Surface-Functionalized Electrospun Nanofibers for Tissue Engineering

Raunak Pandey, Ramesh Pokhrel, Prabhav Thapa, Sushant Mahat, K. C. Sandip, Bibek Uprety, and Rahul Chhetri

Abstract Electrospun nanofibers have been investigated for applications in diverse fields of tissue engineering such as degradable polymers, bioactive inorganics and nano-composites/ hybrids. Poly (ε-caprolactone) (PCL), poly (L-lactide-co-3-caprolactone) (PLLACL) and poly(lactic co-glycolic acid) (PLGA) electrospun nanofibers have been reported to be an effective scaffold for tissue engineering and drug delivery due to high surface-to-volume ratio, tunable porosity, cell affinity, hydrophilicity and ease of surface functionalization. In particular, electrospun fibrous scaffolds prepared by coaxial and co-electrospinning showed promising applications in adhesion, proliferation, elongation, cell growth and apoptosis which is highly desired for human body applications in tissues such as bone, nerve, ligament along with bio-artificial bone graft mimicking and bio-mineralization. Different characterization methods such as FESEM, SEM, FTIR, XRD and wet chemical precipitation have been used for these studies. Furthermore, a wide range of materials suitable for extracellular matrix scaffold has been prepared by electrospinning technique. This review summarizes preparation methods, functionalization and characterization techniques of nanofibers by electrospinning and their wide application in the field of tissue engineering. In addition, challenges pertaining to cell infiltration, low-density growth and inadequate mechanical strength of nanofibers as well as suggestions to mitigate these problems are also pointed out.

Keywords Electrospun nanofibers · Surface functionalization · Electrospinning · Tissue Engineering · Cell infiltration

Full Forms

FESEM	Field Emission Scanning Electron Microscope
SEM	Scanning Electron Microscope

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FTIR Fourier Transform Infrared Spectroscopy
XRD X-ray Diffraction

1 Introduction

Tissue engineering applies the knowledge of nanotechnology, engineering and biology to engineer cells or a combination of cells for replacing the biological tissues that have been damaged due to natural conditions or injuries [1]. The process begins by seeding cells into a scaffold followed by incubation in a growth medium that helps the cells to multiply and grow across the scaffold forming a substitute tissue. The cells forming the substitute tissue are taken from the stem cells of the human body. It is vital for the substitute tissue to be able to mimic the native fibers of the site and to provide essential topographical and chemical cues for regeneration [2, 3]. The scaffolds used for tissue generation are either extracted from donor organs or artificially developed from biodegradable polymers. The lack of sufficient donor organs and the difficult extraction process have necessitated the development of artificial techniques for fabricating scaffolds [4, 5]. Several well-known methods have been developed for producing nanofibrous scaffolds such as self-assembly, solvent casting, gas foaming, phase separation, melt blowing and electrospinning. The scaffolds made from these techniques have good porosity, small diameter, superior mechanical integrity, good biocompatibility (with native tissues at the site) and low cost for application in tissue engineering [6, 7].

Among the different techniques, the electrospinning process produces high surface area fibrous structures with necessary cell attachment, proliferation, differentiation and stiffness properties required for growing a substitute tissue [8]. The electrospinning process uses an electric field to generate nanofibers from a solution of polymer using a setup shown in Fig. 1. In general, a polymer solution is held at the tip of the capillary tube forming the moderate viscous Taylor cone at the tip of the plunger. This polymeric solution is subjected to an electric field which causes a jet of the solution to eject onto the collector metal screen (Fig. 1). As the solution travels through the air onto the collector screen, the solvent evaporates producing a continuous layer of polymer fiber. The orientation and the alignment of the evaporated nanofibers are based on the behavior and type of collector used in the electrospinning process. The magnitude of voltage also determines the orientation. Nanofibers of polyesters [1, 3, 7, 9–17], poly-anhydrides [18–22], polycarbonate [10, 23], poly (ethylene glycol) [24] and natural biopolymers (collagen, gelatin, fibrin, lipids) [25–27] have been produced using the electrospinning process [6]. Synthetic polymers offer easier process-ability compared to natural polymers and provide more controllable nanofibrous morphology. These nanofibers have been investigated widely for use in different sectors such as drug release, bone tissue engineering, skin tissue engineering, bio-sensing, cardiac tissue engineering and other applications [28, 29].

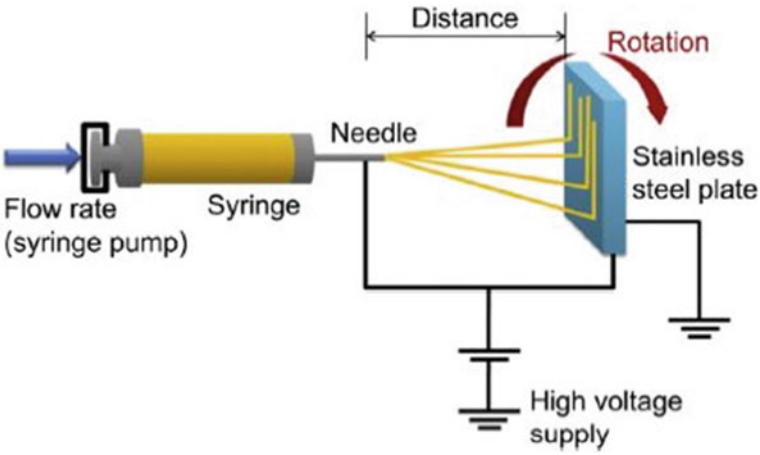

Fig. 1 Schematic illustration of the electrospinning setup. The mandrel can be rotated at various speeds to achieve different fiber orientations. Reprinted with permission from [2]. Copyright 2008, Elsevier

Although electrospun fibers are able to mimic the native tissues of the site, the fibers require surface treatments to match the bio-properties of the native site and to ensure the safety of the host site. In general, hydrophilicity, biocompatibility, stiffness, cell proliferation and antibacterial properties of the electrospun nanofibers are modified using the surface treatment methods. Surface treatment or functionalization is a common method that keeps the bulk properties of the material intact while promoting the biocompatibility of the scaffolds. To this end, plasma treatment, wet chemical approach, surface graft polymerization and surface coating are popular approaches for surface functionalization. Plasma treatment followed by wet chemical methods such as treatment with sodium hydroxide, potassium hydroxide increases hydrophilicity and improves biocompatibility. Wet chemical approach of alkaline hydrolysis and subsequent chlorination of nanofibers produce antibacterial polyacrylonitrile (PAN) nanofibers which enhances the cell attachment, hydrophilicity and mechanical properties [18, 19, 22]. Surface graft polymerization employs coating second material by physical adsorption. This method provides better stability by increasing cell attachment, fiber diameter and proliferation and by enhancing the hydrophilic nature of the cell. To improve their usability and efficiency, the nanofibers are often grafted before the electrospinning process to incorporate biological cues in the scaffolds [30]. The surface coating is usually coating the surface of nanofibers by molten or semi-molten substrates without any action of radiation or plasma sources. This modification enhances attachment, proliferation and other properties which helps to tune the scaffolds. Toward this end, Calcium Phosphate (CaP) coated Nylon-6 nanofibers have been produced which have been shown to promote cell proliferation [23]. Similarly, Polycaprolactone (PCL) fibers doped with silver nanoparticles have been shown to be highly beneficial in reducing bacterial infections in the implanted

devices. Thus, surface treatment is observed to be an effective method to engineer the surfaces of electrospun nanofibers in two and three-dimensional structures to promote cell infiltration and growth [31]. Structural changes of nanomaterials are perceived after the surface functionalization. Increase of hydrophilicity is the main changes that is the nanomaterials will have more affinity toward water. This increase in hydrophilicity will enhance structural changes leading to an increase in water contact angle and wettability, proliferation, biocompatibility and so on. Mechanical strength is also increased, giving certain nanofibers a suitable candidate for use in grafts which are durable and strong. Improvement of covalent cross-linking after functionalization is also seen. Cell adhesion and attachment is an important characteristic which is obtained after surface functionalization. These help the nanofibers to remain at a fixed position. High surface area and increase in fiber diameter after functionalization ensures more contact to the surface of the cell leading to various applications of functionalized nanofibers in bone grafts, cardiovascular systems, cartilages, wound healing and so on.

Electrospun poly (lactide-co-glycolide) (PLGA) scaffolds are commonly used in biomedical engineering and drug delivery fields due to their improved cell attachment, proliferation and adhesion [28]. Anterior Cruciate Ligament (ACL) replacement parts are being developed with the recent advancements in electrospun nanofibers and the surface grafting [32]. Polycaprolactone nanofibers have applications in skin tissue engineering along with bone tissue engineering. Poly-L-lactic acid can help in cartilage tissue regeneration and Poly (L-lactide-co-3-caprolactone) in neural tissue engineering. G-Rg3/PLLA electrospun fibrous scaffolds can be used in wounded skin for patients with deep trauma, severe burn injury and surgical incision having potential application in human skins. Likewise, tendon, muscles, blood vessels and so on also have potential applications of tissue engineering.

The nanofibers are often characterized for their morphological and mechanical properties and product consistency throughout the synthesis process. Scanning Electron Microscope (SEM), Atomic Force Microscope (AFM) and Field Emission Scanning Electron Microscope (FESEM) are used to characterize morphologic properties of nanofibers. Similarly, the chemicals used in producing the electrospun nanofibers and their surface treatments are characterized by Fourier Transform Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR) and Raman Spectroscopy techniques. The bending test and Young's modulus test are performed using the AFM, SEM and TEM techniques to characterize the mechanical characteristics of the nanofiber. X-ray diffraction (XRD) is also used to analyze the structure of nanofibers [33–38].

Thus, the objective of this review is to discuss electrospinning, surface treatment methods, characterization techniques and the applications of electrospun nanofibers in tissue engineering. The future aspects, along with challenges faced, is also pointed out.

2 Electrospinning

Electrospinning is the most widely used process for creating scaffolds for tissue engineering applications among various methods such as solvent casting, gas foaming and phase separation. In particular, the electrospinning process is inexpensive, produces high aspect ratio materials and provides the ease of combining different biomolecules for tailoring the properties of the final structure. Electrospinning is a method of synthesizing nanofibers from a polymer solution by using the electric field. The solution is held at the tip of a capillary tube by surface tension and subjected to an electric field which induces a charge on the liquid surface. The induced charge develops a force directly opposing the surface tension in the liquid, and when this force overcomes the surface tension, a charged jet of the polymer solution is ejected from the capillary tube end towards the metal screen (Fig. 1). The trajectory can be controlled with the help of an electric field. The solvent evaporates while the jet travels through the air producing a continuous layer of polymer fiber that lay randomly in the collector metal screen [39]. There are various types of electrospinning processes such as uniaxial, uniaxial with emulsion, side by side, coaxial, nozzle and melt electrospinning.

Coaxial electrospinning is a leading technology to surface modify the nanofiber scaffolds using multiple feed needles instead of a single feed needle. The two feed needles are connected to the common spinneret that helps to produce the scaffolds of different mixtures. In the two feed systems, one feed usually contains a hydrophobic polymer and second feed contains water-soluble bioactive solutions [1]. Using this technique, substrates of heparin contained poly (L-lactic acid-co-ε-caprolactone) (PLLA-CL), Poly(lactic-co-glycolic) acid (PLGA) and polyurethane nanofibers have been produced for tissue engineering applications [1, 40, 41].

The polymer solution in the emulsion electrospinning process is usually polymers dissolved in an organic solvent. Emulsion electrospinning enables loading of drug solutions into polymer solutions for spinning into nanofibers. The emulsion solution for electrospinning is prepared by mixing drug solution and polymer solution with the help of an emulsifier. The rest of the apparatus set up is the same as the electrospinning process (Fig. 1). Using this method nanofibers with Poly (ethylene oxide)-Fluorescein iso-thiocyanate (PEO-FITC) inside copolymer of Poly (ethylene glycol) / Poly (lactic acid) (PEG/PLA), Doxorubicin hydrochloride (Dox) an anticancer agent inside copolymer of Poly (ethylene glycol) / Poly (lactic acid) (PEG/PLA), proteins in Poly (l-lactide-co-caprolactone) (PLCL) has been prepared [42–44].

Melt electrospinning is the process of electrospinning which uses polymer melt instead of the polymer solution for generating the nanofibers. The polymer melt is generated from the polymer rod by melting the polymer rod. These rods should be non-conductive and viscous [45]. Gases and radiations are mainly used to melt the rod. Radiation is generally not preferred as it leads to poisoning of the polymer and is also economically infeasible. Gases can be used either directly or through steel jackets. Use of gas is convenient and abundant to melt the polymer rod. Also, the

diameter of the nanofibers after electrospinning can be controlled by controlling the gas flow rate, which is advantageous in surface modifications. Nanofibers of PCL (Poly(ε-caprolactone)) and PLA (Poly-lactic acid) have been fabricated using the melt electrospinning [46, 47]. The primary advantage of this method is the exclusion of the use of harmful organic solvents as used in other electrospinning techniques.

3 Components of the Electrospinning Process

3.1 Apparatus

Major components of the electrospinning process include syringe pump, voltage supply, needle and collector. A syringe pump is usually a plunger and plays a vital role to maintain the proper flow rate of the polymer solution. Generally used syringe pump is a single needle that creates core /shell fiber structure [15]. A programmable syringe pump is also available [24]. The voltage supply is between the collector and the needle. Change in voltage will change the acceleration of the electrospinning jet resulting in the change in the volume of the solution. Morphological changes in the scaffolds are experienced if the voltage is manipulated while electrospinning. The process includes DC focused potential (external voltage) to alter the mean free path of electrospun nanofibers, but in some cases, AC potential is also used giving advantages of less accumulation of like charges on the polymer surface [48]. A time-varying electric field can be used when deflection of the electric field is needed to produce nanofibers [49]. The needle is the release point of the polymer solution. For the industrial production, the apparatus is similar, but with little adjustment in the number of needles. Experiments in labs generally use a single needle, while industries use multiple needles to allow scale-up [50]. The collector plays an essential role in the alignment of fibers. The types of collectors are traditional (stationary plate), rotatory drum, parallel disc, rotating flat disc plate, rotating disc sharp and flat edge and conveyer collectors [15, 51]. The materials generally used for making collectors are (Aluminum foil [29, 52]), tin-foil [53] and stainless steel plate [54]. Collectors can also be modified to improve mechanical strength and cell infiltration of the nanofibers by employing drum, disc shape, and sharp edges collectors and so on. Modifications of the collectors can lead to the formation of nano-yarns, which are nanofibers formed from powdered polymers. Two collector system consists of a metal rod as the first collector and hollow metal hemisphere as the second collector. The surface of the collectors is insulated, resulting from deposition of electrospun nanofibers at the tip and the edge [55]. Porous nanofibers can be obtained with the help of non-conductive collectors [51].

3.2 Parameters Affecting Fiber Quality

The characteristics of the nanofibers can be controlled by the flow rate of the solution and applied voltage. In addition, polymer solution properties such as viscosity, conductivity, elasticity, concentration, environmental factors such as temperature and humidity also play an essential role. Voltage is known to affect the diameter of the nanofiber. The average diameter of the fiber initially decreases as the voltage applied is increased. The diameter reduces to the lowest point after which any further increase in the voltage increases the diameter of the nanofiber due to the increase in bead defects. However, some studies have shown that both the high and low voltage can lead to decrease in the diameter of nanofibers [15, 51]. The solution flow rate affects the pore size and the diameter of the nanofibers. Increase in flow rate leads to a rise in both pore size and diameter [51]. Low flow rate is generally preferred because the polymer solution will have more time for evaporation, and uniform nanofibers are formed. Any change in the flow rate is followed by the change in the voltage to obtain uniform nanofibers [56]. Collector distance plays a vital role in the size of nanofibers. Distance between needle and collector should be chosen carefully because too-far can lead to large diameter formation, and too-near can result in inadequate drying [51]. Increase in viscosity causes increases in intermolecular forces leading to an increase in fiber diameter [51]. These forces are challenging to break and hence to draw small nanofibers; the viscosity should be moderate. Volatility is an important factor to produce nanofibers as a low volatile solution cannot evaporate quickly and thus not suitable for forming nanofibers. A more volatile solution leads to an increase in micropores in the nanofiber structure. The increase in micropores leads to an increase in the surface area of the nanofibers, which is desired for increasing the reactivity [51].

4 Importance of Surface Functionalization for Electrospun Nanofibers

Surface modification techniques are the change in physical/surface properties of desired nanoparticles. As multiple processes have been described in the above section, the main goal is to alter the surface chemistry of the desired substrate. The modified surface of biomaterial is the first layer that connects with the body. For instance, in the dialysis process dialyzer will not work if a clot is formed in the surface of dialyzer [57]. The connection of biomaterial with body surface is mainly determined by the surface modification techniques. Where adhesiveness and connectivity is the main factor, the modification should be precise and accurate. In this scenario, the ion beam deposition technique is used. In the case of bone damage, the modified surface of nanomaterials containing osteogenic cell should be precise and accurate. This will lead to the formation of an adequate structure of bone and will give dynamic support to the body. This can be achieved only through perfect

adhesiveness of the foreign material. In this scenario, the ion beam deposited nanomaterials will be a suitable choice. Not only adhesiveness surface modification also enhances the biocompatibility of nanomaterials. The human body can contain a lot of compounds within a small vicinity. There may be lipids, fatty acids, cholesterol and so on. In the case of phospholipids of cell membranes, it contains the phosphate head group and two fatty acid chains. For instance, pure graphene with neutral charge can interact hydrophobically with lipid tails. This will extract all the cholesterol from the cell membrane and damage it. Similarly, it will affect the cytoplasm by penetrating due to their small sizes and sharp edges. But using the right amount and altering the surface chemistry will maintain the cell viability. For instance, it has been reported using graphene oxide with polyethylene glycol, poly (acrylamide) cell viability can be maintained up to 100% at 100 μg/ml [58]. Implanting different functional groups in graphene nanomaterials can be done by different surface modification techniques.

Polymeric nanoparticles are widely used in drug delivery systems due to their stability and ease of surface modification. To enhance the drug delivery, opsonization and prolongs circulation should be minimized. Opsonization refers to the coating of dangerous antigens. The target material such as microbes, molecules are modified and develop a stronger interaction with surface receptors. It invades the specific antigens that can be done only by specific components called as opsonins. Similarly, in vivo circulation of nanoparticles is equally important. It enhances the non-uniform distribution of the deliverance system throughout the target vicinity. To prevent this phenomenon, nanoparticle should be coated with polymer surfactants with biodegradable copolymers. The presence of charge in the surface of nanoparticles agglomerates the particles. In drug delivery system, aggregation of particles should be strictly prohibited. It has been reported, modified nanoparticles with zeta potential will develop stable suspension [59]. Along with the targeted drug delivery, modified nanoparticles should be of good mechanical strength. Large variation between untreated and treated nanofibers has been reported. Increase in tensile strength and young's modulus of sisal/polyester composites nanofibers has been reported after each modification step [60].

5 Surface Functionalization/Modification Techniques for Nanofiber Scaffolds

Nanofibers are incapable of activating specific cell functions and responses such as cell adhesion and proliferation until their surface is modified. These cell functions and responses are important for application in tissue engineering. An ideal scaffold must mimic the chemical and physical properties of the tissues for application in tissue engineering [26]. Surface functionalization/ modifications is a technique which involves changes or addition of some reagents in the surface of the nanofiber so that the fibers can bio mimic the microenvironments surrounding the cells and

tissues. The surface is modified physically or chemically according to the properties of the scaffold after electrospinning. Modification enhances hydrophilicity, mechanical properties, chemical properties, biocompatibility and fiber diameter of the scaffold. Modifications do not compromise the initial physical properties and structures of the electrospun scaffold. Surface treatments are mainly performed after the electrospinning is done. It also points out to those methods which will lead to the improvement of adhesion, biocompatibility, strength and so on which can be used in tissue engineering for regenerative medicines. Surface functionalization generally comprises physical approach and chemical approach. Some of the techniques used for surface modification/functionalization are discussed below.

5.1 Physical Approach

Surface modifications via physical approach method have been widely adopted in the field of nano-engineering. This approach is practical, feasible and simple. Modifications in the surface of nanoparticle are done by bringing physical changes in the substrate. Deposition and layering are the major principles of physical approach. Some of the techniques for the physical approach are given below: (Fig. 2).

5.1.1 Ion Beam Deposition

Ion beam deposition techniques is one of the automated process used for surface modification. It works on the principle of ionization of the target material. It involves ionized inert gases, substrate and target material. Initially, a substrate is placed inside the chamber where low energy inert gas source is used for cleaning of the substrate. A high-intensity ionized inert gas is bombarded to the target material. The ionized inert gas transfers its energy to the target surface. This energy melt vaporizes and alters the target surface. The vaporized surface is deposited on the substrate and forms a new modified substrate's surface [62, 63]. An accelerator is used to accelerate the ionized particle. The ionized particle travels through the mass analyzer. Here, only selective ionized particle with certain mass is passed. In this way, process of deposition is achieved.

Ion beam deposition can be both assisted, induced and sputtered. They can be further classified as Ion Beam Induced Deposition (IBID), Ion Beam Sputtering Deposition and Ion Beam Assisted Deposition (IBAD). Among them, IBAD is most common because it omits the oxide formation in substrate surface and produces compact coating [62, 63]. Ion beam deposition has been widely used because of control over stoichiometric parameters such as sputtering rate and ion current density. It produces uniform deposition with high precision coatings that have low absorption and scatterings over other methods. It improves wearability, adhesive properties and biocompatibility of the nanomaterial. It has been reported that coatings with high densities, high refractive indices, low extinction coefficients and good mechanical

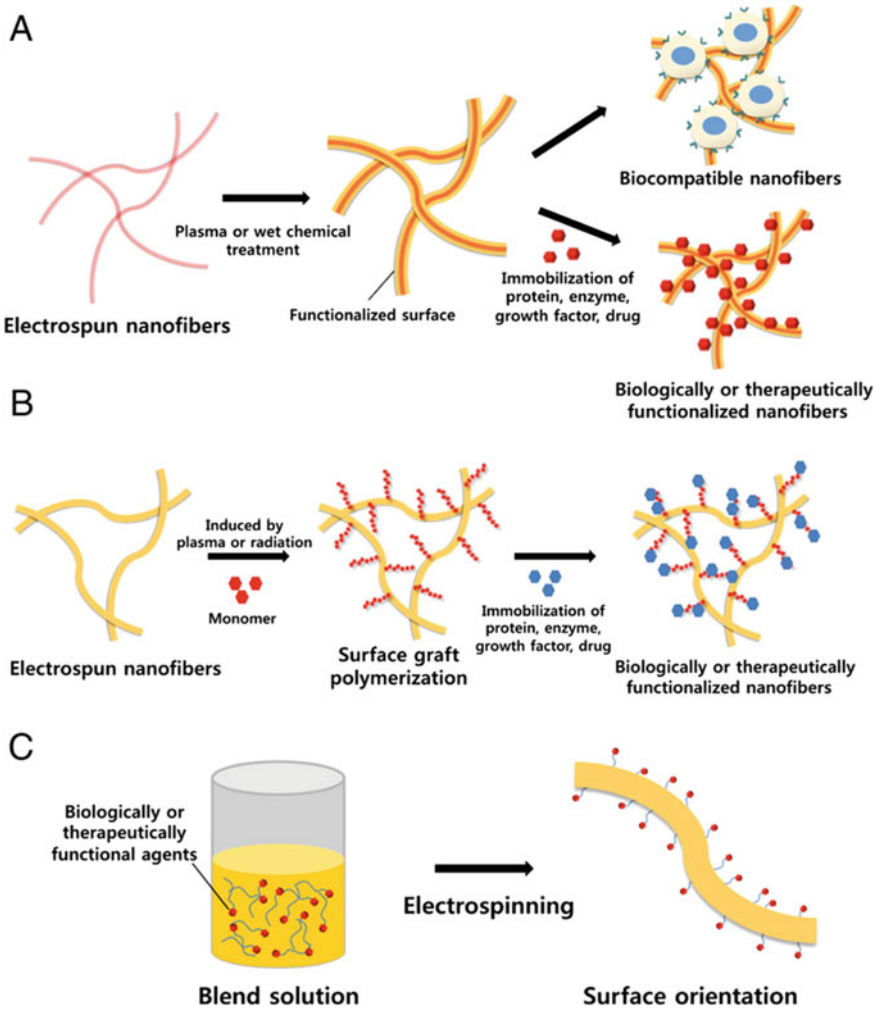

Fig. 2 Surface functionalization techniques of electrospun nanofibers. **a** Plasma treatment or wet chemical method. **b** Surface graft polymerization. **c** Co-electrospinning. Adapted with permission from [61]. Copyright 2009, Elsevier

properties for nanomaterial have been produced [62, 64]. Similarly, deposition of 10 nm INAs quantum dots in gallium arsenide substrate through this process has also been reported [64]. The enhancement of properties related to higher energy promoting rapid cellular adhesion and spreading in polyetheretherketone (PEEK) by ion beam deposition can help in biomedical applications [62]. Ion beam deposition created biomimetic apatite precipitation layers by emerging calcium phosphate thin film on pure titanium, helping to modulate precipitation processes and bioactivity enhancement [63].

5.1.2 Layer by Layer Deposition

Layer by layer deposition process is based on the principle of electrostatic interactions. Initially, a dipping of the charged substrate into a dilute aqueous solution of anionic polyelectrolyte is done. After a certain time, the polymer absorbs the charged particle. Then another layer of cationic polyelectrolyte is placed on the top of anionic polyelectrolyte. This process is repeated from time to time, and the layer of anionic polyelectrolyte followed by cationic polyelectrolyte is achieved. At each step, a new layer is formed, and the modified surface is achieved [65]. The result is the self-assembled layer of opposite charge. In this manner, the chemical stability of the film is achieved due to covalent cross-linking [63].

This deposition method is used where the chemically well-defined structure is not important [65]. Similarly, control over stoichiometric parameters such as deposition rate, deposition thickness cannot be achieved [65]. Though it cannot produce uniform coating, this process is cheap, less time-consuming and practical approach [63, 65]. The working principle is simple and widely used in MSC cells treatment therapy [63]. Deposition of 5 nanometer (nm) nanoparticle quantum dots on a gold substrate have been done by this method [66]. Similarly, deposition of titanium oxide (TiO_2) nanoparticles on multifunctional woven fabrics has also been reported [67]. Collagen/hyaluronic acid (Col/HA) polyelectrolyte multilayer film on titanium-based implants, ultrathin anionic polysaccharide support was created using the layer by layer deposition helping in proliferation, hydrophilicity, increased cross-linking properties on nanofibers [63]. Stainless steel stent with chitosan/heparin (CH/HE) multilayer functionalized with poly l-lysine (PLL), ultra-strong and stiff multilayered polymer nano-composites layer by layer coating on β-tricalcium phosphate/polycaprolactone porous scaffolds (3DP bTCP/PCL) fabricated by 3D printing, antibacterial coating through the deposition of negatively charged gold nanoparticles (GNPs) and positively charged lysozyme (Lys) on highly porous electrospun cellulose nanofibrous mats have been used as functionalized nanofibers using layer by layer deposition [68].

5.1.3 Langmuir-Blodgett Method

Langmuir–Blodgett method is one of the commonly used methods for the deposition of the nanoparticles into a solid substrate. The substance to be deposited in the substrate is in solid-state in other modifications techniques, while in this method it is in a liquid state. Initially, a thin film of required deposition is developed. The development of film is done by adding a few drops of desired film substance in a liquid layer. The desired deposit and liquid makes a bond and film is developed over the liquid surface. A vertical substrate is placed in the middle of the liquid pool. After the complete immersion of the vertical substrate, the distance of barriers within the sides of the pool is decreased. This increases the pressure within the film surface. The substrate is gradually pulled out of the pool. The liquid film is then transferred to the

substrate in this process. After repeating this process for a few times, a homogeneous layer of film is developed in the substrate [63, 69].

This method is based on the absorption of the film in the substrate. It is a powerful method for depositing amphiphilic molecules into a substrate at the air–water interface. Deposition of the film on the substrate depends upon the affinity of the film to the substrate [63, 70]. This method can be used in nano-medicines, nanoelectronics and so on. Formation of carbonated hydroxyapatite films on the metallic surface has been reported [70]. Similarly, fabrication of multifunctional nanoparticle has been done by this method [69]. This method is simple and can be widely used for flat substrate deposition.

5.1.4 Rapid Prototyping

Rapid prototyping is a fabrication method which is fully computerized and based on developing a template. Initially, a computer-aided design is drawn and fed into a system that can prototype without design error. The target metallic powders are melted by high-intensity laser beam and fabricated over the focused laser beam spot. The template is placed over the substrate, and target materials are passed over the substrate. This process is done in multiple types, and the desired prototype is fabricated all over the substrate [63]. It is a simple, inexpensive and rapid method. Nanoparticles between 10 and 100 nm can be fabricated easily. The templates used in this approach can be reusable. It is widely used in the field of nanoelectronics. The nanomaterials developed are uniform, compact and mechanically sound. Scaffold developed in this manner possesses a structured matrix with controlled porosity [63]. Deposition of silver (Ag) nanoparticles on TiO_2 precursors has been reported [71].

5.1.5 Surface Graft Polymerization

Surface graft polymerization is a simple, useful and versatile approach to improve surface properties by introducing radicals or groups like peroxide groups into polymer surface and then covalently bonding and polymerizing the monomers on the surface of scaffolds [48]. Surface graft polymerization improves hydrophilicity by decreasing water contact angle and exposing the peroxides to the polymer surface, increases cell attachment by chemically modifying the interior of porous scaffolds and improves the proliferation rate of scaffolds by instigating the polymer by free radicals through radiation or plasma [12, 18]. Surface modification tunes wettability, changes the chemical composition and creates a suitable environment for adhesion [18]. Surface collagen grafting has also become popular along with surface graft polymerization where collagen is added to the surface of the polymer after the action of plasma or radiation.

Radiation is often used to assist in surface grafting as it helps in the generation of reactive sites to polymer surfaces. This method is relatively simple and used

to initiate the polymerization as it does not require any chemical additives or catalyst on the polymer surface. Penetration depth and graft yield can be easily controlled by gamma-ray irradiation. Radiation method successfully cultivated human mesenchymal stem cells (hMSCs) by grafting acrylic acid on Poly (lactide-co-ε-caprolactone) (PLCL) film [1]. Structural changes may occur, but inner surface modifications are difficult due to variability in penetration depth [1]. Surface graft polymerization, when used on PCL surface by air plasma treatment physically, absorbed the gelatin to PCL surface [12]. The process maintained the expression of key markers typical for gelatin, improved cell attachment and growth of fibers after culturing with endothelial cells [12].

Radiation is a common way of surface penetration followed by grafting but krypton radical (Kr+) irradiation, when done to PLLA nanofibers, improves cell attachment with the cost of cell damage to make the surface thin [8]. However, discarding the damaged cells can lead to a refined larger surface area and spaces for cell attachment [8]. Cell adhesion can also be seen by the induction of new functional groups and carbonization [8]. Also, when argon radical (Ar+) irradiation is done to PLLA, fibronectin adsorption is increased along with an increase in cell attachment and excellent biocompatibility of PLLA nanofiber scaffolds [8]. When ultraviolet (UV) light is passed to nanofiber mats, cell cytotoxicity was decreased, and positive control for cell viability was seen with no morphological changes [72].

Adding collagen and solvents after electrospinning has also become an unvarying method for surface modification techniques. Residual solvents can also affect the biological performance of scaffold. Biological and structural changes during processing are observed in natural collagen, although being relatively non-immunogenic. Type-I collagen, when dissolved in hexafluoropropanol (HFP) has a negative impact on cell culture [25]. Biocompatibility and hydrophilicity of the scaffolds may be increased when collagen is added. The unrestricted somatic stem cells (USSCs) prefer extracellular matrix (ECM) for attachment than in the surface of polyethersulfone (PES). Also, better morphological characteristics can be seen at these collagen sites than plasma-treated sites. Proliferation and attachment of USSCs on natural microenvironment is also managed by the collagen [25, 26]. Grafting of poly trimethylsilyl-propargyl-hexaethylene glycol methacrylate-co-OEGMA (TMS-Prg-HEGMA-co-OEGMA) brushes from styrene-based copolymer surfaces was done for the surface graft polymerization of protected alkyne monomers. The polymerization of these alkyne monomers was through atom transfer radical polymerization (ATRP). The copolymerization of 37, 47 TMS-Prg-HEGMA with OEGMA475 formed polymer brush coating on fiber surface for effective SI-ATRP. The grafting of fiber and de-protection of the collagen was due to the formation of the polymer brush, and this formation confirmed that fiber structure was not altered by the reaction. The observation of minimal contrast between solid electrospun core and polymer brush coating was seen which was not improved by attachment of azide-functional gold nanoparticles and staining protocols [21]. After surface modification using collagen and surface graft polymerization, hydrophilicity, cell attachment and proliferation will increase to help make the scaffolds to be used in tissue engineering [26].

5.1.6 Surface Coatings

Surface coating is a process of reinforcing the surface functions of the substrate by depositing the layer of molten, semi-molten or chemical materials onto the surface [73]. Surface coatings with compounds enhance the physical and chemical properties such as the increase in fiber diameter, proliferation and attachment of the electrospun nanofibers. Hydrophilicity is also enhanced when the polymer surface is coated. The surface coating does not require penetration by plasma or radiation, unlike the surface graft polymerization technique.

Mussel inspired coatings to produce proteins have applications of adhesives in spite of wet conditions. Also, cell attachment and proliferation of endothelial cells were increased when PCL was coated with polydopamine [1]. Cell adhesion rate and proliferation of hMSCs were increased when polydopamine was coated to PLLA electrospun nanofibers [1]. This coating further increased osteogenic differentiation and calcium mineralization of hMSCs [1]. Calcium phosphate (CaP) coating on nylon-6 (N6) fibers also increased the amount of hydroxyapatite (HAp) which enhanced surface roughness and coated layer thickness [23]. These coating increased the fiber diameter and improved the crystalline phase of N6. CaP coatings increases the hydrophilicity of the nanofibers, enhances bone tissue growth and proliferation with an increase in cellular attachment and maintenance of surface morphology [23]. Surface chemical composition, surface roughness, topography and wettability enhances the biocompatibility and biological, environmental functions on Nylon-6 nanofiber when coated with CaP [23]. Tensile strength is increased after coating with CaP on Nylon-6, but the nanofibers become brittle when the HAp layer is increased [23]. For coating poly (lactide-*co*-glycolide) (PLGA)/gelatin nanofiber with CaP coating, soaking time of the nanofiber was increased along with agglomeration [27]. The hydrophilicity was also enhanced when this coating is applied [27]. When N6 nanofibers are coated with hydroxyapatite, homogeneous nucleation, and fast precipitation of hydroxyapatite is experienced, which increases layer thickness resulting from extended reaction time and pressure [10]. The coating of poly-pyrrole on electrospun PLGA nanofibers has an application on neural tissues. These coatings make the nanofiber non-cytotoxic and influence uniformity, conductance and morphologies of the nanofibers. In addition, the fiber diameter is increased [11].

5.1.7 Electrospinning

Electrospinning is a process of synthesizing the nanofiber scaffolds from the polymer solution by using an electric field. Electrospinning is the basic requirement to produce nanofibers for tissue engineering applications, but by different methods of electrospinning, the physical and chemical changes in the nanofibers can be altered and modified. Coaxial electrospinning, co-electrospinning, blend electrospinning are some of the modified techniques of electrospinning to modify the surface of nanofibers. Coaxial electrospinning uses multiple feed system, electrospinning two or more polymer solutions from coaxial capillaries [74]. Co-electrospinning is a process

of simultaneously collecting scaffolds onto a single collection device [18]. Blending drugs with a polymeric solution before electrospinning is blend electrospinning [18]. Localization of biomolecules by co-electrospinning allows sustained release profiles. This method bio-functionalizes the fiber in situ and the interspersed fibers from different molecules are produced from concomitant electrospinning from two or more spinnerets. Co-electrospinning combines the natural and synthetic polymer which has advantages of modulating the chemical, physical, mechanical, bio-resorb ability profile and degradation properties.

Composite scaffolds are obtained from co-electrospinning Poly (L-lactic acid) and gelatin from two distinct spinnerets which improves morphology and mechanical properties [7]. Loss of biological activity of biomolecules by denaturation or aggregation leads to a conformational change in organic environment so, preservation of the protein activity must be done for successful growth factor delivery, and appropriate concentration of growth tissue is needed [1]. Coaxial electrospinning is used to functionalize nanofiber surfaces by generating core-shell structured nanofibers. The good properties of cell attachment and proliferation of smooth muscle cells and endothelial cells are shown by coaxial electrospinning of Poly (L-lactide-co-3-caprolactone) (PLLACL) solution as shell and the phosphate-buffered saline solution containing protein as a core. The average strength of PLLACL was, however, higher than the nanofibers with Bovine Serum Albumin (BSA) at the core [14]. Poly (glycerol sebacate) (PGS) based electrospun nanofibers was produced from coaxial electrospinning by blending PGS with poly (ε-caprolactone) (PCL) [3]. When electrospun fibers containing PGS and poly (L-lactic acid) (PLLA) were produced from coaxial electrospinning, fiber diameter increased having a higher concentration of PGS [3]. The use of PLCL/HA core-shell matrices from coaxial electrospinning reduced the biocompatibility of the scaffolds [17]. The HA concentrations should be higher to enable smooth electrospinning. From coaxial electrospinning, hydrophilicity was improved, which aids on cell attachment, proliferation and differentiation by decreasing the water contact angle of the scaffolds.

5.2 Chemical Approach

Different chemicals require the activation of the surface, i.e., synthesizing the functionality of nano biomaterials on the surface. The amine-functionalized graphene oxide (AGO) provides improved modulus, increased proliferation of hMSCs, increased osteogenesis of stem cells and inhibited biofilm formation compared to PCL graphene oxide composites and reduced graphene oxide. Alkaline hydrolysis, covalent adsorption and the wet chemical process are some of the most common methods for chemically modifying substrates surfaces [63].

5.2.1 Adsorption via Covalent Bonding

In comparison to the physical adsorption process, the substrate and adsorbed molecules are binding in the covalent bonding method or by electrostatic interactions. This approach is more surface-resistant than physical adsorption due to its covalent bonds or electrostatics. In terms of cell growth and bodily fluid movement, it is also strongly bio-monitored.

The covalent linkage between the reactive groups on the surfaces is the primary determinant for the physical and chemical properties of the biomaterial changed by the surface. There have been experiments in which covalently adsorbed peptides incorporated in human vitronectin with oxidized titanium substrate has improved endogenous implant inclusion in the process of bone tissue engineering. Also, it has the ability to regulate the actions of neuronal stem cells for therapy applications by adaptation with different signaling molecules by covalent bonding of conducting PEDOT (poly (3, 4-ethylene dioxythiophene)/GO (Graphene oxide) scaffold. In combination with other polymers for tissue engineering purposes, new biomaterials can also be created through the enzymatic functions of natural polymers, including chitosan. Another study has also shown that in the presence of porcine pancreatic lipase, copolymer (poly-lactic acid chitosan) can be produced that enhances the adhesiveness of cells and can be used as a scaffold in tissue engineering.

5.2.2 Alkali Acid Hydrolysis

In Alkali hydrolysis process, ester bonds are cleaved to cause the formation of carboxyl and hydroxyl functional group by diffusing protons between the polymer chains, which induces hydrolysis of the surface. The hydrophilicity of Poly-Based Scaffolds can be improved by this process. When citric acid is washed, the water contact angle on the PLLA film surface decreases and surface roughness increases significantly.

5.2.3 Self-Assembled Monolayer

Self-assembling is an energy-efficient method used to prepare modeled monolayers on biomaterial surfaces on the substrate. Intermolecular or inter-particle forces at a critical point facilitate a normal molecular arrangement that minimizes the total free energy of the entire surface. In an area from the nanoscale to the micro-scale, molecules or nanoparticles can self-assemble. Many material forms, such as block copolymers, nanospheres, nanoparticles and biomolecules, have already been used to auto assume patterns.

Endothelial cells cultured on a gold substrate covalently adsorbed by the base fibroblast growth factor (bFGF) achieved the highest rate of proliferation when grown on carboxylic-ended self assembled monolayer (SAMs). In stem cell, research nanoparticles also have been utilized that functioned with monolayer SAMs. Also

recently developed, the nanotopography-mediated reverse uptake (NanoRU) delivery system successfully transfers siRNA to neural stem cells (NSCs). The surface charge on SAMs could therefore be concluded to play an important part with respect to the binding characteristics of various substrates and of ligands/biomolecules.

5.2.4 Plasma Treatment

Plasma treatment is the process of immobilizing polar groups such as carboxyl, hydroxyl or amine moieties on the surface of the scaffold by the action of plasma. High energy plasma which impinges on the surface of the scaffold alters the wettability and hydrophilicity of the scaffolds by exposing the polar groups of the polymer and decreasing the water contact angles [15]. Hydrophilicity enhances fibroblast adhesion, proliferation and biocompatibility of the scaffold allowing steady seeding process of the scaffolds [1, 18]. The surface morphology of plasma-treated fibers becomes rough and is able to control surface roughness and to crosslink during the process of graft polymerization and thin-film coating [75]. Plasma treatment with air, oxygen, argon, nitrogen and acetylene is mostly common.

Argon plasma treatments have been performed to remove surface contaminants and produce ex-situ oxygen surface functionalities for poly(L-Lactide) (PLLA) nanofibers [13, 75]. Surface wettability and surface roughness properties of the nanofiber were found to increase with the plasma power [13, 75]. The decrease in average fiber diameter, increase in cell attachment, wettability and modification of fiber morphology to increase biocompatibility were experienced after plasma treatment with argon oxide (ArO_2), nitrogen and hydrogen ($N_2 + H_2$), and ammonia and oxygen ($NH_3 + O_2$) to polycaprolactone (PCL) nanofibers [75]. Oxygen plasma treatment was able to remove residual organic impurities and weakly bound organic contamination on the anatase titania nanofiber mats/ indium tin oxide (TiO_2-NF/ITO) surface. Ultra-clean surface for biomolecules loading was also offered after the plasma treatment with oxygen [76]. Thus, modification of surface hydrophobic or hydrophilic characteristics, etching and nano-texturing of polymer surface and improving mechanical properties by treatment conditions are prominent advantages of plasma treatment [16].

5.2.5 Wet Chemical Method

Wet chemical method involves generating reactive functional groups on random chemical excision of ester linkages on polymer backbone by treatment with mild acids or bases. With the wet chemical method, chemical functional groups are not generated, but carboxylic and hydroxyl groups on conventional hydroxyl-esters are normally seen. Surface-modified nanofibers with minimal change in bulk properties are produced by determining the duration of hydrolysis and concentration of hydrolyzing agent [1]. The wet chemical approach provides flexibility for surface modification and helps to enhance hydrophilicity by decreasing the water contact

angle. Biocompatibility through surface immobilization and an increase in mechanical strength is experienced when electrospun nanofibers are immersed into the acidic or basic medium.

When poly (ε-caprolactone) (PCL) /polyacrylonitrile (PAN) is electrospun to form highly aligned nanofibers while engineering the parallel aligned myoblasts and myotubes, PAN modified the surface of PCL. After modifications, the alignment of the topography of nanofibrous scaffolds on cell viability was prevalent on the myoblasts tissues [18]. An acidic and alkaline solution such as sodium hydroxide (NaOH) can create nanotopography by modifying the surface wettability of electrospun polyesters. Surface wettability is induced by partial surface hydrolysis of the nanofibers. In the case of poly (ε-caprolactone), NaOH hydrolyzes the ester bond by random chemical scission to change the surface morphology in the more agglomerated form which influences cellular attachment and proliferation [9, 12]. This scission exposes hydroxyl and carboxyl groups to the polymer surface, which improves the hydrophilicity and decreases the water contact angle [12, 77]. Polyacrylonitrile (PAN) nanofibers modified by loading ferric oxide (Fe_2O_3) were found to have increased the mechanical strength and uniformity [19]. However, the challenges of agglomeration and particle dispersion were also prevalent [19]. The mechanical integrity of scaffold is also influenced by the concentration of hydrolyzing agents and submersion time [12]. In the case of chitosan/ hydroxyapatite nanofibers, the chemical method showed the spindle-like morphology, which delayed cell attachment and proliferation [78]. When cellulose acetate is hydrolyzed with NaOH better biocompatibility was observed, which resulted in enhanced cell adhesion and proliferation along with improved cellular response [79]. The degree of crystallinity and hydrophilicity of poly (vinyl alcohol) (PVA) increases when treated with methanol, but the reduction of cell proliferation was not seen [72]. Poly (ethylene glycol) (PEG) loading in Poly(lactic acid) (PLA) nanofibers showed an increase in diameter and uniform morphology of PLA [24]. Hydrolysis by potassium hydroxide (KOH) and chlorine loading in polyacrylonitrile (PAN) nanofiber was done where less number of chlorine exhibited effective biocidal functionality, and more number of chlorine resulted in slow cell inactivation [22]. Hence, immobilization of bioactive molecules is mainly done by applying chemical modifications where immobilized molecules are covalently attached to nanofibers and aren't easily leached out.

5.2.6 Click Chemistry

A recent approach in surface modification by click chemistry has been of interest to many scientists in tissue engineering. Click chemistry is 1,3-dipolar cyclo-addition of azide and alkyne to form 1,2,3-triazole applied to functionalize nanomaterials to rapidly create scaffolds necessary for tissue engineering [80]. Click chemistry induces click reactions which can be used as a powerful method for surface modifications due to the high efficiency and excellent selectivity under mild reaction conditions.

4-dibenzocyclooctynol (DIBO)-terminated PCL nanofiber is modified by performing conjugation of azide-containing peptide and azide-containing gold nanoparticles onto DIBO-terminated poly (γ- benzyl-L-glutamate) (DIBO-PBLG) nanofibers. Conjugation is done via the strain promoted azide–alkyne cycloaddition (SPAAC) where SPAAC provided efficient conjugation of the nanoparticles [20]. Utilization of photo-initiators and UV-irradiation for functionalization of alkene bearing nanofibers in click reactions are also prominent. Enhancement of hydrophilicity and anti-biofouling characteristics are provided by TEG groups, and reactive handles are provided by polymeric precursors of polylactide-based copolymer with furan groups when nanofibers are formed. When nanofibers are conjugated with cell adhesive peptide, cRGDfK provided cell adhesion and proliferation. A poly (styrene-co-VBC) macro-initiator was synthesized with smooth morphology, and fiber size also increased to allow cell infiltration into scaffolds [20, 21] (Table 1).

6 Characterization of Nanofibers

Nanofibers are differentiated in the synthesis process by correlating test methods with functional properties of the materials and confirming the product quality. Basic data and information can be obtained from the single fiber characterization for understanding the structural relationship and properties of nanofibers. The method of characterization is obviously still in its initial phase, and the demand for the production of effective characterization techniques has steadily increased. The study focuses on chemical characterization, as well as the physical characterization of nanofibers.

6.1 Physical Characterization of Nanofibers

Physical evaluation of nanofiber scaffolds after surface modification is done to observe the morphological changes in the scaffolds. Morphological properties such as porosity, fiber diameter and length of the scaffold are usually analyzed. A scanning electron microscopy (SEM) scans a focused electron beam to form the image of the sample by producing various signals. The surface of the sample in SEM is sputtered and coated with a thin layer of gold, platinum-coated aluminum or coated palladium-platinum-gold or palladium-platinum or aluminum/palladium [11, 14, 17, 23, 24, 81–87]. Field emission electron microscopy (FeSEM) is applicable to analyze the morphological behavior of the samples at fairly high resolution and provides surface or whole small particles topographical information to determine the elemental composition of the surface of the sample [34–36, 76, 88]. Furthermore, the SEM and FeSEM were used to analyze the structure and morphology of nanofiber for PLA [89], PCL/PEI and PLC [2, 90, 91], PES [26], PLLA [8], PGS-PMGA/gelatin

Table 1 Advantages and disadvantages of functionalized nanofibers [57–70]

S.N		Approach	Advantages	Disadvantages
1	Physical	Ion beam deposition	Good adhesiveness and biocompatibility of deposited material, packing with high densities, high refractive indices, low extinction coefficients, Compact packing and good mechanical properties. A fully computerized process with Easy monitoring of stoichiometric parameters	A complex process with a time-consuming, expensive and lower spatial resolution
2		Layer by Layer Deposition	Simple working chemistry, cheap, Deposition of multiple layers and Less time-consuming	No control over stoichiometric parameters such as deposition rates, deposition thickness with a less chemically well-defined structure
3		Langmuir- Blodgett	Control over film thickness, substrate deposition and barrier pressure with simple, easy, less time-consuming with the cheap process	Mixing of other phases while multiple layering, resulting dis-oriented structure
4		Rapid prototyping	A simple, easy, cheap computerized process with less design error and time Good control over structural integrity and porosity of the matrix	Frequent monitoring of vibration suppression and fiber solidification
5		Surface graft polymerization	Simple, easy and versatile approach for incorporating polymer in solid nanoparticles, with good adhesion of the attached graft. Control over-penetration	Poor graft density in grafting to and grafting through method due to the limited number of polymer chain and slower chain reaction
6		Surface coating	Simple and easy and cheap process with an increase in hydrophilicity. No use of harmful radiations or plasma	Tends to increase the stress in the outer side of nanoparticles that may lead to internal stress

(continued)

Table 1 (continued)

S.N		Approach	Advantages	Disadvantages
7	Chemical	Adsorption via covalent bonding	Provides strong bonding to biomolecules	Loss of functional conformation of biological molecules
8		Alkali acid hydrolysis	Increases roughness of the surface with a short time of functionalization	Requires exact conditions, permanent surface functionalization
9		Self-assembled monolayer	Limits to small nanofibers from nm to microns for fabrication	The high cost of synthesis, complex process
10		Plasma treatment	Low-temperature treatment, low operating costs, environmentally friendly	Adaptation mechanism is effected, depth of plasma penetration is increased.
11		Wet chemical	Versatile and economical, easy scale-up	Difficult to achieve a uniform layer system, the external stimulus is required
12		Click chemistry	High selectivity, efficiency, biocompatibility and stability	Expensive substrate material, the problem of heating requirement issues in certain cases

[3], PLGA/gelatin [92], HEC/PVA [93], PCL-MWCNTs [94], DTX/PDLLA [95] and PLO/PLEY [96].

Transmission electron microscopy (TEM) is technique of microscopy in which a beam of energetic electrons is transmitted through a sample and an image is formed by electrons interacting with the sample. TEM has been widely used in characterizing nanofibers core-shell structure, internal morphology, crystalline structure and membrane elemental information [17, 36, 40, 76, 97]. The samples for TEM observation were prepared by depositing the nanofibers onto a copper mesh or grid [17, 38, 78, 81, 97].

Atomic force microscopy (AFM) is known as a widely used technique for the characterization of nanomaterials which gives information about topography, morphology and particle/grain distribution from the surface of the sample. The topology of nanofibers including polycaprolactone [98] and morphology of Poly (l-lactide) (PLLA) [13], polyvinyl alcohol (PVA) [99] and polycaprolactone [100] has been investigated through AFM images.

Mercury intrusion porosimetry is the technique based on the property of mercury that does not wet the surface of solid materials [41]. The analysis done by the mercury porosimetry indicated that the porosity of the electrospun copolymer nonwoven was more than 80% with a median size of 8 micrometer (mm), total pore area of $5m^2/g$ and pore size of (10–200) mm [101]. Similarly, another study showed porosity ratios,

and pore size PHB ranges varied from 62 to 83% and from 0.4 to 8 μm, respectively [81].

Capillary flow porometry is a well-known process for measuring pore size distributions in polymer membranes and fibrous media. It offers a simple and non-destructive technique that enables rapid and accurate pore size and distribution measurements [102]. Structural pore properties of electrospun PCL/gelatin [102] and PCL with natural polymers [103] were done by capillary flow porometry.

6.2 Chemical Characterization of Nanofibers

The chemical composition, functional groups and chemical bonding of the nanofibers are analyzed to investigate the creation of scaffolds having these characteristics. The Fourier Transform Infrared Spectroscopy (FT-IR) is a method of investigating the composition of organic and inorganic materials based on the chemical bonds and functional groups that have different characteristics of energy levels. FTIR collects and transforms interference pattern data to a spectrum that offers information about molecular transmission and absorption that gives the materials a molecular fingerprint. The FTIR spectroscopy were used to analyze the functional groups of many nanofibers such as PCL [38, 91, 98, 102, 104], H-PCL, PCL-matrigel [91], PVA, PVA/γ-Fe_2O_3 [34], Nylon 6 [23, 35], non irridated and Kr^+ irridated [8], PLLA [3, 83, 105], PLLA/HA, PLLA/collagen/HA [83], (PANI-CNT)/PNIPAm-co-MAA [86], HEC [93, 106], HEC(PVA) [106], TA-g-PCL [105], PLA/GO, PLA/GO-g-PEG [107], mineralized PLGA [27, 36], PCL/P3ANA [108], PLCL and PLCL/HA [17], PLGA/gelatin [27, 92], PANi-CSPA, pure PCL, PCL/PANi [55], PET, PVA/PAA [109], silica/PVP [110], PCL/gelatin [102], CA [109, 111], CA-DA [111], PCL/SF, MeOH PCL/SF [38], PAN, PAN Fe_2O_3 [19], CA/CS/AG, CA/CS/MWCNTs [79], γ-PGA [112], raw DEX, PCL NFMs [113], HA-NPs [6], PolyNaSS, ungrafted PCL [114], AM/ST/PEO [115] and PLO, PLEY [96].

X-ray photoelectron spectroscopy (XPS) is one of the most powerful techniques that measure the chemical composition, elemental and molecular distribution of the material's surface in the range of (5–10) nm. The x-ray in the source stimulates the emission of photoelectrons and photoelectron kinetic energy is measured by the analyzer, and the detection of emitted electrons provides the information for quantitative and qualitative analysis. XPS was used to investigate the chemical composition of the core-shell structure of electrospun nanofibers of PCL, H-PCL, PCL-matrigel [91], SA/PEO [85], Hap /CTS [78], pure PCL and blended PCL/PANi [55] and PPy-PLGA [11] by measuring the binding electrons associated with atoms [85]. The C 1 s core-level spectra of PCL fitted into three peaks components about 284.6, 286.1 and 288.5 eV ascribed to C–H, C–O, O = C–O species, respectively.

Raman spectroscopy is a technique to inspect the vibrational, rotational and other low-frequency modes. From the analysis of vibrations, useful information such as functional group, chain orientation, interfacial properties of polymeric composition can be revealed. Electrospun cellulose [116], carbonization [8], N6 fiber [23],

graphite, synthesized GO, electrospun fibers PLA, PLA/GO, PLA/GO-g- PEG [107], TiO_2-NF [76] and CaP [117] were characterized using Raman Spectroscopy.

6.3 Mechanical Characterization of Nanofibers

Mechanical strength for the durability of the scaffolds to be used in tissue engineering must be tested and analyzed. The tensile test is mainly performed where electrospun nanofibrous scaffolds are cut into rectangular samples [85, 91, 94, 104, 118] to perform the tensile testing. Instron tensile tester [91], universal testing machine [55], tabletop tensile tester [118] are applied to determine the tensile properties such as tensile strength [55, 118], elongation [55, 104, 118], yield point [104] and Young Modulus [55] from the stress–strain curves of electrospun nanofibers.

6.4 Structural and Thermal Evaluation of Nanofibers

Techniques are provided to determine thermodynamic data and the evaluation of whether the nanofibers can endure the thermodynamic changes when applied in tissue engineering. X-ray diffraction (XRD) is a non-destructive technique for characterizing the crystalline materials and also the chemical composition and physical properties of materials. XRD has been used to study the crystal structure in several nanofibers including TiO_2 [33], PVA-γ-Fe_2O_3 [34], N 6 [35], HAp/CTS [78], HEC/PVA [52], PLGA [36], PA-6,6 [37], PCL [38, 98, 100, 104], PCL/SF [38], HA [10, 36], HA/chitosan [88], PCL-MNA [119], HA-NPs [6], TiO_2-NF [76], Fe_3O_4, PUA/ Fe_3O_4, PANI/PUA/ Fe_3O_4 [120], PLLA [121], PCL@MS [104] and AM/ST/PEO, AM/ST/PVA [115]. The X-ray spectrum was performed using Cu Kα radiation with recommended operating conditions [33, 37, 76, 88, 115].

Differential scanning calorimetry (DSC) is a thermal technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. The thermal and crystalline properties (melting temperature [34, 94, 100], crystallization [34, 94, 100, 119] and glass transition [94]) for different electrospun nanofibers such as PVA, PVA-γ-Fe_2O_3 [34], HEC/PVA [93], PCL-MWNTs [94], PCL NFMs[113], PCL-MNA [119] and PCL [100] have been assessed by DSC process.

Thermo gravimetric analysis (TGA) is especially useful for identification of compositions in materials by decomposing all organic polymer content at high temperatures. Properties of decomposition temperature [37, 116], thermal stability [106, 107, 122] and residual weight [35] of electrospun nanofibers such as cellulose [116], Nylon-6 [10, 35], CNC-ZnO [123], PGS-PMMA [3], HEC, PVA, HEC/PVA [106], PLA, PLA/GO, PLA/GO-g-PEG [107], PA-6,6 [37], pristine PVP, precalcined silica/PVP [110], CS/MWNTs, CS/ALG [79], HEC/PVA [93], PCL [122], PAN [22] and PCL@MS [104] have been analyzed by TGA.

7 Applications of Electrospun Nanofibers in Tissue Engineering Scaffolds

Various studies on tissue regeneration with the use of electrospun scaffolds have been performed. These scaffolds are first surface-functionalized and characterized before applications in the human body mainly in bones, cartilages and neural tissues. Some of the applications are discussed in detail below.

7.1 Bone Tissue Engineering

There has been wide research in bone tissues using electrospun nanofibers. PCL nanofibers have been extensively used for bone tissue engineering [38, 98, 104, 124]. It was seen that the core-sheath structured PCL/silk fibroin (SF) nanofibrous scaffolds provide desirable mechanical, biological properties and is potential of controlled drugs release [38].

In addition, the currently developed MS-shelled PCL hybrid nanofiber has also demonstrated outstanding properties for bone [99] favorable such as vitro bone bioactivity, mechanical functionality, osteogenic stimulation of stem cells and the loading and delivery capacity. These outcomes are considered to be useful as nano-bio matrix platforms therapies for the repair and regeneration though the further studies of bone regenerative ability of scaffolds in vivo are still remained [104]. Similarly, electrospun cross-linked HA containing chitosan nanofibrous scaffolds subsequently cross-linked with genipin are potential candidates for cranial and maxillofacial reconstruction [88].

The PANI nanofibers have been found to increase the biocompatibility of PES [125]. Similarly, the nanofibrous membrane of chitosan/PEO with a ratio of 9:1 has retained good structural integrity in water and exhibited better adhesion of chondrocytes [87]. It is also anticipated that the natural bone ECM in terms of nanoscale structure and chemical composition is a good choice of biomimetic bone tissues [36]. Biological in vitro cell culture with human fetal osteoblast (hFOB) cells for up to 15 days has demonstrated that the incorporation of HAp nanoparticles into chitosan nanofibrous scaffolds led to significant bone formation compared to that of the pure electrospun CTS scaffolds [78].

Other electrospun biomimetic PLLA/coll/HA nanofibers [83], PLLA/HAP/PCL nanofibers [124], the addition of gelatin in nanofibers [92, 110] and PLLACL–collagen (3:1) nanofibrous mats [97] have been examined. They have shown excellent bioactivity [124], adhesion [83, 92], hydrophilicity [97], proliferation [83, 92, 97, 98, 124, 125], mineralization of osteoblasts [83], osteogenic differentiation of MC3T3-E1 cells by increasing cell numbers, ALP activity, osteocalcin concentration [98, 124], for bone tissue regeneration. It is worth noting that mineralized

PLGA/gelatin nanofibers are a suitable choice because of their high efficiency of mineralization [27].

7.2 Neural Tissue Engineering

One of the most promising methods to restore nerve systems in human health care is nerve tissue engineering. The role of scaffold is significant in nerve tissue engineering. Many studies of nanofibers are done in nerve tissue engineering.

The Nerve Growth Factor (NGF) released from coaxial electrospun PLLACL nanofibers was studied by observing the differentiation of PC12 cells into neurons, in the presence of the supernatant obtained from the electrospun NGF which revealed that the PLLACL fibers retained at least some degree of bioactivity for up to 10 days [14]. In another study, rat PC12 cells seeded onto PGS-PMMA/gelatin nanofibers showed the potential to induce the differentiation of PC12 cells into neuron-like cells even in the absence of any nerve growth factor or chemical treatment [3]. Likewise, the TS PCL-NFs nanofibers, compared to ES PCL-NFs, have shown a positive impact on the development of neurons due to the enhanced tensile properties [100].

In vitro studies using C17.2 nerve stem cells on aligned PCL/gelatin, nanofibrous scaffolds have shown that the direction of nerve cell elongation and neurite outgrowth is parallel to the direction of fiber alignment. It is seen that the fiber alignment of PCL/gelatin nanofibrous scaffolds is less than the PCL nanofiber scaffolds and also the proliferation of C17.2 was higher on aligned nanofibrous scaffolds in comparison to random nanofibrous scaffolds for both PCL and PCL/gelatin [102]. Similarly, in vitro nerve stem cells culture and the electrical stimulation of Rat nerve stem cells (C17.2) on PLLA/PANi nanofibers revealed that the electrical stimulation of nerve cells could stimulate the differentiation or neurite elongation [118].

Furthermore, in vitro culture of PC12 cells and hippocampal cells on the PPy-PLGA meshes demonstrated that the electrical stimulation of PC12 cells on the conducting nanofiber scaffolds improved the neurite outgrowth compared to the non-stimulated cells [11]. These studies reveal increases in neurite length and percentage of neurite bearing cells will aid to design neuronal tissue interfaces integrated with topographical and electrical cues for use in nerve tissue scaffolds and for neural interfacing.

7.3 Cartilage Tissue Engineering

Bi-layer scaffold of collagen and electrospun poly-L-lactic acid nanofibers (COL-nanofiber) bi-layer in which mesenchymal stem cells were cultured on the bi-layer scaffold revealed that the implantation of COL-nanofiber scaffold seeded with cells induced more rapid subchondral bone emergence, and better cartilage formation,

which led to the better functional repair of osteochondral defects as manifested by histological staining, biomechanical test and micro-computed tomography data [54].

Similarly, the study of PLLA/gelatin nanofibrous scaffolds showed that the chondrocyte growth and differentiation markers emphasized the cartilage tissue growth with PLLA50GEL50 and PLLA70GEL30, providing the best cellular response. Furthermore, the mineralization experiment too suggests useful for cartilage-bone interface tissue engineering [7]. Likewise, the first demonstration of bone and cartilage regeneration was reported by implementing a novel strategy based on a synthetic nanoengineered bio mimicking membrane functionalized with nano-reservoirs of a growth factor (bone morphogenetic protein 2, BMP-2) [117].

On the other hand, electrospun nanofibers such as novel PPDO/ PLLA-b-PEG copolymer [101], composite CS/PEO [99] and PCL grafted with polyNaSS [114] were also reported for cartilage regeneration.

7.4 Skin Tissue Engineering

Skin is the largest body organ, functioning as a barrier to harmful mediums, preventing pathogens from entering into the body. A wound is a result of physical, chemical, mechanical and/or thermal damages. The natural healing process of the skin is complex and continuous.

Alfalfa carries genistein, which is a major phytoestrogen known to accelerate skin repair. In vitro cell culture on PCL/alfalfa nanofiber scaffolds promote cell growth and sustain biocompatibility for epidermal keratinocytes (KCs) and dermal fibroblasts to accelerate skin tissue regeneration in both mouse and human skin, without requiring additional proteins, growth factors or cells [82]. Similarly, in vitro by seeding with human KCs on GT/PCL, nanofibrous membranes were investigated which was further evaluated by transplantation of engineered epidermis into a wound-healing model in the nude mouse showed that the repaired skin at day 14 in the epidermis-treated groups, multiple layers of epithelial cells covered the wound area enhancing a suitable scaffold for tissue engineering [126].

Likewise, the use of G-Rg3/PLLA electrospun fibrous scaffolds rapidly minimizes fibroblast growth and restores the structural and functional properties of wounded skin for patients with deep trauma, severe burn injury and surgical incision [121]. In another study, pbFGF-loaded electrospun fibrous mats showed that the gradual release of pbFGF polyplexes revealed significantly higher wound recovery rate with collagen deposition and maturation, complete reepithelialization and skin appendage regeneration as well as accelerate the healing of skin ulcers for patients with diabetes mellitus [127].

Other electrospun nanofibers such as PCL [38, 91], PVA/γ-Fe_2O_3 [34], PPDO/ PLLA-b-PEG [101], TA-g-PCL, PLLA [105] and PVA, PVA/HA, PEO [99], also support skin tissue engineering. Moreover, the addition of matrigel or nanoparticles in the nanofibrous scaffolds holds the better potential for skin than only neat electrospun nanofibrous scaffolds [34, 91].

7.5 Clinical Perspective

The creation of biological replacements that can replace or revitalize human is optimistic in regenerative medicine. Although electrospinning is considered to be a simple and efficient process for the preparation of electrospun nanofibers, it is the clinical application on the market not yet fully utilized [128]. Recently, AVflo ™, a vascular access graft, was developed by Nicast using medical grade polycarbonate urethane nanofibers for use as a subcutaneous, arteriovenous conduit for blood access [129]. In the same way, SURGICLOT ®, electrospun-based nanofibers containing protein has been developed by St. Teresa Medical, Inc ®, to promote blood clotting [130]. On the other hand, there are still some economic and technological concerns regarding the safety and effectiveness of electrospun nanofibers in terms of clinical perspective. Since, due to low production yield and requiring highly skilled human resources, it doesn't seem very easy from the economic viewpoint. Furthermore, the large quantities of products in a continuous way are challenging. Since electrospun nanofibers have massive potential in tissue engineering so, the problems should be resolved to have its wider use [131].

7.6 Others

In addition to the above-mentioned issues, the feasibility of using nanofibrous scaffolds to culture stem cells [104, 118], and tissues such as muscles [5, 84, 118], tendon, ligaments [5, 132], and blood vessels [121, 127, 133] have been reported.

8 Challenges and Future Prospects

8.1 Challenges

Scaffold formation for tissue engineering is a delicate and complex process done mainly by electrospinning. While these methods have proved to be successful in the field of tissue engineering, challenges remain for successful regeneration of the tissue. In addition, challenges of deposition and degradation [32], mechanical integrity and porosity [134] and three-dimensional scaffolds formation [134] are faced by many scientists and engineers working on tissue engineering.

The balance between de novo tissue deposition and scaffold degradation is a common challenge of tissue engineering. Quick degradation of scaffolds will affect the mechanical integrity of the scaffolds. So, in order to balance the rate of degradation of scaffolds, optimization in spatial and temporal growth factor needs to be modified for the acceleration of de novo tissue deposition. The ability to withstand physiological loads needs to be ensured. Although regeneration of new bone tissue

has been observed, strong mechanical properties were not shown by the human cancellous bone [32, 134, 135].

Fabrication of three-dimensional biomimetic bone tissue scaffolds and alignment of the nanofibers in contact with the liquid environment is a major challenge. Layering and contacting the structure to the liquid environment is difficult, which creates issues of change in size/shape and cell infiltration [134, 135]. Growth of new blood vessels to deliver oxygen and nutrients to the implanted tissue is a long process but implanting these cells to vascular beds of liver, spleen, bone and so on will decrease the growth time, eventually consuming oxygen to engineered tissue at a fast rate.

The diffusion rate of genes and proteins from scaffolds needs to be in an accepted range of physiology to be able to use in human applications. So, a challenge of maintaining this range has also risen. Regulating cell behavior, offering alternatives for enhancing scaffold performance, covalently incorporating growth factors can be done. Specific instruction is required for tissue regeneration in vivo for guided growth of nerve, bone, blood vessels or corneal epithelia for critical injury sites.

Computer simulations for predicting how the cells bind to the extracellular matrix cannot always be used, and the challenge of determining the correct quantity of adhesion is a challenge. If adhesive ligands are less, cells cannot bind effectively for movement, but if there are more adhesive ligands, cells bind firmly to get stuck. So, intermediate adhesion is required for cell movement.

The scale-up process of three-dimensional tissue engineering is a complex challenge as it is limited to laboratory product. Formidable regulatory issues, separate culture for each new patients, high cost are some of the issues which need to be addressed [135].

Spinal injury results in loss of axonal connections and motor functions. Regeneration of injured site is a challenge due to too complex inhibitory environment. Scaffolds promote connections of these axons for regeneration and functional recovery. Finding consistent, quantitative and replicable treatment in preclinical trials for the spinal cord is a challenge.

In the case of the brain, it doesn't promote regeneration capacity, so the fabrication of scaffolds needs to have properties of cell infiltration, degradation and regeneration. Glial scarring should be prevented and thus, reducing the inflammatory response to neuron survival while coating brain implanted devices. Alternative creation of autograft in the peripheral nervous system for the clinical standard is a big issue in tissue engineering. Implantable scaffolds posing as a bridge for long gaps producing results same as autograft without harvesting autologous donor tissue has also become an issue. Biodegradable scaffolds can be the solution for this, capable of fully recovering the damaged tissue.

Thus, the complex task always promotes complex challenges which need to be addressed [136]. These challenges, when addressed accordingly, will give the right solution, thus, helping in modifying the complex structure of tissue engineering basics.

8.2 Future Work

Electrospun nanofibers can be considered a feasible way of generating nano scaffolds for various applications. It is good in every aspect, but still, there is room for improvement. Researchers can give more focus on melt electrospinning. Though solution spinning is easy and convenient, the use of organic solvent can lead to solvent toxicity when implanted in the human body. If more advancement in the scaffold can be done, the work in the future will be easy.

Similarly, cartilage injury, arthritis in bone joints is common nowadays. Repairing of cartilage tissue depends on the collagen orientation. Control tissue growth of damaged joints can be obtained when there is no foreign body involved after the injury. Efforts can be focused to mimic the tissue structure and to fabricate the nano scaffolds exact and biocompatible [137]. When an inert protein gradient is created, it can help to create a masking gradient and conserve the amount of bioactive protein along with the generation of self-assembling peptides [138].

9 Conclusion

This paper presents a review on surface-modified nanofibers produced through electrospinning process and its applications in tissue engineering, particularly on bone tissue regeneration and drug delivery system. The electrospun nanofibers are biocompatible, biodegradable, easily fabricated, and are able to support cell adhesion, proliferation, and differentiation and have emerged as a promising material for constructing replaceable biological components. The first and foremost step of the electrospinning process is the selection of proper polymer solutions with intrinsic functions that can be enhanced by employing several modifications strategies. The properties of electrospun nanofiber such as its morphology, fiber diameter and porosity depend on the flow rate of the polymer solution and the electric voltage applied. The scaffolds prepared through the electrospinning process are usually surface treated before application in the human body to match the bio-properties of the native site and to ensure the safety of the host site. Surface treatment by use of plasma, using chemicals, grafting after plasma treatment, coating and click reactions is mainly performed. Plasma treatment of PCL, PLLA, nylon-6 has increased the hydrophilicity and wetting characteristics of the polymer. The wet chemical method by using NaOH on PCL influenced cellular attachment and proliferation. PAN treated with Fe_2O_3 increased mechanical strength and uniformity. Surface graft polymerization of PCL after air plasma treatment increased cell attachment and growth. CaP coating in PLGA/gelatin increased hydrophilicity and agglomeration. Co-electrospinning PLLA improved fiber morphology, and mechanical properties and coaxial electrospinning of PLLACL increased cell attachment and proliferation. Other physical and chemical functionalization techniques involving the use of various nanofibers

were also discussed. The electrospun and surface-modified nanofibers are characterized using SEM, TEM and diffraction before application inside the human body. Scaffolds or nanofiber produced by electrospinning possess characteristics which closely resemble the natural bone (Extra Cellular Matrix) ECM in nanoscale structure and chemical composition; so scaffolding is a good choice for bone tissue engineering. The mechanical properties and cell response of aligned electrospun nanofiber bundles is a promising scaffold for orthopedic tissue engineering applications. Bio-composite scaffolds facilitated hMSC (human mesenchymal stromal cells) colonization and bone formation. The highly porous nanofibrous film with a high surface area is used for the processes of tissue regeneration as it provides more structural space for the accommodation and attachment of cells and enables the efficient exchange of nutrients and metabolic waste. Neural tissues can be designed by incorporating scaffolds with directional cues, bioactive to promote regeneration and repair, neural/progenitor cells for the release of growth factors, and functionalized polymers for better neuro-compatibility. Tissue engineering is mankind's attempt to replicate functioning tissues and organs whose working prototypes are available in nature. Thus, electrospun scaffolds have the potential to provide both a structural and functional mimicry of the native tissue through intelligent biomaterial scaffold design, enhanced time course and the functional outcome of endogenous tissue repair.

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