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# Nanomedicine

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Application of Nanofibers in Drug Delivery

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**Abbreviations**

NSF National Science Foundation

PLA poly(lactic acid)

PLGA poly(lactic-co-glycolic acid)

PCL poly(caprolactone)

SAPNs Self-Assembled Peptide Nanofibers

**Abstract**

There is a great interest in polymeric nanofibers for drug delivery due to their remarkable micro and nano structural characteristics that include high surface area, small pore size, and the production of three-dimensional structures that enable the development of advanced materials with a plethora of applications. Electrospinning is the most simple and economical method of nanofiber fabrication. The electrospinning set-up consists of a syringe with a nozzle, an electric field source, a counter electrode or a grounded target and a pump. The electrospinning process is controlled by many parameters, classified generally into solution parameters, process parameters, and ambient parameters. There are many ways to characterize the produced nanofibers: imaging analysis (optical microscopy, scanning or transmission electron microscopy and atomic force microscopy), porosity and pore size measurements (mercury porosimetry and capillary flow porometer), geometrical characterization (diameter, diameter distribution, fiber orientation and morphology) and chemical characterization (molecular structural characterization by Fourier transform infrared spectroscopy, nuclear magnetic resonance and differential scanning calorimeter). Nanofibers can be used in many biomedical applications including drug delivery (delivery and detection of Vitamins DNA and siRNA delivery, frowth factor delivery, oral mucosal and per oral drug delivery), biosensors, tissue engineering, regenerative medicine and wound dressings.

## 1. Introduction

In the last decade nanomedicine has experienced a high rate of advancement due to nanofibers enormous potential in biomedicine [1]. The scientific publication reviews show that the interest in polymeric nanofibers for drug delivery is enormous due to their remarkable micro and nano structural characteristics, high surface area, small pore size, and the possibility of producing three-dimensional structures that enable the development of advanced materials with sophisticated applications. It is estimated that the global market for nanofibers products will grow the coming decades and there will be many companies worldwide likely to be involved into their production.

The National Science Foundation (NSF) defines nanofibers as materials that have at least one dimension of 100 nm or less. Nanofibers are solid fibers with a large surface area to mass ratio, high porosity, flexibility, theoretically unlimited length and remarkable mechanical performance. Nanofibers are used in the healthcare system as a tool for drug delivery in various diseases, as they enhance efficacy and safety of the drugs by controlling the rate, time and site of their release.

Three distinct techniques have proven successful in routinely creating nanofibrous tissue structures: self-assembly, phase separation, and electrospinning. Of these three processing methods available, nanofibers are most often prepared by electrospinning, as it seems to be the simplest, most economical and productive way. Compared with other fabrication techniques, such as drawing, template synthesis and phase separation, electrospinning is a reproducible, continuous and scalable technology with the ability to produce nanofibers from a wide variety of polymers.

Using various electrospinning techniques scientists can employ a number of different drug loading methods, coatings and drug encapsulation. Due to flexibility in materials selection, the delivery of various drugs (antibiotics, anticancer, proteins, vitamins and DNA-acting) is possible. In addition, biodegradable or non-degradable materials can be used to control drug release that could be either due to diffusion alone or due to diffusion and scaffolds' degradation.

This work reviews the advancements on using nanofibers in drug delivery systems. The examples of nanofibers presented herein, show that these systems have a profound impact on clinical outcomes [2-6].

## 2. Nanofibers fabrications techniques

### 2.1 Electrospinning

The first documented practice of the electrospraying phenomenon dates back to the 17$^{th}$ century, when William Gilbert observed that a water droplet close to an electrically charged amber formed a cone shape and small droplets were ejected from the tip [De Magnete p.1628). By the end of the 19th century, there had been numerous reports in the literature of electrical spinning and its first trial materials, include shellac, beeswax, sealing-wax, gutta-percha, and collodion [7]. The first patented electrospinning process appeared in 1900 [7]. Nevertheless, electrospinning was not fully explored for producing nanofibers until the early 1990s. Several research groups have been working on the electrospinning technique, demonstrating that many organic polymers can be electrospun into nanofibers [8-16]. Since then, more and more efforts have been devoted to electrospinning. Polymeric nanofibers are produced by electrospinning, which spins fibers of diameters ranging from 10 nm to several hundred nanometers. Electrospinning can be carried out from polymer melts or solutions.

#### 2.1.1 Electrospinning set up

The electrospinning set-up involves a syringe with a nozzle, an electric field source, a grounded target or a counter electrode and a pump. The process is based on the electrostatic repulsion forces, developed in a high electrical field; these forces are utilized in the nanofiber synthesis. The solution to be electrospun is held in a syringe nozzle and a high electric field is generated between the nozzle and the counter electrode. As the solution is ejected, the solution droplet at the nozzle adopts a cone shape due to the potential difference between the nozzle and the grounded target. As the charged jet

accelerates towards the counter electrode, the solvent evaporates, leading to the formation of solid continuous nanofibers on the grounded target. The physical properties of the electrospun nanofibers are heavily dependent on the solution properties (e.g. conductivity, viscoelasticity, surface tension, etc.), on environmental factors, like temperature, humidity, etc., and on technical variables (e.g. tip-counter electrode distance, applied electrical potential, flow rate, etc.) [17].

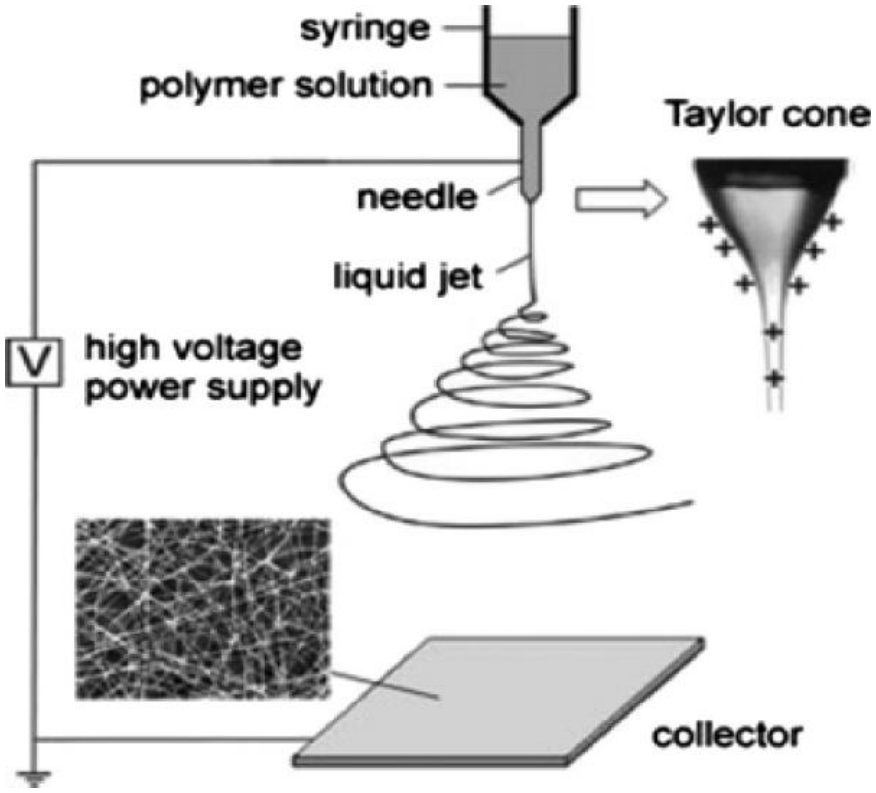


**Figure 1.** Basic electrospinning setup (reproduced from [18])

Several variations of this conventional electrospinning method have been developed during the past years. Multineedle or needleless electrospinning techniques have been utilized to enhance the productivity of the conventional electrospinning, while co-electrospinning is used to generate single layer and bilayer nanofibers and co-axial

electrospinning has been used in the synthesis of coreshell/multilayer nanofibrous structures with additional functionalities and improved quality [19].

*Co-electrospinning*: The nanofibrous structures have limited assembling configurations and functionalities. As such, by increasing the number of layers of nanofibers, the resultant connectivity of the composites and their functionalities can be improved. A recent report suggests that multifunctional nanofibers with more than two layers may be prepared via co-electrospinning [20]. Electrospinning can also be used to synthesize multilayer nanofibers [21]. By following the conventional electrospinning and selectively removing components, porous nanofibers with high specific surface area can be constructed using the same method [22].

*Sol-gel-based electrospinning*: Another intriguing variation of electrospinning is the sol-gel-based electrospinning method, which is a combination of the electrospinning and sol-gel techniques.

### 2.1.2 Effects of various parameters on nanofiber shape in electrospinning

The electrospinning process is controlled by many parameters, classified in general into solution, process, and ambient parameters. Solution parameters include the polymer selection, viscosity, conductivity, molecular weight, and surface tension. Process parameters include applied electric field, tip to collector distance and feeding or flow rate. Each of these parameters significantly affects the fibers morphology obtained as a result of electrospinning, and by proper manipulation of these parameters nanofibers of the desired shape and diameters can be obtained. In addition to these, ambient parameters include the humidity and temperature of the surroundings, which play a significant role in determining the shape and diameter of electrospun nanofibers [23].

**Solution parameters**

*Polymer selection*: The selection of the materials used plays an important role in the nanofibers' design. The ideal material should be biocompatible, biodegradable, non-toxic, hydrophilic and with the suitable mechanical strength. Many different polymers have been used for the construction of nanofibers and can be broadly classified as either synthetically or naturally derived. Among the synthetic biopolymers the most commonly used are poly(vinyl alcohol), poly(ethylene oxide) and biodegradable aliphatic polyesters, such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA) and poly(caprolactone) (PCL). These synthetic materials are not expensive, easily electrospinnable and have proper physicochemical properties that can be controlled through production; however, their main flaw resides on non cell recognition sites, leading to poor similarity for cell attachment. Among the natural polymers the most commonly used are chitosan, alginate, collagen, gelatin, hyaluronic acid and silk. The natural polymers are more suitable due to their similarity with the biological environment. However, the nanofibers' preparation is more challenging due to their polyelectrolyte nature and their high viscosity at low concentrations. To circumvent these difficulties researchers often use natural polymers blended with synthetic ones [1, 24].

*Concentration*: In the electrospinning process, for fiber formation to take place, a minimum solution concentration is required. It has been found that at low solution concentration, a mixture of beads and fibers is obtained. As the solution concentration increases, the morphology of the beads changes from spherical to spindle-like and finally uniform fibers with increased diameters are formed, due to the higher viscosity resistance [25-30]. There should be an optimum solution concentration for the electrospinning process, as at high concentrations the formation of continuous fibers is prohibited because of the inability to maintain the flow of the solution at the tip of the needle resulting in the formation of larger fibers [31]. Researchers have attempted to find a relationship between solution concentration and fiber diameter and they found a power law relationship, which indicates that increasing the concentration of the solution, the fiber diameter increases with gelatin electrospinning [29, 32].

*Polymer molecular weight*: The molecular weight of the polymer has a significant effect on the rheological and electrical properties (viscosity, surface tension, conductivity and dielectric constant) [30]. High molecular weight polymer solutions provide the desired viscosity of fibers and larger fiber average diameters, while low molecular weight solutions form beads rather than fibers. In general, the molecular weight of the polymer mirrors the number of entanglements of polymer chains in a solution, thus the solution viscosity.

*Solvent selection in electrospinning*: The selection of the solvent with the optimal blend, for each polymer or polymers, plays also an important role in electrospinning. Moreover, solvent selection is essential in determining the critical minimum solution concentration in making electrospinning possible, as well as contributing to the solution surface tension and conductivity, thus affecting the solution's spinnability and the shape of the electrospun nanofibres. Frequently used solvents for nanofibers construction are tetrahydrofuran, dimethylformamide, chloroform, acetic acid, acetone, ethanol, and distilled water [1].

*Solution's viscocity*: The viscosity of the solution plays an important role in determining the fiber morphology and size during spinning of polymeric fibers. It has been established that with very low viscosity there is no continuous fiber formation and with very high viscosity there is difficulty in the ejection of jets from polymer solution [31].

*Surface tension*: The surface tension and the nature of the solvent of the solution plays an important role in the electrospinning process. By reducing the surface tension of a nanofiber solution, fibers void of beads can be obtained. Different solvents may contribute to different surface tensions. In general, the high surface tension of a solution inhibits the electrospinning process because of instability of the jets and the generation of sprayed droplets [11]. The formation of droplets, beads and fibers depends on the surface tension of the solution and a lower surface tension of the spinning solution enhances electrospinning at a lower electric field [30].

*Conductivity/surface charge density*: Polymers are mostly conductive, with a few exceptions of dielectric materials, and the charged ions in the polymer solution highly affect the jet formation. Solution conductivity is mainly determined by the polymer type,

the solvent used, and the availability of ionisable salts. It has been found that increasing the electrical conductivity of the solution, the diameter of the electrospun significantly decreases. Researchers have showed that solutions of high conductivity are extremely unstable in the presence of strong electric fields, resulting to a dramatic bending instability as well as a broad diameter distribution [33]. Generally, electrospun nanofibers with the smallest fiber diameter can be obtained with the highest electrical conductivity of the solution and it has been found that the size of the fibers decreases, due to the increased electrical conductivity. It was observed that the jet radius varied inversely with the third power of the electrical conductivity of the solution [19, 30, 34-39]. Natural polymers, for example gelatin, are generally polyelectrolytic in nature. The ions increase the charge carrying capacity of the jet, thus subjecting it to higher tension with the same applied electric field. Thus, the fiber forming ability of the gelatin is less as compared to the synthetic polymers.

**Processing parameters**

*Applied voltage*: The voltage applied to the solution is also a significant process parameter. Researchers have found that when higher voltages are applied, there is more polymer ejection, which facilitates the formation of a larger diameter fiber [40].

*Feed rate/Flow rate*: The flow rate of the polymer from the syringe is an important process parameter, as it influences the jet velocity and the material transfer rate. A lower feed rate is more desirable, as the solvent will get enough time for evaporation [41]. Scarce studies have investigated the relationship between solution feed or flow rate on fiber shape and size [37]. High flow rates result in beaded fibers, due to incomplete drying prior to reaching the collector [38-39, 41].

*Types of collectors*: The electrospinning process also depends on the type of collector used. The collector serves as a substrate, where the nanofibers are collected. The collector, mostly used, is an aluminium foil, but other collectors such as, conductive paper, conductive cloth, wire mesh, pin parallel or grided bar, rotating rod and rotating wheel have also been used.

*Tip to collector distance*: The distance between the tip and the collector has been examined as another approach to control the fibers' diameter and morphology. It has been found that a minimum distance is required to give the fibers sufficient time to dry before reaching the collector, since, with distances that are either too close or too far, beads could be formed [42].

**Ambient parameters**

Apart from the solution and the processing parameters, there are also enviromental parameters, such as humidity, temperature, etc, that play an important role in fibers' construction. Studies, that have been conducted in order to examine the effects of the environmental parameters, concluded that there is an inverse relationship between viscosity and temperature. The variation in humidity, while spinning polystyrene solutions, has also been studied and found that by increasing humidity there is an appearance of small circular pores on the surface of the fibers, while further humidity increase leads to the pores integration [43].

**Table 1.** Effects of various parameters on nanofiber morphology in electrospinning [44]

| Electrospinning parameters | Symbol | Effects the morphology and structure | Effects on the diameter |
|---|---|---|---|
| **Solution properties** | | | |
| Concentration | C↑ | Increasing concentration leads to increase in fiber diameter. | Nanofiber diameter↑ |
| Viscosity | η↑ | Increasing viscosity leads to thicker nanofibers without beads, but too high viscosity causes generation of beads. | Nanofiber diameter↑ |
| Solution conductivity | σ↑ | Increasing conductivity leads to thinner nanofibers. | Nanofiber diameter↓ |
| Surface tension | γ | No conclusive correlation has been established between the surface tension and the nanofiber morphology. | - |
| Molecular weight of polymer | Mr↑ | Increasing polymer molecular weight leads to formation of a nanofiber with fewer beads. | - |
| Volatility of solvent | $\alpha_{solvent}$ | Higher volatility requires higher flow rate and leads to formation of a nanofiber with fewer beads. | - |
| Solution relative volatility | α | Porous microstructure appears because of higher volatility. | - |
| Dielectric constant | $E_r$ | Sufficient dielectric constant of the solvent is needed for successful electrospinning. | - |
| **Process parameters** | | | |
| Flow rate | Q↑ | Higher flow rate results in thicker nanofibers. Too high flow rate causes the generation of beads. | Nanofiber diameter↑ |
| Applied voltage | V↑ | Higher applied voltage leads to thinner nanofibers. | Nanofiber diameter↓ |
| Needle diameter | $D_{needle}$ | | |
| Needle tip to collector distance | D↑ | Minimum distance required to obtain dry nanofibers. Beaded morphology occurs when the distance is too short and the electric field is too strong. | Nanofiber diameter↓ |
| Geometry of collector | - | Metal collectors with conductive frame or rotating drum are preferred. | - |
| **Environmental conditions** | | | |
| Relative humidity | ϕ | Porous microstructure appears due to evaporation-cooling effects. Lower humidity enables higher flow rate and the generation of beads is reduced. | - |
| Temperature | T↑ | Higher temperature leads to thinner nanofibers. | Nanofiber diameter↓ |

### 2.1.3 Nanofiber characterization

There are many ways to characterize the produced nanofibers. The imaging methods that are used include the optical (light) microscopy in the visible range, the scanning or transmission electron microscopy and the atomic force microscopy. Mercury porosimetry and capillary flow porometer can be used for porosity and pore size measurements. The geometrical characterization includes fiber diameter, diameter distribution, fiber orientation and shape (e.g. surface roughness and cross-section morphology). The chemical characterization refers to the molecular structural characterization of a nanofiber by various techniques, like Fourier transform infrared spectroscopy, nuclear magnetic resonance and differential scanning calorimetry [1].

**Geometrical characterizations**

Physical characterization is associated with structure and morphology of the sample and the nanofibers internal structure, which basically determines the physical and mechanical properties. Geometric properties of nanofibers include fiber diameter, diameter distribution, fiber orientation, and fiber morphology (e.g. cross-section shape and surface roughness). For the characterization of the geometric properties, techniques, such as scanning electron microscopy, field emission scanning electron microscopy, transmission electron microscopy, and atomic force microscopy are employed [8]. Scanning electron microscopy (SEM) has been used by many researchers to observe the morphology of the fibers produced, [8-10, 35] as it pictures fiber diameters and shapes, *albeit* the fact that the resolution is less at extreme magnifications. Atomic force microscopy is used to determine fiber diameter and to observe any type of surface morphology and exact descriptions of the fiber surface. To obtain information regarding the crystallinity of the sample, other forms of characterization must be used, such as polarized light microscopy, which is perhaps the easiest method, and determination is done by the amount of birefringence produced. Polarized light microscopy is a very cost-effective and quick method that can be performed on the collected fibers [37,45]. X-ray diffraction, both wide angle and small angle, and differential scanning calorimetry are used to measure the crystallinity of produced nanofibers.

Porosity is another geometric parameter and the porosity and pore size of nanofiber membranes are important in applications, like filtration, template for tissue engineering, protective clothing, etc. [18, 26, 46]. The pore size measurement can be conducted by a capillary flow porometer and mercury porosimetry.

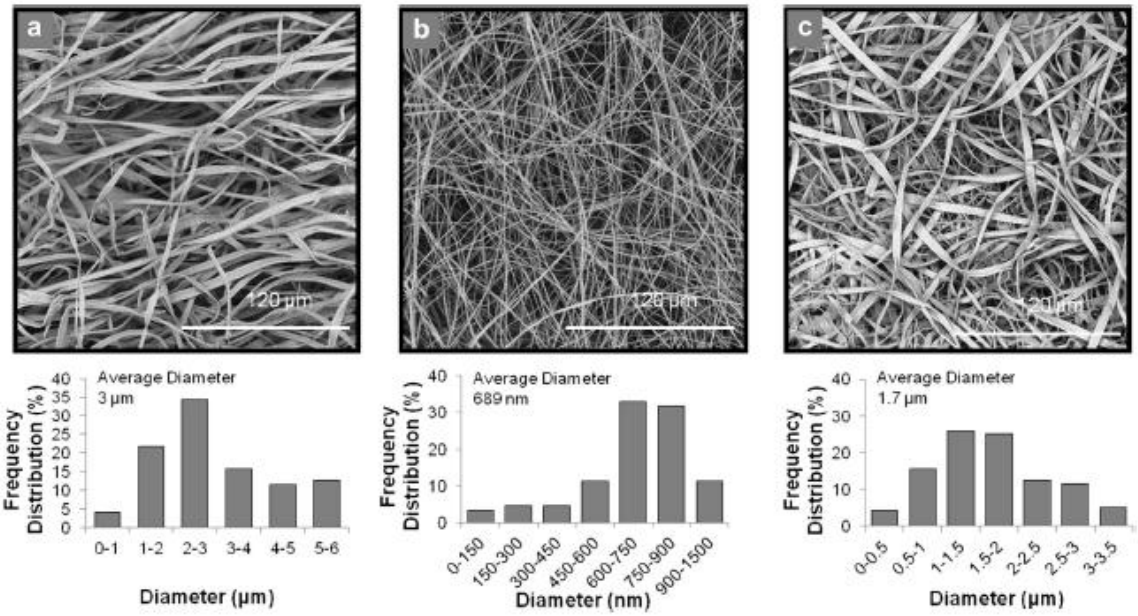


**Figure 2.** SEM images and average diameter distribution histograms of nanofibers (modified from [47])

**Chemical characterizations:**

The characterization of the molecular structure of a nanofiber can be performed by Fourier transform infra red and nuclear magnetic resonance spectrometry [36]. Supermolecular structures relate the configuration of the macromolecules in a nanofiber, characterized by optical birefringence, wide angle X-ray diffraction, small angle X-ray scattering and differential scanning calorimetry [46, 48]. Surface chemical properties of nanofibers can be evaluated by its hydrophilicity, which can be measured by the water contact angle analysis of the nanofiber membrane surface [36]. Researchers in the field

have used Raman spectroscopy and Fourier transform infrared spectroscopy for the changes that may be taking place at the molecular level [45].

**Mechanical characterizations**

Precise measurement of mechanical properties of the nanofibrous mats is very important, as they must be able to withstand the forces exerted by growing tissue or during physiological activities and related biomechanics, e.g., pulsed blood flow in biomedical applications. Mechanical characterization is achieved by applying tensile test loads to nanofibers [36].

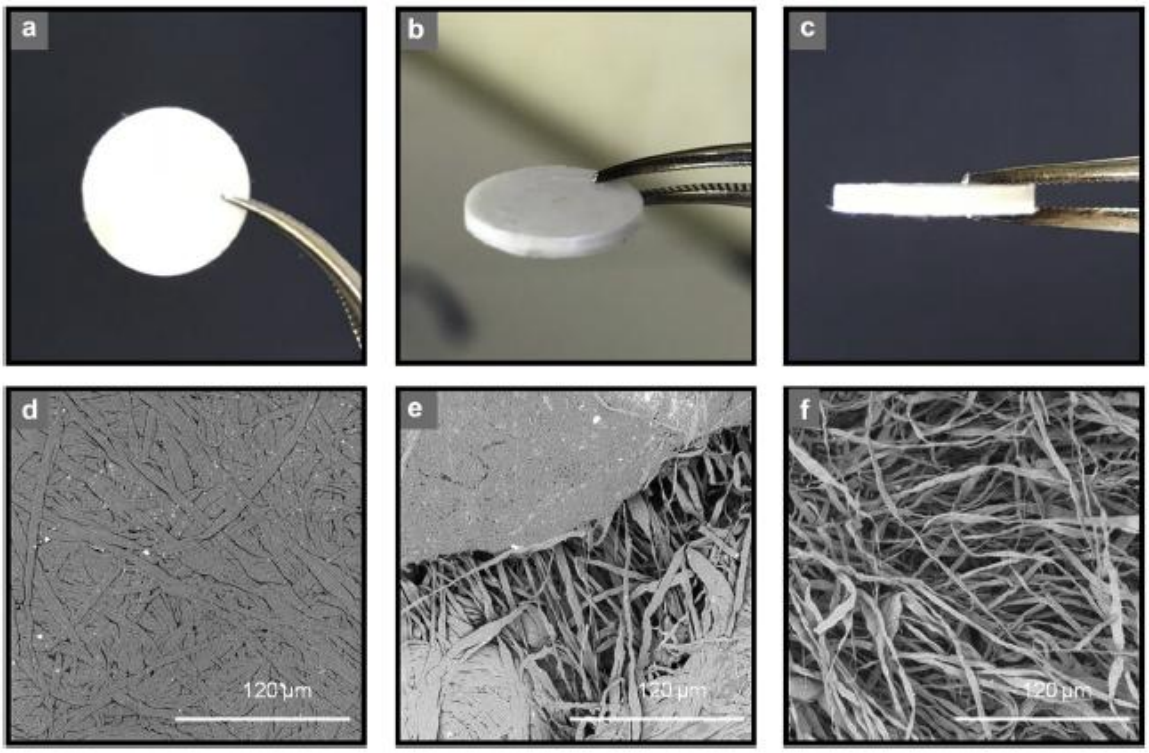


**Figure 3.** Photographs from different perspectives (a, b, and c) and SEM images of the surface (d) and inner part (e and f) of the nanofibrous –based tablets (modified from [47])

### 2.1.4 Methods for Drugs Incorporation

As previously mentioned, the electrospinning technique is an easy and cost-effective method offering great advantages, as the selected materials can be from a wide range of sources, they have high loading capacity and high encapsulation efficiency, which makes it suitable in medical and drug related applications. There are many methods of drug loading in the polymeric solution for electrospinning, which include blending, surface modification, emulsion, multi drug delivery, and multilayer coating.

Blending is the primary method for incorporating drugs into the polymer solution by dissolving or dispersing the drug and subsequently subjecting the solution to electrospinning. This method is simple and easy, but the physicochemical characteristics of the drug and the polymer need to be considered, as these affect the encapsulation efficiency. Lipophilic drugs should be dissolved in a lipophilic polymer and hydrophilic drugs in a hydrophilic polymer for better encapsulation. When the drug is not dissolved properly in the polymer solution, a dispersion is obtained, which might lead to burst release if the drug migrates to the nanofiber surface [49]. To obtain a sustained release of the drug from the electrospun mats, in order to enhance the drug-loading efficiency and to reduce the burst release, different combinations of mixtures of hydrophilic and hydrophobic polymers can be used [50-54]

Surface modification is the technique in which the therapeutic agent is bound or conjugated to the fiber surface to structurally and biochemically mimic the tissue. The drug release will be attenuated, and the functionality of the biomolecules will be protected [54]. The burst release and short-term release will be moderated with this strategy, making it highly applicable for slow and prolonged delivery of gene or growth factors. Incorporation of DNA, growth factors and enzymes, conjugated to fibers, maintains their bioactivity and functionality [54, 55]. The modulation of drug release can also be obtained if surface modification is done on blended electrospun fibers [56].

Another approach is the process of forming an emulsion for electrospinning, where the drug solution is emulsified within a polymer solution. This governs the distribution behavior of the molecule in the nanofiber, which in turn modulates the release profile, the structural stability and bioactivity of the encapsulated biomolecules. The success of

this process is mainly dependent on the ratio of the aqueous solution to the polymer solution [57-59].

Multi-drug delivery is a recent approach in which multiple drugs with or without similar therapeutic effects are combined and subjected to electrospinning with the appropriate polymer(s). Many researchers have used drug-loaded polymeric nanoparticles for the core and drug-loaded polymer for the sheath, in order to obtain a chain-like structure with a distinct release behavior, enabling a “programmed” release of multiple agents [60-61]

Multilayer coating is another innovative method of incorporation and delivery of drug, as it combines the large surface area of electrospun fibers with polyelectrolyte multilayer structures. Such a multilayer uses either electrostatic or hydrogen bonding or acid-base pairing in the layer-by-layer adsorption of polymers [62].

**2.2 Self-assembly**

This is a method which involves the spontaneous organization of the individual components into an ordered and stable structure with pre-programmed non covalent bonds. The self-assembly of natural or synthetic macromolecules can produce nanoscale supramolecular structures. Therefore, this method can produce thinner nanofibers (diameter: 5-8 nm) when compared with the electrospinning technique, but requires much more complicated procedures. Other limitations of this method include the low productivity, the production of nanofibers with a very short length. Moreover, it is restricted to only a few polymers [1].

**2.3 Phase separation**

This method is frequently used to prepare 3D tissue-engineering scaffolds. Removal of the solvent through freeze-drying or extraction can produce porous polymer structures. Polymer scaffolds, obtained by the usual methods, have a sponge-like porous shape with

microscale spherical pores. Phase seperation is a simple technique, which does not require specialized equipment and batch-to-batch consistency can be easily achieved. The alteration of the polymer/porogen concentration can modify nanofibers' mechanical properties and architecture. The main limitation of this method is that it is effective with only a limited number of polymers and is strictly a laboratory scale technique [1].

## 3. Results and Discussion

As previously mentioned, during the last years researchers are more interested in studying nanotechnology and the characteristic properties of nanoscale materials. Nanofibers are nanoscale fibers that offer various advantages, like high surface area to volume ratio, tunable porosity and the ability to manipulate their composition in order to get the desired properties and function [63]. They are useful in many biomedical applications, including drug delivery, biosensors, tissue engineering, and regenerative medicine.

### 3.1 Delivery and detection of Vitamins

Vitamins are very important organic compounds, which are needed in small quantities to sustain life; thus, many researchers have studied their entrapment in nanofibrous scaffolds.

In a recent report the common derivatives of the unstable vitamins **A** and **E** were studied; vitamin A palmitate and vitamin E TPGS, were successfully incorporated into biodegradable gelatin nanofibers *via* electrospinning. Nanofibers containing vitamin A or E alone showed a sustained release profile over more than 60 hours. Nanofibers incorporating both vitamins showed similar release profiles, but the release for vitamin A was increased [64]. Other researchers reported the use of electrospun mats of cellulose acetate nanofibers as carriers for delivery of the model vitamins, A acid and E. Results indicated that in most cases, the vitamin-loaded mats exhibited a gradual increase in the vitamins' release [65].


Figure 4. Vitamin B12 loaded polycaprolactone nanofibers (modified from [66])

**Vitamin D** is crucial for the human body, due to its role in calcium and bone metabolism, preventing bone diseases; rickets in growing children and osteomalacia in adults. Vit-D is commonly present in two forms as Vit-D2 and Vit-D3, which are also known as ergocalciferol and cholecalciferol. Low Vit-D levels have been linked to neuropsychiatric disorders, such as depression, Parkinson and Alzheimer disease [67]. An approach towards the fabrication of novel architecture of electrochemical biosensors has been attempted for the fast, simple and cheap quantification of Vit-D3, which is an important factor to evaluate their potential use in clinical practice. Magnetite nanoparticles incorporated into polyacrylonitrile nanofibers were produced using electrospinning and directly deposited on indium tin oxide surface. Results showed improved biosensing parameters for Vitamin-D3 detection, such as sensitivity, limit of detection and detection range [68].

Vitamin E is commonly used in drug delivery and wound dressing applications, especially due its antioxidant property and $\alpha$-tocopherol is the most biologically active form of **Vitamin E** [65, 69]. However, the poor water solubility and oxygen, light and alkali pH sensitivity, create limitations during its practices. Cyclodextrins have drawn interest in order to circumvent the limitations of Vitamin E, by inclusion complexation. In a recent work, scientists have demonstrated the fabrication of nanofibers from Vitamin E/HP-$\beta$-CD inclusion complex in the form of free-standing nanofibrous webs without using any

polymeric carrier matrix during the electrospinning process. The rapid dissolution of Vitamin E/HP-*β*-CD nanofiber sample in water was achieved mainly due to the high surface area nanofibrous morphology; the water solubility of Vitamin E was greatly enhanced for Vitamin E/HP-*β*-CD-IC nanofiber sample due to the inclusion complexation. Vitamin E/HP-*β*-CD-IC nanofibe complex has shown an effective antioxidant activity due to presence of Vitamin E and its high aqueous solubility. The photo-stability test, under UV-light, and the prolonged shelf-life stability for 3-years storage of Vitamin E/HP-*β*-CD-IC nanofiber confirmed that Vitamin E was structurally stable and kept its antioxidant activity due to the inclusion complexation with cyclodextrins molecules [70-72]. In another study, dextran nanofibres were produced as vitamin E carriers. The results revealed that electrospinning can be used for the production of dextran ultra thin fibers to entrap hydrophobic compounds [73].

Agents of unstable nature or short half-life, like vitamins, they can be incorporated in nanofibrous structures, as nanofibers may have a variety of cosmetic applications, such as facial masks, deodorants, antiperspirants and perfumes, but also transdermal vitamin delivery. In this case, transdermal drug delivery refers to the administration of vitamins through the skin and controlled rate transportation into the systemic circulation to enable patient compliance. The penetration of drugs *via* stratum cornermen into dermal capillary network happens by two ways, i.e. between the intercellular cells or through transcellular cells. The solubility of drugs is very important for the ease, which the drug traverse the skin [74]. The drug release behavior has been studied from nanofiber-based transdermal systems from various researchers. Specifically, a hybrid electrospun nanofiber system was developed for transdermal delivery and characterized for drug release by incorporation of active ingredients, like vitamins, curcumin and diclofenac. Madhaiyan *et al* evaluated the sustained release of vitamin B12, which embedded in hydrophobic polymer, such as polycaprolactone (PCL) that promotes a reservoir storage, thus preventing a sudden burst release. The percentage of cynocobolamin released, 30%, was seen in the case of the hydrophobic polymer PLC-Nanofiber, whereas 95% was achieved in the case of the surface modified hydrophilic PLC-nanofiber. Thus, the amount released through hydronic polymer *per day* was equivalent to the effective dose for vitamin B12 deficiency [75].

As for **Vitamin A** and **C**, in their research, other scientists developed nanofibers, which were used as face masks with active composites, like ascorbic acid, retinoic acid, gold and collagen succeeding moisturizing and anti wrinkle effects [76].

### 3.2 Tissue engineering and Nanofibers

In 1988 researchers showed that electrospinning could be used to produce nano- and submicron-scale polystyrene and polycarbonate fibrous mats specifically intended for use as *in vitro* cell substrates. This early use of electrospun fibrous scaffolds for cell culture and tissue engineering showed that Human Foreskin Fibroblasts, transformed Human Carcinoma, whilst Mink Lung Epithelium would adhere to and proliferate upon these fibers.

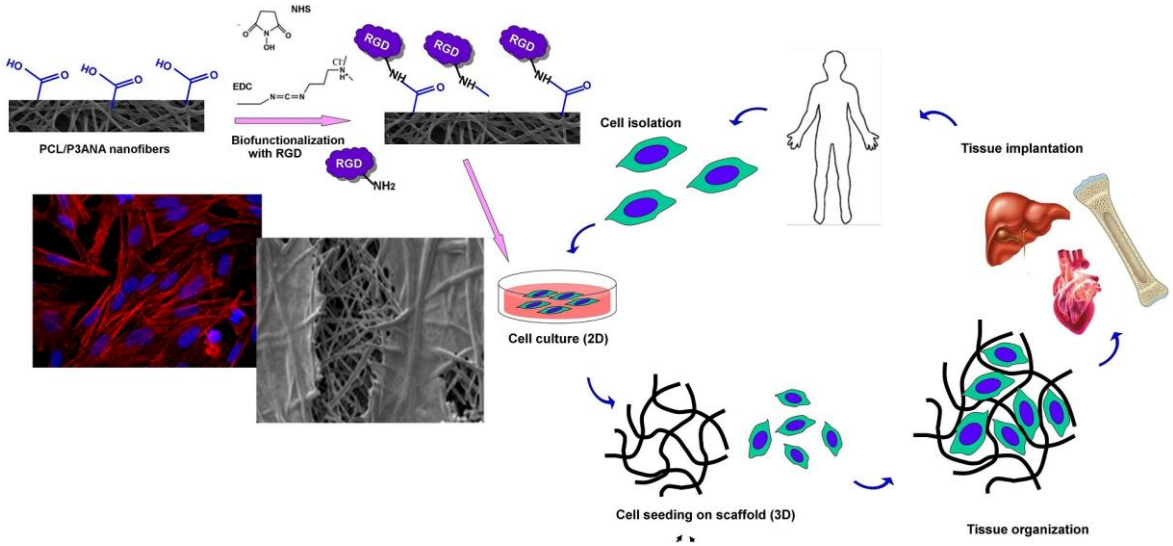


**Figure 5.** Bioactive Poly(m-antranilic acid)-Polycaprolactone-RGD Nanofiber Scaffold for Tissue Engineering (modified from http://www.techin2b.com/techpremium.php?profile_id=0IGcVe8Qm3xO&&log=0T40I1OGTV)

In tissue engineering, a highly porous artificial extracellular matrix is needed to support and guide cell growth and tissue regeneration. Synthetic and natural biodegradable polymers have been used to create those types of lattices. Nanofiber scaffolds are used in bone tissue engineering to mimic the natural extracellular matrix of the bones. The bone tissue is adjusted either in a compact or trabecular pattern and synthesized from organized structures that vary in length from the centimeter range to the nanometer scale. Non-mineralized organic component (i.e. type 1 collagen), mineralized inorganic component (i.e. hydroxyapatite), and a lot of other non-collagenous matrix proteins (i.e. glycoproteins and proteoglycans) from the nanocomposite network of the bone extra cellular matrix [77]. The organic collagen fibers and the inorganic mineral salts ensure toughness and flexibility.

In a research work on bone engineering scientists aimed to fabricate bead-on-string mats based on poly(lactide-co-glycolide) releasing *β*-carotene as a natural osteogen[78, 79]. The appearance of beads on fibers is usually considered as a negative effect during the electrospinning process, but they manage to develop a well nominated scaffold [80]. The mats showed a small burst of *β*-carotene during the first day and a sustained slow release up to 21 days. Results indicated good attachment of the cells on the nanofibrous network [81]. The scaffold loaded with 4% *β*-carotene not only induced the early phase of osteogenesis, but also advanced the differentiation to the osteoblast maturation phase. [82]. Thus, these bead-on-string scaffolds can be used as a substrate for direct bone tissue engineering.

### 3.3 Tissue engineering and regenerative medicine

By combining various biological and engineering techniques, using the three fundamental entities of cells, biomolecules, biomaterials, tissue engineering and regenerative medicine seek to restore or regenerate the normal tissue and organ functions. The nanofiber-based scaffolds are amongst the most actively researched biomaterials because they emerge as versatile alternatives for tissue engineering and regenerative medicine applications.

With their extremely high surface-to-volume ratio and porosity, nanofibers offer a high loading capacity for biological substances. Furthermore, with their interconnected network of micropores mimicking the native *in vivo* topographic features of extracellular matrix, nanofibrous scaffolds present a favorable route for cellular growth, proliferation, and differentiation [83]. The specific selection of materials depends very much on the types and properties of the tissues to be regenerated as well as the small duration of the regeneration process. An increasing number of studies on the applications of nanofibrous scaffolds for tissue engineering have been reported lately. In a study, self-assembled chitin nanofibers were synthesized for the fabrication of biodegradable and flexible substrates, seeded through replica molding for engineering cell sheets [84]. On the substrates, the seeded fibroblast cells attached and aligned along the primary axis of the micropatterned features, leading to the formation of ultrathin and free-standing ordered cell sheets, which were flexible and could be easily controlled for the construction of complex tissue networks. In another study, scientists synthesized gelatin nanofibers *via* electrospinning as the scaffolds for the growth of myoblast, specifically, for an improvement in the formation of aligned myotubes with enhanced contractibility [85]. The activation of mechanotransduction-related genes was up regulated and the myotube maturations and contractions were improved through the presence of the hybrid scaffolds. In a research study, electrospun PLGA nanofibers were functionalized with adhesive peptides for cardiac tissue engineering application, and specifically for improving the adhesion and contraction of cardiomyoctes [86]. Moreover, biodegradable electrospun polycaprolactone nanofiber-based scaffolds were coated with platelet-rich plasma and managed to enhance the adhesion and proliferation of mesenchymal stem cells [87]. Additionally, multifunctional osteoinductive hybrid peptide nanofibers were synthesized based on the self-assembly of three bioactive peptide molecules and then utilized as an implant coating to promote bone-like mineralization on a medical grade titanium substrate surface [88]. Moreover, chitosan/silk combined nanofibrous membrane scaffolds were synthesized based on electrospinning for bone tissue engineering, and particular, for enhancing the proliferation and osteogenic differentiation of mesenchymal stem cells [89]. Apart from mesenchymal stem cells, nanofibrous scaffolds were also used for supporting the differentiation of neural stem cells. In the context of a study, collagen nanofibrous scaffolds were prepared for facilitating the

presynaptic maturation of neuralstem cells – derived neurons towards the formation of a neural network [90]. More recently, a unique hybrid polycaprolactone-grapheneoxide nanofibrous scaffold has been demonstrated to provide instructive physical signals in guiding the specific differentiation of neural stem cells into mature oligodendrocytes in the absence of chemical inducers.

Nerve regeneration following the injury of nerve tissue remains a major issue in the therapeutic medical field. Various bio-mimetic strategies are employed to direct the nerve growth *in vitro*, among which the chemical and topographical cues elicited by the scaffolds are crucial parameters, primarily responsible for the axon growth and neurite extension involved in nerve regeneration. In a study, researchers fabricated both random and aligned nanofibers of poly(3-hydroxybutyrate-co-3-hydroxyvalerate; PHBV) and composite PHBV/collagen nanofibers with fiber diameters in the range of 386–472 nm and 205–266 nm, respectively. To evaluate the potential of electrospun aligned nanofibers of PHBV and composite scaffolds as a substrate for nerve regeneration, they cultured nerve cells and studied the biocompatibility effect along with neurite extension by immunostaining studies. Results showed high cell proliferation of nerve cells on nanofibers that also provided contact guidance to direct the orientation of nerve cells providing elongated cell morphology required for nerve regeneration. Therefore, nanofibers are promising substrates for application as bioengineered transplant implants for nerve tissue regeneration [91].

### 3.4 Polymeric Nanofibers in Tissue Engineering

Tissue engineering utilizes three key approaches: scaffolds, cells and biochemical or mechanical stimulus. Nanofibers scaffolds have the potential of tunable drug loading efficiency, drug release modulation and biodegradability. They can also provide physicochemical cues to modulate differentiation and cell adherence, promoting tissue regeneration, while preserving biocompatible properties. Nanofibers can be employed to base tissue growth and as carriers for bioactive factors' delivery. Moreover, they also prolong tailored mechanical property to cover up the wound bed from collapse and mechanical mismatch between host tissues and scaffolds. By replicating host tissue

matrices, nanofibers can regulate stem cell differentiation and promote cell growth. Since polymeric nanofibers are suitable for such applications, they are gaining popularity in tissue engineering and are currently used in many attempts to regenerate a variety of tissues [93-94].

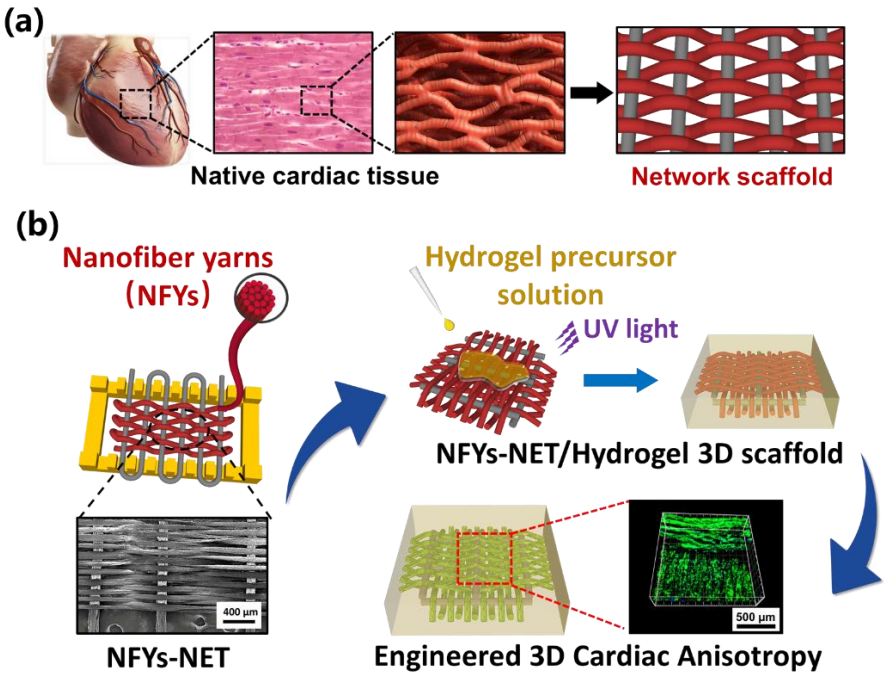


**Figure 8.** Researchers Improve 3D Scaffold for Cardiac Tissue Engineering (modified from http://pubs.acs.org/doi/abs/10.1021/acsnano.7b01062)

### 3.5 Wound dressings with nanofibers

It is estimated that in the USA alone, over 290,000 surgical site infections occur within 30 days of an operation and kill more than 13,000 people each year [94-96]. These infections account for nearly 10 billion US$ annually in additional healthcare costs [85] and comprise 22% of all healthcare-associated infections and represent the most common problem among surgical patients [94-96]. These postsurgical infections increase the hospitalization, the rates of re-admission to the hospital, expenses and rates of death [97]. The current treatment includes wound dressings that deliver antibiotics, but their use can induce survival of drug-resistant pathogens [98]. The increasing frequency of multidrug-resistant bacterial species underscores the need for novel approaches with

modes of action different from current antibiotics to bolster up the antimicrobial regimens used to prevent surgical site infections [99].

Therefore, researchers have focused on electrospun nanofiber wound dressings that offer significant advantages over hydrogels or sponges for local drug delivery. A group of scientists aimed at the development of a nanofiber-based dressing capable of local sustained delivery of 1$\alpha$,25-dihydroxyvitamin D3 (1,25$(OH)_2$D3) and augmenting human CAMP induction. Nanofibrous wound dressings containing 1,25$(OH)_2$D3 were successfully prepared by electrospinning. Nanofibrous dressings could enhance innate immunity by inducing antimicrobial peptides [100]. Thus, these nanofibrous dressings could enhance innate immunity by inducing antimicrobial peptide production. This strategy could possibly dampen multidrug resistance and improve wound healing [101].

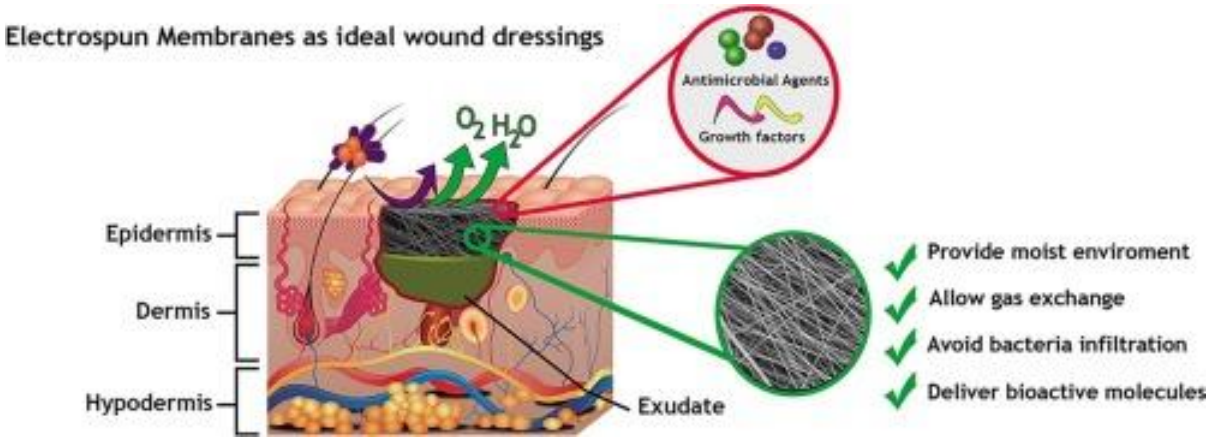


**Figure 6.** Electrospun membranes as ideal wound dressings (modified from [102])

### 3.6 Electrospun membranes in wound dressing

Wound dressing plays an important role in the management of specific types of wounds, including thermal, chronic, and traumatic wounds, as well as in facilitating the regeneration of epidermal and dermal tissues in wound healing. An ideal wound dressing should possess important dual properties: (1) the promotion of exudate absorption for rapid and improved epithelization and wound healing and (2) the inhibition of external

infectious microorganism growth, including those of antibiotic resistant bacteria. The use of nanoscale biomaterial-based wound dressings in aiding wound healing has increased in the past last years. Later advances in nanotechnology have enabled the preparation of nanomaterials possessing architectural features and shapes mimicking those of *in vivo* extra cellular matrices. Electrospun nanofibrous scaffolds with their large surface area and highly porous configuration support the adhesion and proliferation of skin cells, such as fibroblasts and keratinocytes, and facilitate the secretion of critical extracellular matix components, like collagens and growth factors, for the synthesis of new natural extracellular matrices and the subsequent repair of damaged tissues. Nanofibrous networks have been prepared from a wide range of biomaterials, such as biocompatible and biodegradable natural biopolymers. Chitosan and sericin, have been widely used for this purpose, due to their excellent biocompatibility, biodegradability, and importantly, their broad spectrum of antibacterial activity coupled with low immunogenicity. Applications of nanofibers with excellent antimicrobial activity in wound dressings have been actively reported in the last few years [103, 104]. Indicatively, continuous uniform polyurethane/dextran nanofibrous mats loaded with the drug, ciprofloxacin.HCl, have been electrospun as a wound dressing material with antimicrobial properties against both Gram-positive and Gram-negative bacteria [104]. The cellular attachment and viability of the seeded fibroblast cells were enhanced with the use of the composite nanofibrous mats. Also, Ag nanoparticle (AgNP)-functionalized chitosan nanofibers were synthesized via electrospinning for wound dressing application and exhibited excellent antibacterial activity against Gram-negative *P. aeruginosa* and Gram-positive *S. aureus* bacteria, demonstrating the potential of these materials for topical antimicrobial use in wound care. In the context of another study, antimicrobial wound dressing nanofibrous mats comprised of a combination of the colloidal dispersions of Ag nanoparticles embedded inchitosan and the polymer poly vinyl alcohol (PVA), were developed. The multi-component chitosan/AgNPs/PVA nanofibrous system showed good antimicrobial performance against *E. coli*, one of the most prevalent infectious bacteria. Furthermore, based on two natural biopolymers, *i.e.*, chitosan and sericin, the continuous uniform hybridchitosan/sericin nanofibers were electrospun for wound dressing application wth no toxicity [103]. These systems also enhanced cell proliferation, displayed excellent bactericidal property against both Gram-positive *B. subtilis* and induced a more effective

and improved rate of healing and wound recovery. Recently, chitosan/polyethyleneoxide/green tea extract nanofibers with con-trollable diameter were prepared through electrospinning and investigated as a wound dressing scaffold [105]. The fabricated polymeric composite nanofibers exhibited antibacterial characteristic against Gram-positive and Gram-negative bacteria.

### 3.7 DNA and siRNA delivery

DNA and small interfering RNA (siRNA) are biomacromolecules with the ability to interfere with cellular processes by altering specific signals (*i.e.* cell transfection, secretion of growth factors or other signaling bioactive molecules). Thus, they can be helpful in regenerative medicine. The development of novel and more efficient carriers, for viral and non-viral delivery, is necessary to benefit from their advantages, such as transfection efficiency and cell viability. Nanofibers' high surface area, high porosity, interconnected pores are beneficial for cell adhesion/proliferation and oxygen/nutrient transferral, making them ideal for nucleic acids' delivery. Many researchers aimed at DNA-functionalized nanofibrous networks production for gene delivery in tissue engineering [106-113]. Cao et al published the first report on encapsulating siRNA within PCL nanofibers. The results indicated higher cellular uptake and successful transfection of human embryonic kidney cells in a significantly slow rate [114-117]

### 3.8 Growth factor delivery

Growth factors are a group of biomacromolecules that regulate biological processes by transferring signals between cells and their extracellular environment. Threfore, they play an important role in regulating proliferation, migration, and differentiation of cells enhancing tissue regeneration [118]. These newly developed scaffolds could be of great benefit especially if the damaged tissues lack the required potential for regeneration [119]. Although the controlled release of growth factors has been achieved, their instability hampers the successful development of Growth factor-loaded tissue-

engineering scaffolds. Various techniques (blending, specific or non-specific surface modifications, coaxial electrospinning, emulsion electrospinning, etc) have been utilized by many researchers for the growth factor incorporation into nanofibrous scaffolds. Ten years ago, a group of scientists produced silk nanofibers blended with epidermal growth factor that promoted a burst release mainly due to the hydrophobic nature of the epidermal growth factor. [120].

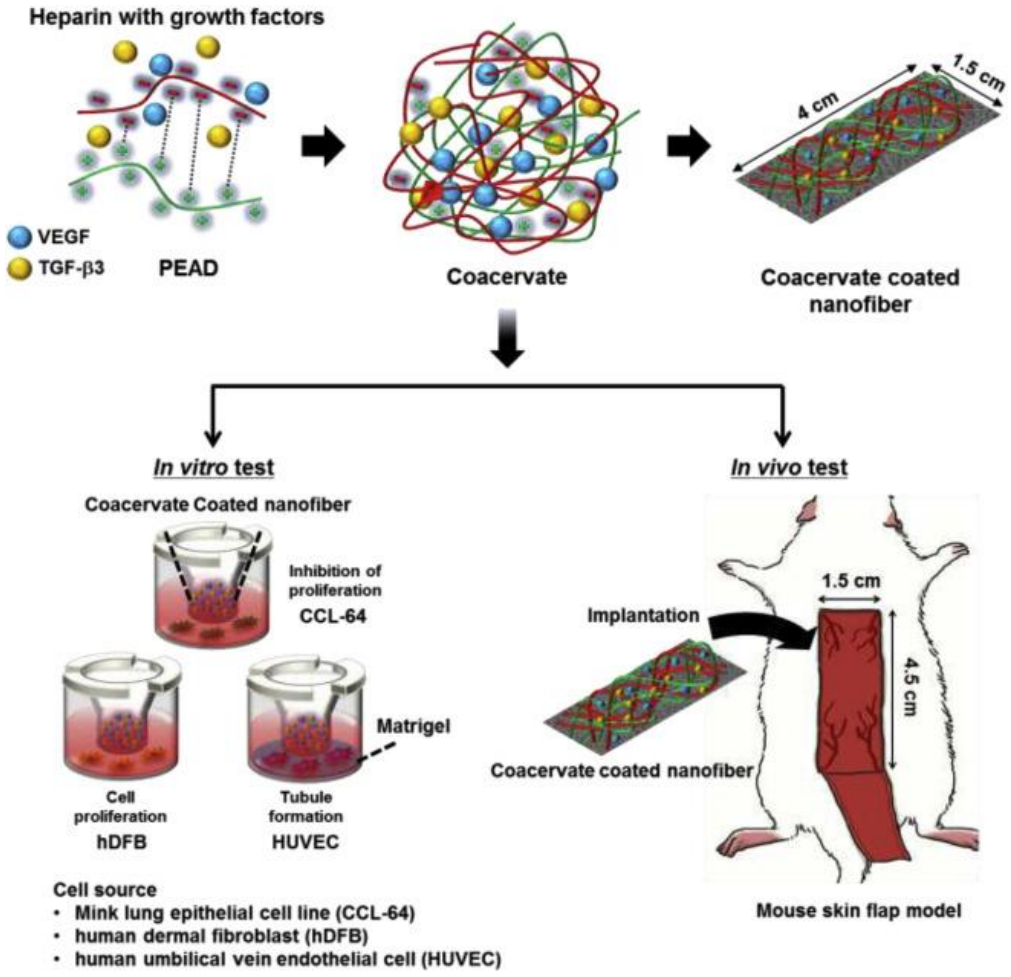


**Figure 7.** A schematic illustration of the preparation of coacervate-coated nanofibers and the design for the further in vitro and in vivo experiments. (Dual delivery of growth factors with coacervate-coated poly(lactic-*co*-glycolic acid) nanofiber improves neovascularization in a mouse skin flap model [121]

Other researchers have used a surface-modified nanofibrous network for growth factor delivery that showed promising results mainly due to its morphological and biochemical similarity to the physiological tissues [122]. In other studies, scientists have attempted growth factor immobilization onto scaffolds using polysaccharides and/or heparin [122, 123]. The results indicated growth factor's 30 days' zero-order release due to the preservative properties of heparin against the proteolytic and chemical inactivation of the growth factors. In another work, scientists have developed a network by combining hydrogels and electrospun nanofibers, merged into a sandwich structure, to provide a better control over nerve growth factor delivery as well as to preserve its bioactivity. The results indicated that this combination reduced the growth factor burst release [124].

### 3.9 Nanofiber Scaffolds for Oral Mucosal Drug Delivery

Fast-dissolving formulations are novel dosage forms, which disintegrate rapidly in the oral cavity providing a quick drug release and therefore, an immediate therapeutic action. They are beneficial especially for paediatric and geriatric patients as they do not require swallowing and they are easily acceptable. These delivery systems can be designed and formulated in the forms of chewing gum, patches, hydro gels, adhesive films and microspheres to provide sustained drug release into the oral mucosa and rapid onset of action [125]. Formulations designed for drug delivery to the oral mucosa need to be biocompatible, mucoadhesive, have good stability, no immunogenicity, and capability of sustained drug release to retain therapeutic levels over an extended period of time [126]. Nanofibers scaffolds can also accommodate more than one drug, releasing them with different release kinetics in response to environmental stimuli [127]. Irrespectively of the nature of each formulation, the ideal scaffold should have well-defined morphology, tunable degradation rate, sufficient mechanical strength for its intended application and a porous structure that has properties similar to those of the native extracellular matrix [128].

### 3.10 Self-Assembled Peptide Nanofibers (SAPNs)

Self-Assembled Peptide Nanofibers are a category of peptides which undergo autonomous assembling process into ordered nanostructures such as nanofiber, nanorods, nanotubes, nanospheres, nanofibrils, and nanotapes [129]. The main driving forces which are involved to start assembling process are van der Waals, electrostatic, hydrogen bonding, and π–π stacking interactions [129]. Synthetic polypeptides which are usually derived from natural amino acids are the more preferred building block. A specific type of peptide amphiphiles could be self-assembled into the supramolecular structures under physiological conditions, mainly nanofibers with a cylindrical geometry.Recently, the use of SAPNs as “smart” drug delivery platforms which can release the therapeutic components in response to environmental cues is very attractive. The amphiphilic peptide with pH-responsive self-assembling nature can be utilized as a pH-responsive drug delivery system that can release the drug content in response to environmental pH changing [130].

Based on the literature, SAPNs have been used to deliver the wide range of drugs and biomolecules such as anticancer agents, growth factors, and nucleic acids. For instance Ashwanikumar et al. developed a SAPN sustained-release DDS based on the RADA-F6 peptide with pH-responsive self-assembling nature to deliver 5-fluorouracil (5-FU) as an anticancer drug at basic pH [131].Wang et al. developed a RGD peptide-based SAPN hydrogel for sustained drug delivery to the rabbit-eye posterior segment that was degraded gradually and exhibited great biocompatibility [132]. SAPNs are utilized to encapsulate the hydrophobic chemotherapy agent, e.g., camptothecin, to improve their solubility [133]. Studies also have reported the potential of SAPNs as the tissue engineering scaffold to deliver growth factors or bioactive biomolecules [134].

### 3.11 Nanofibers in per oral drug delivery

The most conventional oral drug products are formulated to release the active drug immediately after oral administration. Many researchers have used nanofiber scaffolds to incorporate various ingredients, like *Angelica gigas* Nakai (AGN) extract [135], nebivolol [136], spironolactone [137], loratadine [138], caffeine, riboflavin [139], paracetamol, caffeine [140], meloxicam [141] and ketoprofen [142]. Scientists have utilized diverse techniques to make dosage forms that incorporate electrospuns in minitablets [143]. They also used cyclodextrins (CDs) [144-148] and various polymers, like eudragits [149], polyethylene glycol diacrylate (PEG-DA575) [150] and polycaprolactone (PCL) [151] for the development of immediate release dosage forms.

**Table 2.** An overview of the electrospinning technique applications in immediate peroral drug delivery

| drug release behavior | delivery system | API | excipient (s) | electrospinning technique | ref |
|---|---|---|---|---|---|
| immediate | mats | Angelica gigas Nakai (AGN) extract | poly (vinyl alcohol) and Soluplus | blending | 135 |
| | mats | nebivolol | | blending | 136 |
| | Nano-miocro-fibers | spironolactone | PVP K90 | blending | 137 |
| | mats | loratadine | PVP | blending | 138 |
| | mats | caffeine, riboflavin | polyvinyl alcohol | blending | 139 |
| | mats | paracetamol, caffeine | | blending | 140 |
| | mats | meloxicam | polyvinylpyrrolidone (PVP)/cyclodextrin | blending | 141 |
| | mats | ketoprofen | Eudragit L and Eudragit S | blending | 142 |

Apart from immediate release formulations, researchers have developed electrospun nanofibers for the management of disorders that need special drug release patterns, like oral modified/controlled drug release systems [152-157], delayed release systems [158-161], colon-targeted drug delivery systems [162-169], that provide biphasic drug release profiles [170-176] and dual drug delivery systems [177].

Table 3: An overview of the electrospinning technique applications in modified peroral drug delivery [modified from 44]

| drug release behavior | delivery system | API | excipient (s) | electrospinning technique | ref |
|---|---|---|---|---|---|
| controlled | mats | ampicillin sodium salt | amyloid-like bovine serum albumin | blending | 152 |
| | mats | tetracycline hydrochloride | poly(lactic acid), poly(ethylene-co-vinyl acetate) | blending | 153 |
| | mats | curcumin | polyvinyl alcohol, β-cyclodextrin | blending | 154 |
| | multilayered gelatin mesh | piperine | gelatin (type A), acetic acid | multiple blending with sequential crosslinking using glutaraldehyde | 155 |
| | mats in hard gelatin capsules | melatonin | cellulose acetate, polyvinylpyrrolidone and hydroxypropylmethylcellulose | blending | 156 |
| | mats in 3-layered tablets | melatonin | cellulose acetate polyvinylpyrrolidone | blending | 157 |
| delayed | mats | 5-fluorouracil | Core: poly(vinylpyrrolidone), ethyl cellulose, methacrylic acid copolymer S100 or drug alone<br>Shell: methacrylic acid copolymer S100 | coaxial | 158 |
| | gelatin nanofibers | piperine | gelatin (type A), acetic acid | sequential crosslinking using glutaraldehyde | 159 |
| | nano-fiber packed capsules | uranine and nifedipine | methacrylic acid copolymer S100 | blending | 160 |
| | mats in tablets | acetaminophen | methacrylic acid copolymer S100 | blending | 161 |
| colon targeted | mats | diclofenac sodium | methacrylic acid copolymer L100-55 | blending | 162 |
| | mats | ferulic acid | Core: shellac<br>Shell: N,N-dimethylformamide | coaxial | 163 |
| | mats | indomethacin | methacrylic acid copolymer RS100 and S100 | blending | 164 |
| | mats | indomethacin | methacrylic acid copolymer RS100 and S100 | blending | 165 |
| | mats | celecoxib | pectin, methacrylic acid copolymer RS30D, polycaprolactone | blending | 166 |
| | milled mats | budesonide | methacrylic acid copolymer S100 | blending | 167 |
| | nanofilm | bovine serum albumin | Core: chitosan<br>Shell: alginate | coaxial | 168 |
| | mats | doxorubicin | polydopamine, poly-ε-caprolactone | blending | 169 |
| biphasic | mats | ketoprofen | Core: ethyl cellulose<br>Shell: polyvinylpyrrolidone | coaxial | 170 |
| | mats | ketoprofen | Core: zein<br>Shell: polyvinylpyrrolidone | coaxial | 171 |
| | tri-layered mesh | ketoprofen | Core: zein<br>Shell: polyvinylpyrrolidone and graphene oxide | sequential coaxial | 172 |
| | gelatin coated | ciprofloxacin | Mg-Ca alloy | blending with crosslinking using glutaraldehyde | 173 |
| | mats | resveratrol | polycaprolactone | blending | 174 |
| | mats | ampicillin | Core/Shell: polycaprolactone | coaxial | 175 |
| | mats | piroxicam | hydroxypropylmethylcellulose | blending | 176 |
| dual | mats | aceclofenac/ pantoprazole | zein/methacrylic acid copolymer S100 | blending | 177 |

## 4. Conclusion

Electrospinning is a simple, flexible, and affordable technology, which is used for producing nanofibers with a high surface-to-volume ratio. The nanofibers produced by electrospinning have many advantages, such as high encapsulation efficiency, controlled release of the encapsulated material, and high thermal, light, and storage stability with increased protection of bioactive compounds [178-181]. Nanofibers can be used in many biomedical applications including drug delivery (delivery and detection of Vitamins DNA and siRNA delivery, frowth factor delivery, oral mucosal and per oral drug delivery), biosensors, tissue engineering, regenerative medicine and wound dressings [182, 183].

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