineering.

### 3. NANOFIBERS APPLICATIONS IN BIOMEDICAL TISSUE ENGINEERING

A variety of methods has been reported for the fabrication of scaffolds to be used in tissue engineering. Nanofibers can be successfully use in musculoskeletal tissue engineering. Attempts are made to regenerate bone tissue, cartilage, ligament or skeletal muscle. In case of bone tissue it is the most important to recreate 3D structure and appropriate physical and mechanical properties like mechanical strength, pore size, porosity and hardness. Cartilage is more problematic than bone tissue because of its specific construction. Moreover nanofibres can be used to build structures for skin or blood vessels regeneration. Nanofibers also can be used as a drug delivery system to improve the therapeutic efficiency and safety of drugs. By virtue of high surface area and porosity of nanofibrous systems, they have the potential to provide enhanced cell adhesion and by virtue of the similarity of their 3D architecture to natural ECM, they provide an excellent micro/nano environment for cells to grow and perform their regular functions [23, 24]. Therefore, nanofibrous systems have been strongly pursued as scaffolds for tissue engineering applications. As one of the most actively researched biomaterials, nanofiber-based scaffold emerges as a versatile alternative for tissue engineering applications. With their interconnected network of micropores mimicking the native in vivo topographic features of extracellular matrix (ECM), nanofibrous scaffolds present a favorable avenue for cellular growth, proliferation, and differentiation. For the particular application of tissue engineering, biodegradable and biocompatible natural or synthetic polymers are typically used as the nanofiber materials [25]. The specific selection of materials depends very much on the types and properties of the tissues to be regenerated as well as the duration of regeneration. Although the combined use of different types of polymers can significantly improve the physical and biological properties of nanofibrous scaffolds, further modification of their surface with specific bio-functional groups is often needed in order to improve their usage. Specifically, surface modification of nanofibers with proteins or peptides is essential to develop functional nanofibers with more desirable biological features for tissue engineering applications. Conceptual scheme of (a) surface modification, (b) physical adsorption of bioactive molecules and (c) covalent immobilization of bioactive molecules on nanofibers is shown in Figure 8.

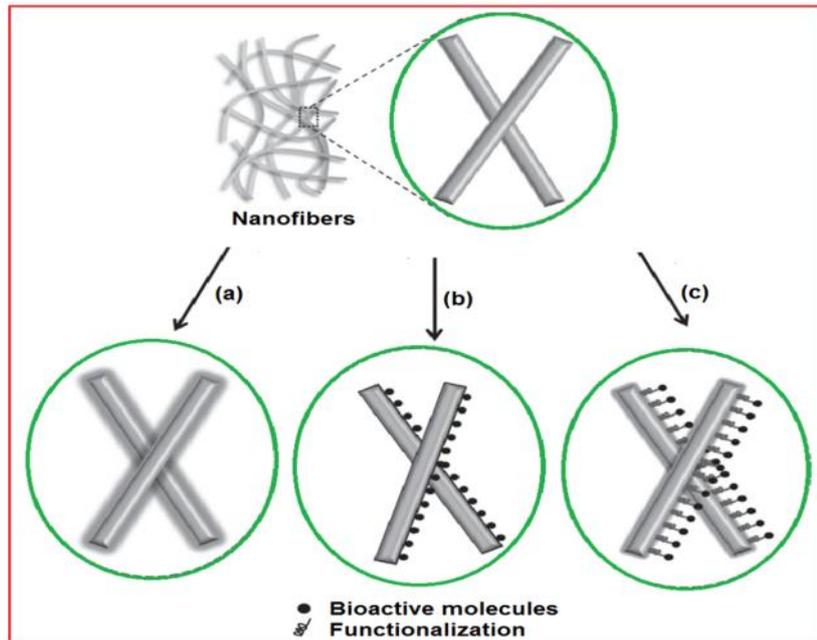


Figure 8: Schematic of mechanism of modification of nanofibers with bioactive molecules

Surface modification techniques have been developed to improve the surface properties of the nanofibers by introducing a variety of functional groups. In order to apply nanofibers to biomedical uses, their surfaces need to be chemically and physically modified after being nanofibers prepared. This process allows the nanofibers to provide biomimetic microenvironments to surrounding cells and tissues. Various surface modification techniques change the chemical composition of the surface of the nanofibers and enhance the surface hydrophilicity, which provides a more favorable environment for cellular adhesion. Moreover, surface modification enhances the biocompatibility of polymeric materials and introduces various functional groups, which can render secondary bioactive molecules immobile. In order to immobilize bioactive molecules on the surface of electrospun nanofibers, chemical modification is favoured. The immobilized molecules are covalently attached to the nanofibers and they are not easily leached out. There are various types of molecules used as surface bioactive molecules, such as ECM proteins, and ECM-derived peptides. For example, collagen, one kind of ECM protein, was immobilized onto the nanofiber surface [26]. Neural stem cells were cultured on the collagen-immobilized nanofibers and the results indicated that the collagen-modified nanofibers enhanced the attachment and viability of the neural stem cells. In another study, the surface of nanofibers was modified by the air-plasma treatment, and then laminin was covalently attached [27]. Many materials (natural and synthetic) have been explored as nanofibrous scaffolding materials for bone, cartilage, ligament, and skeletal muscle tissue engineering [28-30]. Although nanofibers have been studied as scaffolds for multiple tissue types, musculoskeletal tissue is probably the most well studied.

### 3.1 Nanofibers scaffolds for bone tissue engineering

Bone is a hierarchical structure. It consists of a dense compact shell called cortical bone and a porous core called spongiosa or trabecular bone. The major components of bone tissue are collagen fibrils, hydroxyapatite particles, and proteoglycans. Collagen has a helical shape with a length of 10 nm; it can self-assemble into fibrous structures with varying diameter ranging from 50 to 500 nm and plays a critical role in the mechanical and biological properties of bones. Bone defects and injuries pose significant medical challenges. However, bone tissue engineering presents a versatile way for bone tissue regeneration and repair [31]. As a complicated and dynamic process, this technique regulates bone cell migration, proliferation, and differentiation and accelerates bone matrix formation, resulting in shorter healing time compared to traditional procedures. Unlike permanent implants, scaffolds are first supposed to provide temporary support for cells adhesion. Figure 9 shows the concept of bone tissue engineering: (a) damaged bone, (b) nanofibers scaffold implanted

into bone, (c) new bone tissue formation on the scaffold, and (d) degradation of scaffold and complete regeneration of bone tissue. Scaffolds deliver bioactive agents that promote tissue regeneration, and they are able to mimic the intricate fibrillar architecture of natural extracellular matrix (ECM) components. Biomimetic scaffolds provide a synthetic osteogenic microenvironment to facilitate the ossification process and improve clinical therapy [32-34].

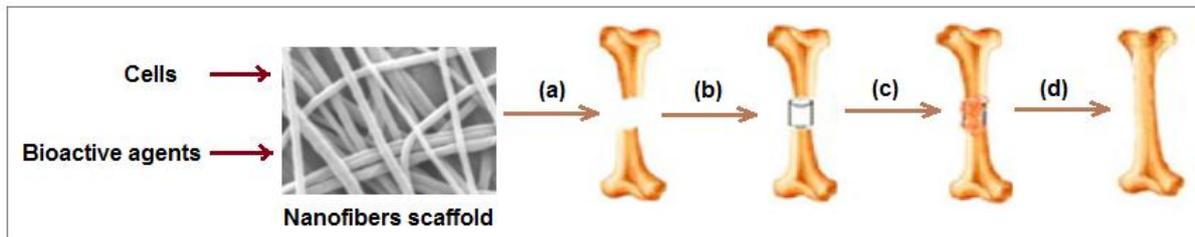


Figure 9: Schematic of nanofibers scaffold for bone tissue engineering

### 3.2 Nanofibers scaffolds for neural tissue engineering

In the nervous system, degeneration of neurons or glial cells or any unfavorable change in the extracellular matrix of neural tissue can lead to a wide variety of clinical disorders. Neural tissue repair is a daunting challenge because almost all neural injuries lead to an irreversible loss of function [35]. Neural tissue engineering aims to repair neural tissue by employing biological tools such as normal or genetically engineered cells and ECM equivalents along with potent synthetic tools such as biomaterials for scaffold design and/or drug delivery systems. More recently, a unique hybrid polycaprolactone-graphene oxide (PCL-GO) nanofibrous scaffold has been demonstrated to provide instructive physical cues in guiding the specific differentiation of neural stem cells (NSCs) into mature oligodendrocytes in the absence of chemical inducers [36]. In the study, biocompatible and biodegradable polymeric PCL nanofibers were synthesized through electrospinning, followed by oxygen plasma treatment. GO, the oxygenated derivative of graphene [37–41], was then uniformly coated on the hydrophilic surface of PCL nanofibers. Schematic illustration of the synthesis and application of the hybrid PCL-GO nanofibrous scaffold in guiding and enhancing the specific differentiation of NSCs into mature oligodendrocyte lineage is shown in Figure 10. NSCs cultured on the GO-coated PCL nanofibers exhibited extensive branching characteristic of oligodendrocytes. Further gene expression investigations revealed that cells grown on PCL-GO scaffolds exhibited significant increase in their mature oligodendrocyte marker expression.

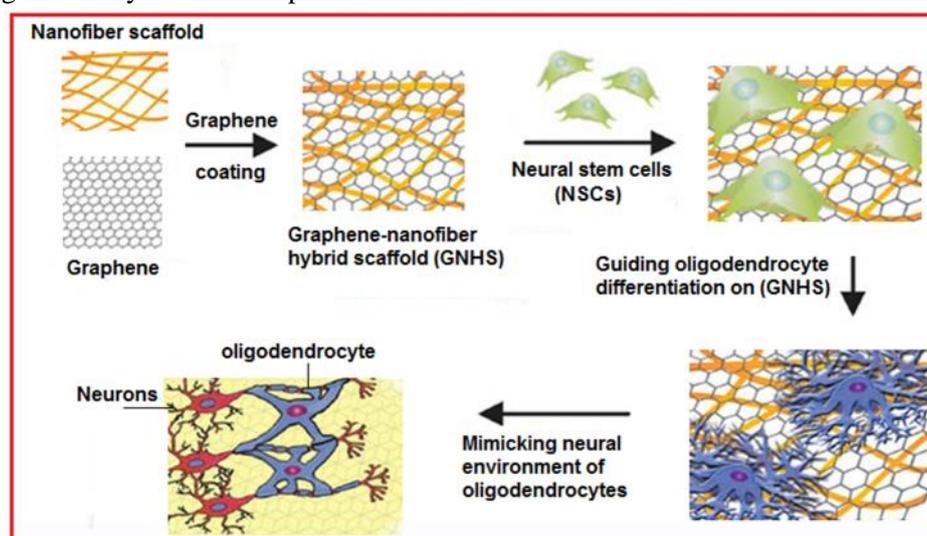


Figure 10: Schematic illustration of the synthesis and application of the hybrid PCL-GO nanofibrous scaffold for neural tissue engineering

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### 3.3 Nanofibers scaffolds for cartilage tissue engineering

Articular cartilage tissue has a limited capacity for repair due to the reduced availability of chondrocytes and complete absence of progenitor cells in the vicinity of the wound to mediate the repair process. PCL-based nanofibrous scaffolds were seeded with fetal bovine chondrocytes (FBC) and studied for their ability to maintain chondrocytes in a mature functional state. Their results demonstrated that FBCs seeded on the PCL nanofibers were able to maintain their chondrocytic phenotype by expressing cartilage-specific extracellular matrix genes like aggrecan, collagen type II and IX, and cartilage oligomeric matrix protein [42]. In a more recent study, bone marrow-derived MSCs along with PCL nanofibers was tested if the nanofibrous scaffolds supported in vitro MSC chondrogenesis. These results indicated that PCL nanofibers in the presence of a member of the transforming growth factor- family caused the differentiation of MSCs to chondrocytes that was comparable to that caused by cell aggregates or pellets [43]. However, since the PCL nanofibrous scaffolds possess better mechanical properties than cell pellets, they show potential to be developed as a scaffolding system for MSC delivery and hence cartilage tissue engineering.

### 3.4 Nanofibers scaffolds for skin tissue engineering

Skin wounds normally heal by formation of epithelialized scar tissue rather than by regeneration of full skin. There are two layers of skin, epidermis and dermis, the epidermis has less capacity to heal; however, when large areas of the epidermis need to be replaced, normal regeneration is lacking. Further, the dermis has an enormous capacity to regenerate. The scar tissue that forms in the absence of dermis lacks elasticity, flexibility, and strength of the normal dermis. Consequently, scar tissue limits movements, causes pain, and is cosmetically undesirable. Therefore, engineered skin tissue would be an excellent alternative, not only to close the wound but also to stimulate the regeneration of the dermis. Along with collagen, several other natural and synthetic polymers have been explored for skin tissue engineering; however, the use of these biomaterials as nanofibers has been very limited. Min et al developed nonwoven silk fibroin nanofibers by electrospinning for skin tissue engineering [44]. Due to their high porosity and high surface area to volume ratio, fibroin nanofibers coated with type I collagen were found to promote keratinocytes/fibroblast adhesion and spreading. Therefore, the silk fibroin nanofibers show potential to be developed as a scaffold for skin tissue engineering.

## 4. CONCLUSIONS

Nanofibers possess extremely high specific surface area and surface area-to-volume ratio and therefore have emerged as exciting one-dimensional nanomaterials for a broad spectrum of research and commercial applications. They are capable of forming networks of highly porous mesh with remarkable interconnectivity between their pores, making them an attractive choice for a host of advanced applications. Mimicking the architecture of ECM is one of the major challenges of tissue engineering. Amongst all the approaches used to prepare ECM synthetically, the approach using nanofibers, irrespective of their method of synthesis, have provided for scaffolds to have a significant effect on cell adhesion, proliferation, and differentiation. Hence nanofibrous matrices are currently being explored as scaffolds for musculoskeletal tissue engineering including bone, neural, cartilage, and skin tissue engineering. The results of all these studies clearly indicate that nanofiber-based scaffolds show excellent potential to be developed for a variety of tissue engineering applications.

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