Recent advances in nanofibers fabrication and their potential applications in wound healing and regenerative medicine

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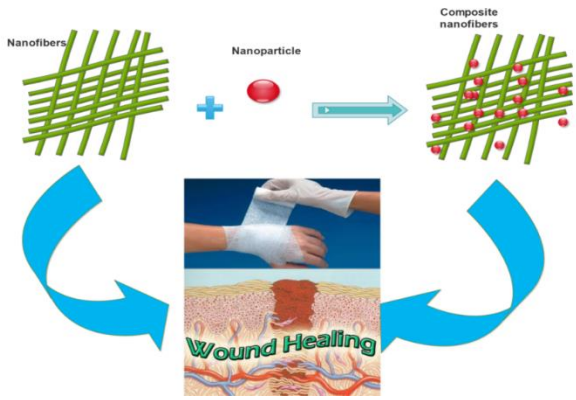
Abstract

Nanofibers fabrication is generating considerable interest in terms of their biomedical applications. Recent development in nanofibers fabrication techniques resulted in controlled manipulation of nanofibers characteristics, such as their high surface to volume ratio, high porosity and their ability to encapsulate bioactive molecules. Development of biocompatible, polymer coated nanofibers can also provide an optimal environment for cell adhesion, proliferation and differentiation. This paper presents an overview on different applied techniques for nanofibers fabrication, in addition to the process variables to tailor their physico-chemical characteristics. Furthermore, the current review sheds a new light on the application of nanofibers on treatment of diabetic foot ulcers and artificial skin reconstruction.



Keywords: Nanofibers, polymeric**,** wound healing**,** inorganic composite nanofibers.

Introduction

The world gross sales for wound healing products are expected to increase from $ 15.6 Billion in 2014 to $18.3 Billion by 2019. The wound healing products are generally categorized into traditional, basic and advanced products. Among various categories of wound care products, the advanced wound healing products comprised the largest share in 2014. Factors such as the increased awareness towards the advanced technology for wound healing and the increased incidence of obesity, diabetes and cardiovascular diseases are the driving forces toward the growth of the wound care market [1].

The process of wound healing is complex as it comprises three distinct phases: inflammation, proliferation and maturation [2]. The inflammation phase immediately occurs following the skin injury and lasts from 2-5 days. This phase initially starts with hemostasis due to formation of a platelet clot then followed with a secondary inflammatory stage due to dilation of the blood supply to the wound area. In the proliferative phase, the wound healing passes through three consecutive steps: granulation, contraction and epithelization. This phase lasts from 5 to 21 days and comprises the formation of new collagen and new blood capillaries. The last phase, the maturation phase, involves collagen formation; which enhances the mechanical properties of the wound tissue, followed with the formation of a scar tissue and reorganization of the extracellular matrix. Chronic wounds arise from physiological defects in the regular wound healing process. Diabetic foot ulcers represent one of the chronic wound conditions; which are commonly developed in 25% of diabetic patients [3]. Chronic wounds associated with diabetic ulcers, psoriasis and malnutrition are usually associated with underproduction of nitric oxide [3]. Nitric oxide has been recently reported to enhance growth factor-mediated wound healing mechanism [4]. Sildenafil was reported to decrease the breakdown of cyclic guano monophosphate (cGMP) thus prolonging the vasodilator effect of NO [5]. The combination of chitosan with Sildenafil in spray-dried powder formulations was successful in acceleration of wound healing through improving blood supply, angiogenesis and formation of new tissues [6].

Conventional wound dressings are originally applied to counteract against development of infection at the wound area [7]. Unlike the traditional wound dressing such as gauze and cotton, nanofibers can be designed to have a biological activity on its own or to deliver active therapeutic molecules to the wound area [8]. In acute wound healing, the incorporated drug can either have an effective role in the curing process such as removing necrotic tissues or an indirect role such as antimicrobial agent or growth factor to indirectly help the healing process. In chronic wounds, the patients usually undergo long term treatment. Therefore, a controlled system with prolonged drug release is preferred to provide a better patient compliance and therapeutic outcomes [9].

Several publications demonstrated that growth factors are important in controlling various cellular processes involved in the development of extracellular matrix (ECM) [2, 10 , 11]. The present article reviews some of these growth factors, their functionalities in wound healing and the reported growth factors-loaded-nanofibers for wound healing and skin regeneration. Proteoglycans and fibrous proteins are two main constituents of the ECM. The nanofiber diameters of the natural ECM were reported to be in the range of 50 - 150 nm, depending on the tissue type [12, 13]. Studies demonstrated that the type of polymeric nanofibers and morphology can substantially affect the cell attachment and proliferation during the different phases of wound healing [14-16]. Therefore, the current review explores the different manufacturing processes for fabricating nanofibers focusing on the electrospinning technique as a simple and least expensive mean to get ultra-fine nanofibers of various synthetic and natural polymers.

Techniques applied for the development of nanofibers

Electrospinning

Engineered nanofibers possess unique properties and hold a great potential in the pharmaceutical industry of wound care products. Nano fibrous materials are generally fabricated by phase separation [1], self-assembly template synthesis, mechanical drawing and electrospinning methods [17-19]. Electrospinning is the most commonly applied method for preparation of nanofibers, due to its simplicity and the possibility of producing multilevel structured ultrafine nanofibers. The mechanism of electrospinning is a direct extension of the electro-spraying technique. In both techniques, the liquid droplet is passed through a syringe at an applied potential gradient. A schematic illustration for the setup used for electrospinning is presented in **Fig. 1**. A typical setup for electrospinning is composed of a source of high voltage (1-50 kV), solution reservoir and a collector of nanofibers. The solution is first injected through a syringe; that is connected to a source of high voltage direct current. The produced nanofibers are targeted towards the collection plate; that is also connected to a source of high voltage direct current [19].

Electrospinning is a simple, inexpensive and scalable technique used for producing nanofibers. Various polymers, metals and carbon can be utilized to produce nanofibers by electrospinning [16]. In addition, all soluble high molecular weight polymers can be applied as a starting material to produce nanofibers. With the exposure to the high voltage direct current, the semi-spherical polymer drip is expanded to form a conical-shaped droplet (Taylor cone). The distribution of electrical charges on the droplet surface leads to the formation of a liquid jet. The exposure to high voltage results in the expansion of a liquid jet to produce ultrafine threads [2]. There are various process parameters that can be controlled to manipulate the diameter and morphology of the produced nanofibers [19].

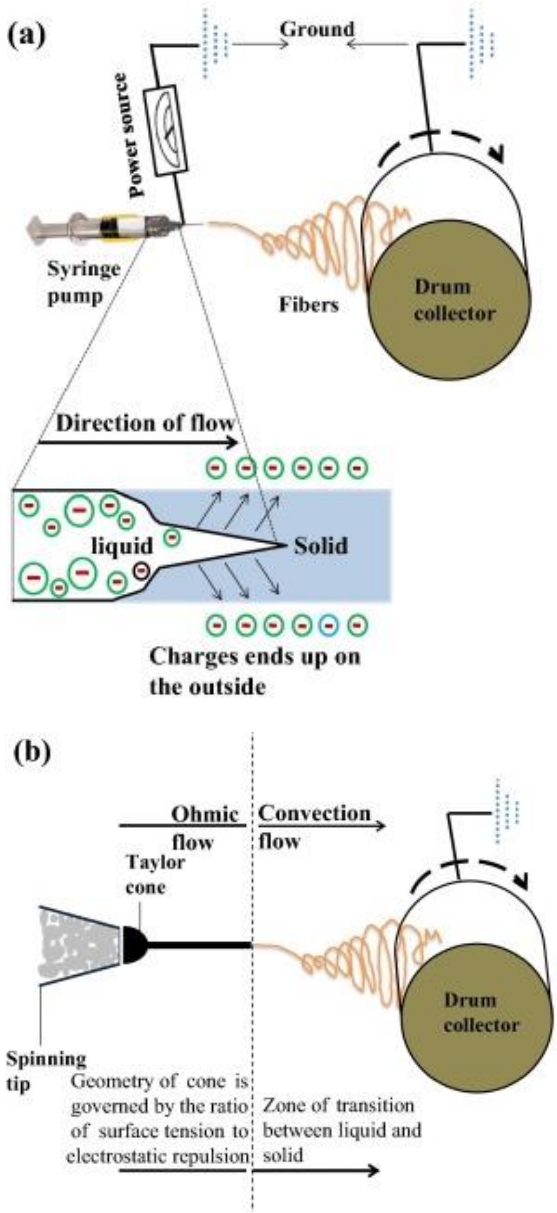


Fig. 2. (a) Electrospinning setup and (b) phenomenon of Taylor cone formation. Reference no. 19.

The diameters of the produced nanofibers are primarily affected by the size of the spraying nozzle and the polymer concentration. Studies recognized that during the formation of the Taylor cone, the spray jet splits into different jets. This results in the formation of nanofibers with different diameters. However, the fiber diameter could be also affected by the polymer viscosity and the applied voltage. Higher polymer viscosity or voltage produces nanofibers with larger diameters [17]. **Fig. 2** exemplarily demonstrates the formation of polymeric nanofibers with different diameters and pore sizes [20].

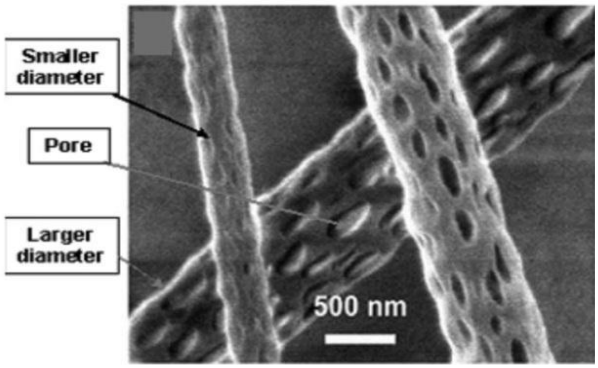


Fig. 2. Polymeric nanofibers with different pore sizes and diameters, Reference no. 20.

Rotary jet-spinning

Despite the popularity of electrospinning techniques, the application of a high voltage direct current, the low production rate and the sensitivity of the characteristics of the electrospun nanofibers to both formulation and process parameters limits its application.

Therefore, there is an increasing need for a more reliable method to produce a 3 D, smooth, uniform nanofibers with a well-defined alignment.

In the rotary jet-spinning technique, the polymer jet is formed by exploiting a high speed rotating nozzle instead of the application of a high voltage direct current. In this method, the polymer droplets undergo extensive stretching before drying at the collector plate. The setup for rotary jet-spinning composed of a reservoir for polymer solution connected to a central motor. The electrospun nanofibers are collected on a flexible air foil placed above the reservoir [21]. Similar to electrospinning, the process of rotary jet-spinning consists of three main steps: (i) jet initiation; which involves the flow of polymer solution through the orifice, (ii) jet extension, in which the surface area of the polymer stream is increased, and (iii) solvent evaporation and formation of nanofibers due to the shrinkage of the polymer jets. An illustration of the rotary jet-spinning setup is presented in **Fig. 3**. **Fig. 3a** presents the different compartments of the setup, including the rotating reservoir with an internal volume of 700 µL and external diameter of 12.5 mm, the flexible air foil and the collector. The steps involved in jet-spinning technique are schematically presented in **Fig 3b.**

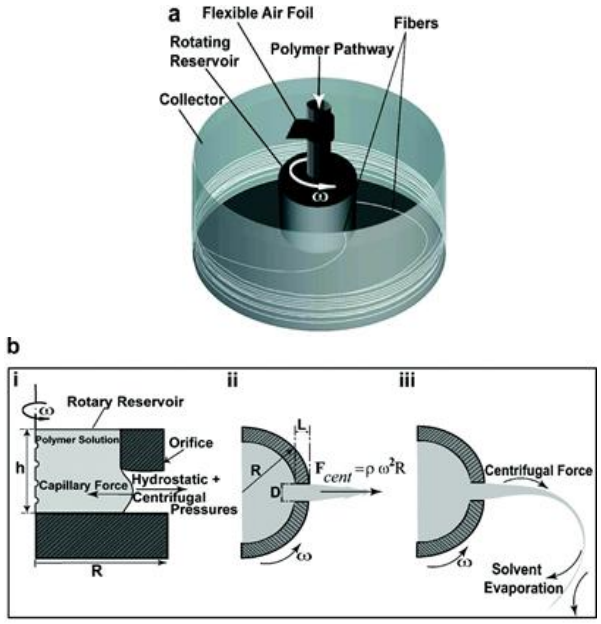


Fig. 3. Rotary jet-spinning process: (a) Rotary jet-spinning setup and (b) the process of producing nanofibers.

Melt spinning

In the melt spinning technique, the polymer solution is initially melted in a heat treatment chamber operated at a temperature up to 700ºC. The molten solution is subsequently injected onto a rotating copper wheel of a 240 mm diameter, a quartz crucible orifice (0.6 mm diameter) at a variable speed of rotation (20 - 40 m/s) [22]. The fabrication of coated melt spun acrylonitrile-based sutures was reported for sustained release of NO [23]. In this study, nanofibers of acrylonitrile-co-1-vinylimidazole were produced by melt spinning and coated with polycaprolactone (PCL). The produced nanofibers showed a high tensile strength. The coated melt spun nanofibers showed a release of 46 µmoL/g of copolymer after 24 h. Cui et al. [23], reported a novel approach for producing micro-nanofibers for bone tissue engineering by the melt spinning technique. They produced nano fibrous network which is essential for cell attachment. Besides, the loose microstructure of the nanofibers was necessary to provide the enhanced mechanical strength [23].

Air jet spinning

In the air jet spinning technique, the polymer solution is sprayed using an airbrush atomization device at a predetermined temperature and relative humidity. The utilized atomization nozzle has a double action with an internal mixing capability. Air pressure is applied for atomizing the polymer solution and the distance between the nozzle tip and the collector plate is being adjusted in the range of 30 - 40 cm [24]. A presentation of the air jet spinning technique is illustrated in **Fig. 4.** Abdal-hay et al. [24], developed a simple method for preparing 3D composite nanofibers of polyvinyl acetate-hyaluronic acid by the air jet spinning technique. The produced nanofibers offered an effective

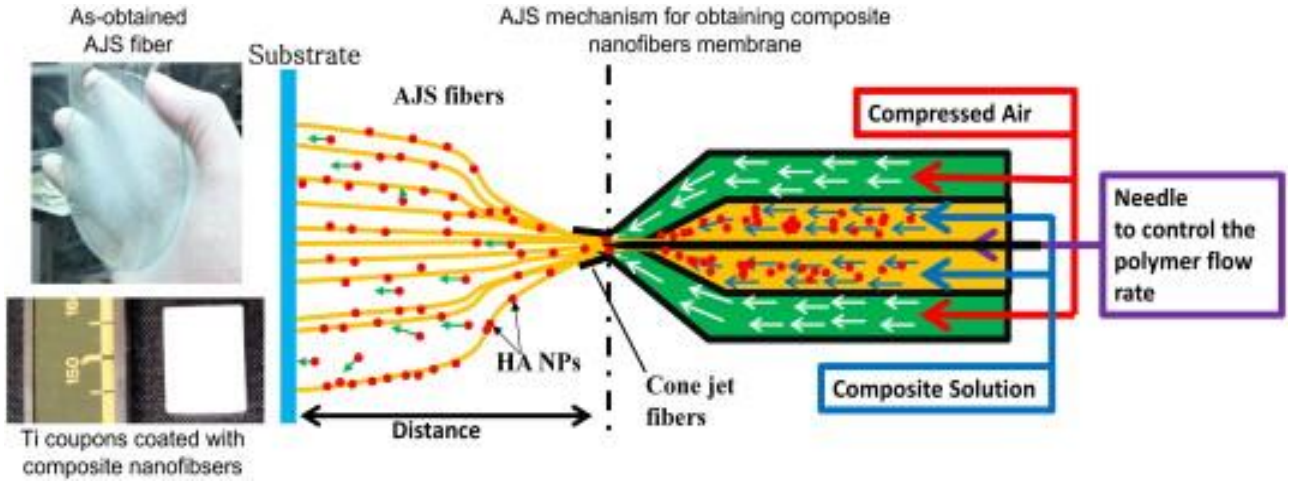


Fig.4. A schematic representation of the air jet spinning process (AJS) for producing hydroxyapatite nanoparticles (HAp NPs) composite nanofibers on Ti substrate. Reference no. 24.

and uniform coating for Ti substrate. Air jet spinning provided an efficient and high production rate technique for nanofiber preparation with an enhanced cell proliferation [24].

Impact of process variables on the physicochemical properties of nanofibers

Variables related to the electrospinning technique

Externally applied voltage

At a critical voltage value, the flow of current is the driving force for the formation of ultrafine nanofibers and this value varies according to the type of starting polymer. The increase in the applied voltage leads to the formation of nanofibers with a smaller diameter. This could be attributed to the stretching of the polymer droplets due to the increased charge repulsion within the polymer jet [25]. Further increase in the voltage value resulted in the formation of beaded nanofibers. The increase in nanofibers diameter with the increase in the applied voltage could be referred to the decrease in the size of polymer droplets with an increase in the jet velocity. The formation of poly ethylene oxide beaded nanofibers was reported by Dietzel et al. [26],with a high applied voltage.

Polymer flow rate

The morphology of nanofibers is significantly affected by the flow of polymeric solution through the metallic needle. Uniform nanofibers could be attained at a critical flow rate value. Beyond this value, the increased flow rate resulted in the formation of beaded nanofibers. For instance, uniform nanofibers of polystyrene could be formed at 0.07 mL/min, while increasing the flow rate to 0.1 mL/min led to the formation of beaded nanofibers. This is due to the incomplete drying of the liquid droplets in the space between the needle and the collector plate [27].

The charge distribution on the droplet surface is another important factor that can affect the size and morphology of nanofibers. Theon *et al.* [28], investigated the effect of reduction of the charge density on the morphology of nanofibers. The increased polymer flow rate led to the reduced surface charge density; which led to the merging of electrospun nanofibers in the space between the needle and collector plate [28].

Distance between the needle and collector plate

The morphology of nanofibers is significantly dependent on the distance between the needle tip and the collector plate. The separating distance affects the evaporation rate and deposition time of the polymer droplets. Similar to the effect of applied voltage, there is a critical value for the distance between the needle and collector plate; at which smooth uniform electrospun nanofibers could be obtained [19].

Polymer viscosity and conductivity

Electrospinning is based mainly on the phenomenon of stretching of charged droplets. Therefore, polymer viscosity plays a crucial rule on the morphology of electrospun nanofibers [19]. The reduced polymer viscosity resulted in breaking the entangled polymer fibers into fragments and hence the formation of beaded structures. The increased polymer density led to the formation of smooth non-beaded nanofibers. However, further increase in the polymer viscosity hindered the flow of polymer through the needle tip. In the same way, the conductivity of the polymer solution affected the diameter of electrospun nanofibers. The increased polymer conductivity produced a stretched Taylor droplet; which led to a smaller diameter nanofibers. The effect of different salt solutions (NaCL, KH_2PO_4 and NaH_2PO_4) on the morphology of electrospun poly lactic acid nanofibers was studied by Zong et al. [29].

Variables related to the rotary jet-spinning technique

As per the process of jet-spinning, a combination of the hydrostatic pressure and centrifugation forces leads to

the formation of polymer jet. The outwards centrifugal force stretches the polymer jet as it is projected to the collector wall. Polymer stretching is a crucial factor in reducing the jet diameter. Also, the volatility of the solvent affects the evaporation rate and solidification of the polymer jet into nanofibers. A highly volatile polymer leads to the formation of thicker nanofibers, due to the fast evaporation, solidification and decreased time for polymer stretching [21].

Classification of composite nanofibers for wound healing

Metal Nanofibers

Titanium oxide (TiO_2)

The photoactive property of TiO_2 can catalyze DNA damage where the release of biomolecules can be triggered by ultraviolet light or X-ray radiation [30]. Sheikh et al. [31], synthesized a combination of electrospun titanium dioxide (TiO_2) nanofibers incorporated with hydroxyapatite (HAp) NPs and the antimicrobial silver NPs.

Archana et al. [32], prepared a composite chitosan-pectin nanofibers loaded with TiO_2 NPs. The composite dressing acquired a good antimicrobial effect with a better biocompatibility than the conventional wound dressings. The composite nanofibers showed a high wound healing rate due to the high water retention rate.

The incorporation of TiO_2 NPs into composite PVP-chitosan nanofibers resulted in improving their mechanical strength. The NPs-loaded composite nanofibers showed an excellent antibacterial properties and good biocompatibility towards fibroblast cells. Additionally, these nanofibers resulted in a higher rate of wound healing compared to the chitosan treated groups [33].

Zinc Oxide (ZnO)

Zinc oxide (ZnO) has attracted a considerable interest as a wound healing biomaterial due to its antimicrobial properties. Composite nanofibers of zinc oxide-sodium alginate-polyvinyl alcohol were prepared by Shalumon et al. [34]. The concentration of ZnO was optimized to get the optimum nanofibers diameters, least toxicity and maximum antibacterial properties. Another study imbedded ZnO NPs into cellulose acetate nanofibers. The composite nanofibers showed an accelerated wound healing due to the incorporation of ZnO [35]. The clinical evaluation of ZnO NPs loaded onto cefazolin nanofiber mats was reported [36]. The 1:1 weight ratio of ZnO : cefazolin resulted in an accelerated wound healing and a good antimicrobial effect. This could be attributed to the enhanced cell adhesion and a more efficient collagen synthesis.

Magnetic oxide (Fe_3O_4)

Magnetic nanoparticles (Fe_3O_4) have recently been applied in both diagnostic and targeted therapeutic applications. Because of their biocompatibility, magnetic NPs are recommended for utilization in composite NPs-nanofiber systems [37]. Fe_3O_4 NPs have been used to enhance the mechanical properties of polycaprolactone (PCL) nanofibers resulting in an enhanced Young's modulus (32% increase) at a 15% loading of NPs [37]. In addition, Fe_3O_4 NPs were recently reported for their antibacterial efficiency [38]. Therefore, composite magnetic-polymeric nanofibers can provide complementary mechanical and antibacterial effects for wound healing. Another study reported the preparation of composite Fe_3O_4-chitosan-gelatin (Fe_3O_4-CS-GE) nanofibers by electrospinning at a 0.8 mL/h feed rate, 15 kV voltage and a 15 cm distance between the needle and collector [39]. The enhancement of the mechanical and antibacterial properties was significantly observed due to introducing Fe_3O_4 NPs into the nanofibers matrix. Increasing the loading content of the NPs from 1 - 4 w % leads to 37% enhancement of Young's modulus with a 22% augment of tensile strength. Further increase in the % loading resulted in falling down in nanofibers tensile strength [39]. Implementations on the stress transfer from rigid NPs into the composite nanofibers matrix were also reported by Wei et al. [40]. In their approach, magnetic-polyvinyl alcohol-chitosan nanofibers (Fe_3O_4-PVA-CS) were prepared with enhanced mechanical properties at 5% rigid NPs filler content. The evaluated Young's modulus was apparently increased from 48% to 57.4% on increasing the NPs loading content from 0 - 5 wt%. The enhancement in dynamics of cell attachment and growth was also observed due to the incorporation of magnetic NPs [40].

Gold

Gold NPs exhibit unique physical, chemical, optical and electrical properties. The antioxidant and anti-inflammatory properties make gold NPs a good candidate for composite nanofibrous mats [41]. Leu et al. [42], studied the wound healing activity of gold NPs and reported the enhancement of cell proliferation and healing of mouse cutaneous tissues. However, gold NPs -composite-nanofibers are mostly applied in trans-differentiation of myocardium cells. Sridhar *et al.* [43], proposed a synergistic approach of electrically stimulated gold NPs and PCL nanofibers for controlling trans-differentiation of myocardium cells. The incorporation of gold NPs prevented the cell loss and provided a more site-targeted wound healing mechanism [43, 44].

Mesoporous silica (MSNs)

There are several advantages of using electrospun nanofibers loaded with MSNs ; mainly, the high surface area, high pore volume and enhanced adsorption of drugs through their nanoporous structures [45, 46]. Studies confirmed that MSNs did not show any cytotoxic effect [47, 48]. Kim et al. [49], investigated the possibility of dual drug delivery from PLGA

electrospun mats. The effect of pH and temperature variation on the drug release from MSNs has been reported [50, 51]. Song et al. [52], proposed the approach of stimuli-responsive MSNs-composite nanofibers for drug delivery. The designed composite nanofibers showed a dual drug delivery system with consistent release profiles for the two model drugs (fluorescein (FLU) and rhodamine B (RHB) [52].

Silver

Silver NPs have been recognized for several advantages in wound healing, such as antibacterial effect and low systemic cytotoxicity. Silver NPs can be incorporated by physical means into a variety of dressing mats, e.g. cotton fabrics. **Fig. 5** presents the incorporation of silver NPs on cotton fabrics [53, 54].

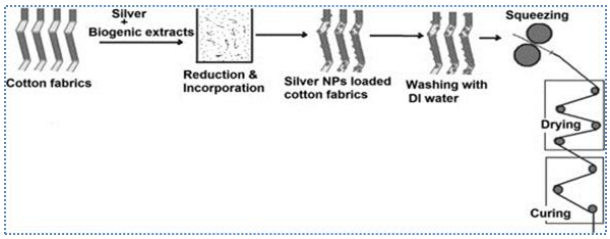


Fig. 5. Incorporation of Silver NPs on Cotton Fabrics. Reference no. 54.

Wound dressing pads were prepared from gelatin nanofibers incorporated with silver NPs. The nanofibers were prepared by electrospinning and possessed a high surface to volume ratio, high pore volume and were efficient for wound healing and drug delivery applications [55, 56]. In another study, silver NPs were impregnated into bacterial cellulose in order to impart antimicrobial property to the dressing pads. The produced dressing showed an enhanced antibacterial effect against gram-negative and positive bacteria [57]. Miller et al. [58], proposed a chitosan nanocrystalline silver dressing for wound healing. The suggested dressing showed a higher rate of healing compared to the conventional silver sulfadiazine. Importantly, the dressing showed a reduced incidence of elevated silver concentration in the blood circulation [58].

Polymeric nanofibers

Chitosan

Many publications have emphasized the application of chitosan as a wound healing material due to its property in accelerating wound healing in addition to its biocompatibility [59, 60], biodegradability [61], haemostatic [62] and anti-infection activity [63]. Chitosan is a linear polysaccharide that will gradually decompose into N-acetyl-D-glucosamine units; which assists the ordered arrangement of collagen and stimulates the synthesis of hyaluronic acid and so, promotes the formation of wound scar tissues [64]. The application of chitosan in wound healing has been recognized due to the similarity between the morphology of chitosan nanofibers architecture and the extracellular matrix; which could promote cell attachment, proliferation and differentiation [65].

The presence of positive charge on its surface supports an efficient cell growth in addition to its role in accelerating induced thrombosis and blood coagulation [66]. Moreover, the presence of free amino group on chitosan surface enables the formation of polyelectrolyte complexes with the acidic components of the cellular elements of blood. Chitosan as an antimicrobial agent has demonstrated an activity against a broad spectrum of bacteria, higher killing rate and lower toxicity to mammalian cells as well [32].

Nevertheless, derivatization of chitosan might be useful to overcome the difficulty of its electrospinning. This is due to its high crystallinity, high ability to form hydrogen bonding in addition to the presence of the rigid D-glucosamine skeleton [65-67]. Several studies on electrospinning of chitosan derivatives have been reported, such as chitosan-polyethylene oxide [68], chitosan-gelatin [69], chitosan- silk fibroin [70] and chitosan-polyvinyl alcohol [71, 72]. Silver and zinc oxide NPs have been utilized for fabricating multicomponent chitosan nanofibers/silver or zinc oxide NPs mats for wound dressing [69]. The in situ synthesis of silver NPs within chitosan nanofibers has been also reported [69,71,73,74]. However, chitosan could be dissolved in an aqueous salt solution, such as hydroxyl benzotriazole in order to avoid the use of organic solvents [75].

Alternatively, chitosan nanofibers can be manipulated via depolymerization, chemical or radiation treatment in order to improve its solubility and hence, enhance its electrospinning ability [76-79].

Polyvinyl alcohol

Polyvinyl alcohol (PVA) has been widely utilized for the production of nanofibers for wound dressing owing to its chemical stability and water solubility. Electrospun PVA nanofibers were applied for the preparation of ultrafast release polymeric mats [80]. The cell affinity towards PVA nanofibers has been also investigated [81]. Normally, a mat of randomly aligned nanofibers was obtained upon electrospinning of PVA using the conventionally recognized techniques. A novel technique has been proposed by Chuangchote and Supaphol [82], for developing aligned nanofibrous mat for wound healing. In this method, a dual vertical wire setup was proposed for the development of uniaxial-aligned nanofibers. The novel setup comprised two parallel stainless steel wires with a charged needle and a grounded collector plate in between. The uniaxial-aligned nanofibers of PVA were collected at a vicinity of the parallel wires, while the randomly organized ones were collected near the collector plate. The proposed method provides a successful technique to prepare aligned nanofibers in a short time. Several process parameters have been also manipulated to enhance the mechanical properties and thermal behavior of the obtained nanofibers [82].

Polyacrylonitrile (NO releasing polymer)

Many publications have shown the effect of nitric oxide (NO) on wound healing [83, 84]. Incorporating NO into biomedical materials and devices has shown potential on wound healing applications. The process of wound healing involves a variety of cells capable of producing NO including, platelets, inflammatory cells, fibroblasts and epithelial cells. These cells can produce NO spontaneously or in response to inflammatory stimulation, via the production of NO synthase enzyme. Therefore, inhibition of this enzyme can impair the wound healing process. Diabetic chronic wound and psoriasis are physiological conditions that are associated with underproduction of NO [84, 85]. Accordingly, NO has been recognized as an endothelial-derived relaxing factor (EGRF) [86]. Although the daily production of NO ranges from 100 pM to 9 mmoL [87, 88], the amount needed for wound healing was reported to be in the range of 1 nM to 1 μm [88]. Hence, the exogenous application of NO in wound healing mats has been reported to have valuable effects [83].

The development of biomaterials that can locally release NO has recently become the focus of many researches. Pegalajar-Jurado *et al.* [89], reported the development of NO-releasing polysaccharide derivative as an effective antibacterial in wound healing. The proposed biomaterial exhibited 8-log reduction against E. coli, Acinetobacter baumannii and Staph. aureus [89]. Quin *et al.* [90], summarized the different techniques involved in the development of NPs as a delivery vehicle for nitric oxide [90]. These NPs include silica, metal oxide, polymer-coated NPs, micelles, dendrimers and star polymers for antibacterial and wound healing applications. Zeolites and metal organic frameworks (MOFs) have been also investigated as a delivery vehicle of NO [54, 87]. A good candidate for wound healing nanofibers would be a polymer that can be easily electrospun and covalently bonded to NO. Lowe *et al.* [83], has reported the fabrication of polyacrylonitrile nanofibers for the delivery of NO. The results showed a prolonged release of 79 μmoL/g of NO for two weeks, which suggested the application of NO-releasing bandage as a new potential wound therapy [83].

Hyperbranched polyglycerol (HPGL)

Recently hyper branched polyglycerol (HPPG) have gained much interest as a biomaterial for wound healing [91]. HPGL has a unique topology; which allows the formation of a molecular capsule that can act as a vehicle for bioactive molecules [92]. HPGL has a bio adhesive, swelling and biocompatible properties which make it a good candidate for wound dressing. The underlying mechanism of HPGL as a dressing mat starts with hydrating the necrotic tissue and consequent adsorption of wound exudates. On the cellular level, HPGL improves the degree of cell attachment and increases the cell growth rate, so it plays an important role as a wound healing accelerator [93]. In addition, HPGL can act as a delivery vehicle for therapeutic agents to the wound site. Torres Vargas *et al.* [91], have studied the preparation of electrospun HPGL nanofibers for the delivery of the wound healing and anti-inflammatory agent, Calendula officinalis. The results obtained showed a fast release of C. officinalis from HPGL nanofibers; which was anticipated to the high swelling ability of the nanofibers in addition to their high porosity. The results of the in vivo experiment demonstrated the potential application of HPGL-loaded- nanofibers as a wound healing mat.

Poly lactic acid, Poly ε-caprolactone, polyurethane and methacrylate

Biodegradable and biocompatible polymers such as poly(ε-caprolactone), poly (lactic) acid (PLA) and polyurethane are good candidates for wound healing owing to their low cost of synthesis, good mechanical properties and desirable cytocompatibility [94]. PLA is one of the most studied biopolymers in wound healing. PLA is a biocompatible and biodegradable linear polyester. Nanofibers prepared from PLA have shown some limitations, such as brittleness and low thermal stability. Therefore, blending with reinforcing compounds as cellulose nanofibers has been proposed for improving the mechanical and thermal properties of PLA nanofibers [95].

Poly (ε-caprolactone) (PCL) has been approved by the FDA as a biodegradable and biocompatible polymer [96]. Composite blends of gelatin-PCL nanofibers have been investigated to enhance the hydrophilicity, cell adhesion of PCL; which are essential for wound healing process [97].

Polyurethane (PU) is another synthetic polymer that is prepared by the poly-addition polymerization of isocyanate and polyol [89]. The properties of PU can be manipulated by controlling the molecular weight of the polyol (soft) segment and the content of the soft to hard segment [99]. Hydrophilic PU can be prepared by introducing a hydrophilic polymer into its soft segment [100, 101]. Hydrophilic PU has shown potential as a wound dressing biomaterial because of its high water permeating properties in addition to the high adsorption of wound exudates [99]. Pyun et al. [99], reported the preparation of PU foams as a dressing material loaded with varying amounts of the recombinant human epidermal growth factor (rh EGF). The study investigated the applicability of (rh EGF)-PU foams in wound healing and accelerated regeneration of diabetic ulcers.

However, the most reported limitation of these polymeric materials is the non-specific protein adsorption; which might lead to bacterial infection and pain upon removing the wound dressing. Therefore, Yang et al. [102], has proposed a novel copolymer composed of carboxy betaine methacrylate. This zwitterion copolymer exhibits an excellent blood compatibility with improved suppression of platelet adhesion. The proposed copolymer has shown an

enhanced resistant to cell adhesion both in vivo and in vitro [103]. Carboxy betaine methacrylate has been also reported for their antibacterial properties and resistance to cell attachment and protein adsorption; which made this zwitterion copolymer a good candidate for non-adherent wound dressing [94, 104].

Zein

As a plant protein, zein is a biodegradable and biocompatible polymer with high thermal resistance, great oxygen barrier, excellent film-forming capabilities and of low cost. Zein showed a low tendency to accumulate in organs; which favors its application in drug delivery systems [105].

Zein is widely employed for electrospinning being a protein having a high solubility in organic solvents [106]. These nanofibers have a unique extracellular matrix-like network allowing their application for drug and gene delivery. However, their major limitations are poor mechanical strength and morphological stability. Strength improvement was achieved by using other polymers or chemical cross-linking agents. Jiang and Yang [107],used citric acid as a cross-linker for electrospun fibers of zein. The cross-linked fibers kept their ultrafine structure after dipping in phosphate buffer saline at 37°C for 15 days. Sodium hydroxide and glycerol were used as cross-linking extenders glycerol to enhance the strength of the prepared nanofibers [107, 108].

Unnithan *et al.*[109], studied the electrospun poly-urethane (PU)-cellulose acetate(CA)-zein composite nanofibers. PU was used as the foundation polymer to achieve a good cell attachment and haemostasis. CA and zein improved the mats hydrophilicity and retained humidity necessary for wound healing. Cui *et al.* [110], prepared electrospun nanofibers of polyvinyl alcohol-stilbazol (PVA-SbQ) and zein. The composite nanofibers showed improved tensile strength, surface wettability and biodegradability. Dashdorj *et al.* [111], prepared zein nanofibrous mats (350–500 nm) by electrospinning where Ag NPs (20 nm) were in-situ precipitated into surgical pads. The composite nanofibers demonstrated a good cell compatibility and attachment with a high antibacterial performance.

Multifunctional nanofibers for wound healing and regenerative engineering

Nanofibers for delivery of growth factors

Tissue regeneration has been evolved since the early 1990s as an interesting research field for the development of functional replacements for damaged tissues [112]. Tissue regeneration aims to enhance the repair of living tissues using biomaterials, cells and growth factors alone or in combination [14]. The typical function of growth factors is binding to a specific transmembrane receptor and to regulate numerous cellular processes [113]. Recently, the new advances in regenerative therapy has been recognized as a complementary research to biomaterials and this new research direction has been proposed as "regenerative engineering" [114]. The key for successful development of biomaterials for tissue regeneration is to design them with biological domains in order to target growth factors and cells [115]. This approach requires the fabrication of materials that simulate the unique structure and characteristics of natural tissues. Furthermore, the development of new tissues or healing of injured tissues involves many signaling pathways that involve a large number of growth factors and signaling molecules. Therefore, another component of regenerative engineering is to manipulate the signaling pathways involved in healing or neo-tissue development [115].

Furthermore, core-sheath structured nanofibers could be also fabricated, with a great promise in the encapsulation and controlled release of drugs [116]. As the core fluid does not have to be electrospun, various proteins, growth factors and genes could be dissolved in the core fluid and eventually injected into the coaxial electrospinning tube [114, 117].

Recently, the development of nanofibers with encapsulated growth factors has been emerged as a promising approach in neo-tissues applications. The proposed nanofibrous systems provide a novel approach to both simulate the extra-cellular matrix for cell adhesion and also for localized delivery of signaling molecules and growth factors [118-120]. Growth factors could be loaded into nanofibers using different techniques including physical adsorption, covalent bonding or encapsulation **(Fig. 6)** [121, 122]. However, caution must be taken during the loading process of growth factors due to the possibility of denaturation as a result of the harsh organic solvent, cross linking and low/high pH conditions [122].

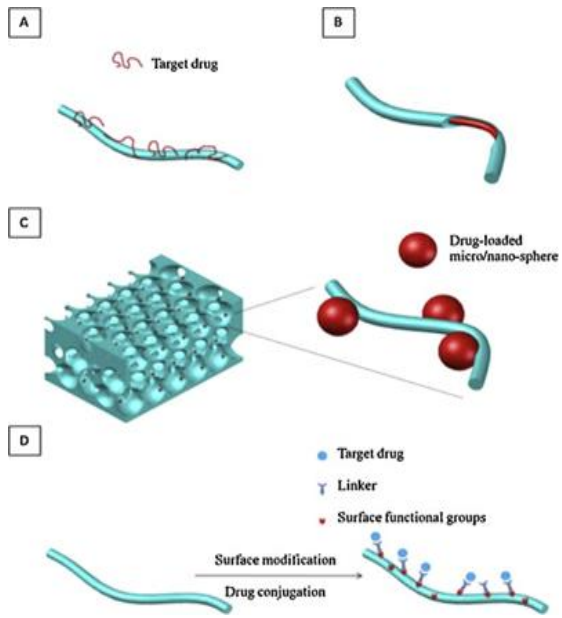


Fig. 6. Reported nanofiber-based growth factor delivery strategies: (A) physical adsorption; (B) coaxial electrospinning; surface immobilization of growth factor / NPs: (C) pore and (D) surface. Reference no. 122.

Nanofibers encapsulated with growth factors such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF) and platelet-derived growth factor (PDGF) have shown their therapeutic efficiency in regeneration of musculoskeletal tissues [123]. FGF has been incorporated onto the surface of polyethylene glycol functionalized based nanofibers fabricated with low molecular weight heparin. The results indicated that the binding of FGF to the PEG functionalized nanofibers was much stronger. This proved the ability of PEG functionalized nanofibers to control the release of FGF for a longer period [124]. Nanofibrous scaffold systems had shown a promising capability for delivering nerve growth factor (NGF) for nerve regeneration [125] and tendon repair [126]. PLGA/ nanofibers have been hybridized with fibrin/heparin-based hydrogel for the delivery of platelet-derived growth factor (PDGF) and adipose derived stem cells (ASCs). The system showed an enhanced tendon healing in a large tendon animal model [126]. Another study reported the development of PLGA/ NPs with chitosan-polyethylene oxide-based nanofibers for dual delivery of vascular endothelial growth factor (VEGF) and PDGF[127]. The results revealed a relatively fast release of VEGF from the nanofibers and a sustained release of PDGF from the NPs. The fabricated hybridized nanofibers showed an enhanced in vivo wound healing in a full thickness skin animal model [128]. For induction of cell migration, platelet-derived growth factor (PDGF) with BSA as a carrier protein was incorporated into an electrospun PLGA/PEG-PLA composite scaffold for tissue regeneration and wound healing. Approximately 20% of the incorporated growth factor was released from the scaffold over 5 days, as determined by ELISA with a preserved bioactivity [123] .

Nanofibers for diabetic ulcers and artificial skin reconstruction

Electrospun nanofibers may offer an effective therapeutic option for patients suffering from diabetic ulcers and permeant skin damage [129]. However, it is difficult for epithelial cells to infiltrate nanofibers due to the small pore size and lamellar-like organization [16]. Therefore, electrospun nanofibers with a matrix metalloproteinase have been developed for encapsulating plasmid human epidermal growth factor (ph EGF). The study suggested that the proposed nanofibers accelerated the wound healing process and significantly enhanced the epithelization of tissues [130]. Another study suggested a facile and efficient method for producing 3D silk-fibroin nanofibers by a cold-plate electrospinning technique presented in **Fig. 7A;** in which the collector was connected to an ice chiller (-90° C). The produced nanofibers were freeze dried and then immersed in 95% v/v ethanol for subsequent crystallization **(Fig. 7B).** The nanofibers produced by traditional electrospinning (TE), solid leaching electrospinning (SLE) and cold-plate electrospinning (CPE) were systemically investigated for their porosity, swelling and water uptake **(Fig. 7E)**. The 3D NF scaffolds showed an enhanced cell attachment and infiltration as a result of the high porosity and easy contouring the facial shape. The suggested 3D scaffolds can be considered as an ideal candidate for artificial skin reconstruction [16].

PCL and PEG were electrospun to biocompatible nanofibers with functional amine groups on the surface. The epidermal growth factor (EGF) was then immobilized on the electrospun nanofibers for the treatment of diabetic foot ulcers. The expression of keratinocyte-specific genes was increased with the application of EGF-conjugated nanofibers. It was shown that EGF-conjugated nanofiber could be used for increasing cell proliferation [130].

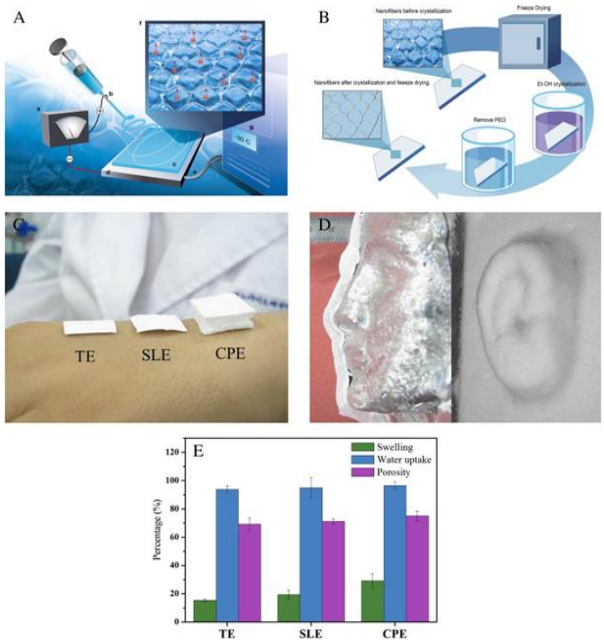


Fig.7. (A) Cold-plate electrospinning technique. (B) crystallization of 3D electrospun silk fibroin nanofibers. (C) gross finding of the TE, SLE and CPE techniques. (D) photographs of the full-thickness 3D bionic face and ear fabricated via the CPE technique. (E) swelling ratio, water uptake and liquid porosity of the nanofibers fabricated via TE, SLE and CPE techniques. Reference no. 16.

Future perspectives and industrial consideration for the scale up of the spinning techniques

Electrospun nanofibers have many industrial applications because of their high surface to volume ratio. The modification of their surface properties, e.g. charges, chemical structure and biocompatibility could widespread their applications. Since most of the electrospinning methods are very time-consuming, it is required to develop a direct method for the fabrication of different types of nanofibers with tailored morphologies. Extensive research now is focused on the preparation of composite nanofibers with optimum properties unattainable with one component of the composite. The combination between inorganic component properties (hardness and thermal stability) together with polymer properties (flexibility and

toughness) would extend the composite nanofibers biomedical applications. The major challenge is evaluating the mechanical properties of nanofibers. Few publications were reported in this concern; which implies the development of a newer technique. The development of finely tuned nanofibers is a challenging approach because of the difficulty in controlling the release of encapsulated signaling molecules, as well as guiding the organization of the new tissue.

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