



Review article



Gelatin nanofibers: Recent insights in synthesis, bio-medical applications and limitations

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ABSTRACT

The use of gelatin and gelatin-blend polymers as environmentally safe polymers to synthesis electrospun nanofibers, has caused a revolution in the biomedical field. The development of efficient nanofibers has played a significant role in drug delivery, and for use in advanced scaffolds in regenerative medicine. Gelatin is an exceptional biopolymer, which is highly versatile, despite variations in the processing technology. The electrospinning process is an efficient technique for the manufacture of gelatin electrospun nanofibers (GNFs), as it is simple, efficient, and cost-effective. GNFs have higher porosity with large surface area and biocompatibility, despite that there are some drawbacks. These drawbacks include rapid degradation, poor mechanical strength, and complete dissolution, which limits the use of gelatin electrospun nanofibers in this form for biomedicine. Thus, these fibers need to be cross-linked, in order to control its solubility. This modification caused an improvement in the biological properties of GNFs, which made them suitable candidates for various biomedical applications, such as wound healing, drug delivery, bone regeneration, tubular scaffolding, skin, nerve, kidney, and cardiac tissue

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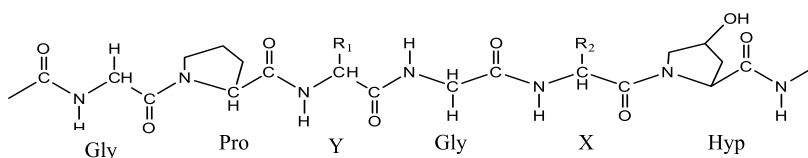
engineering. In this review an outline of electrospinning is shown with critical summary of literature evaluated with respect to the various applications of nanofibers-derived gelatin.

1. Introduction

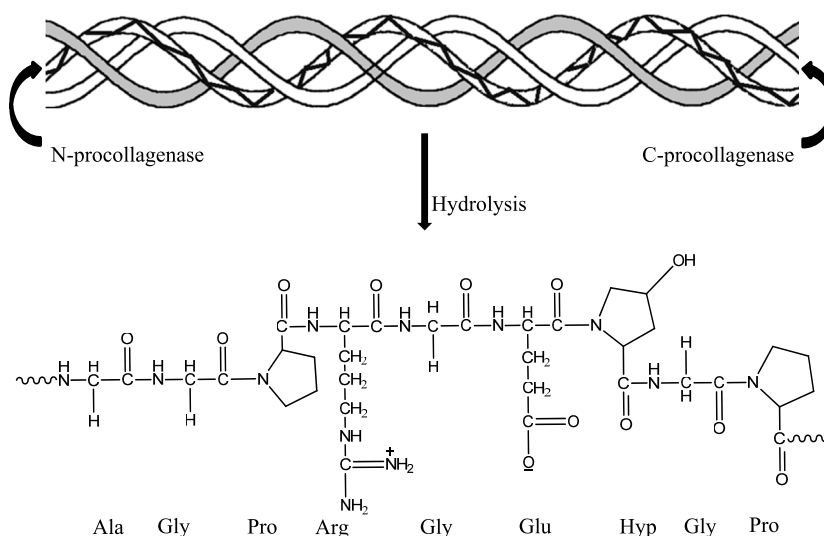
Electrospinning is a valuable electrohydrodynamic approach to the manufacture of micro or nanofibrous structures of non-woven scaffolds from polymer solutions such as chitosan, silk fibroin, collagen, polylactic acid (PLA), and polycaprolactone (PCL). A wide range of biocompatible natural or synthetic polymers, or combinations of both, can be electrospun [1]. Each polymer has distinct mechanical efficiency, rate of biodegradation, physical characteristics, and interactions. The electrospinning of appropriate soluble polymers is a promising approach to synthesize easily dissolved nanofibers [1]. In comparison with other techniques, electrospinning has several advantages including simplicity, production of fibers occurs in a single step, and very thin fibers with high surface areas can be generated [2]. The advantages of electrospinning include superior mechanical qualities of the manufactured fibers, easy processing, and the possibility of large-scale production [1]. Nanoscale materials such one dimensional (nanofibers) have attracted much attentions in various applications due to their unique properties such as high surface area, high aspect ratio and good mechanical properties and can be produced using different approaches [3,4]. However, electrospun nanofibers are versatile, and applicable in various fields, such as energy, catalysis, biotechnology, environmental engineering, defense and security (as chemical and biological protection sensors), nanofluidics, healthcare, and medicine [5]. The biomedical field is considered one of the most active fields in the area of electrospinning, primarily for the regeneration of spinal cord and generation of nanofibrous biotextiles [6,7].

Nanofibers can function via the immobilization, encapsulation, blending, coating, or grafting of compounds. Their biological activity can include the delivery of enzymes, growth factors, and proteins, which can act as antioxidants, antibacterial agents, antibiotics or flavors and fragrances [8]. The possible structures of nanofibers include core/shell structures, multilayer mats, nanocomposites, compositional gradients, and drug-loaded nanofibers, and are characterized by useful mechanical features or extended pores. Micro/nanofiber systems are important in biomedical fields including drug delivery systems (DDSs), the immobilization of enzymes,

(a) Primary amino acid sequence of collagen I



(b) Triple-helix structure of collagen I



Gelatin

Fig. 1. Schematic illustration of the hydrolysis mechanism converting collagen to gelatin [25].

wound healing, and the formation of scaffolds for hard or soft-tissue engineering [1].

In tissue engineering, nanofibers are used as scaffolds in bones, nerves, cartilages, blood vessels and dermal tissue [9]. In cosmetics, membranes of nanofibrous structures are employed as wound dressings, as well as skin care masks. For wound healing, these membranes can participate in improving wound fluid absorption, transmitting vapor, and increasing the transfer rates of additives and drugs [10].

Gelatin is a type of protein produced via the hydrolysis or denaturing of collagen, and is a highly water-soluble biopolymer. It is distinguished by its availability and natural biodegradability in physiological environments, in addition to its bio-affinity, formability, and cost-effectiveness [11]. Gelatin is a polyelectrolyte polymer that has many ionizable groups, while synthetic polymers are typically nonionic. Gelatin has significant applications within the biomedical and environmental fields, so the biodegradability of the polymer is important [11]. It has been commonly used in cosmetic, food, and medical applications, as pharmaceuticals, adhesives, and wound dressings in clinics. Gelatin can be generated in various shapes according to its application, including nanoparticles [12], micro-particles, films, hydrogels [13], microfibers, and the nanofibers which are the focus of this review. It has low electrospinnability so suitable solvents must be used to produce electrospinnable solutions of gelatin such as 2,2,2-trifluoroethanol (TFE), acetic acid, 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) and formic acid [14]. Mixtures of gelatin with synthetic and natural polymers have desirable biocompatibility, physicochemical, and biomechanical characteristics. Nanofibers have large surface area to volume/mass ratios, high porosity in mats, and the surface functionalization is flexible [15]. Owing to the unique nature of gelatin and the distinct features of electrospun nanofibers, electrospun gelatin nanofibers (GNFs) are ideal materials for transdermal or topical drug delivery, wound dressings, as carriers and scaffolds for cell and tissue culture [15]. One of the major applications of gelatin have been biomineralization, meaning the formation of nanomaterials targeted towards the several applications of the biomedical fields, like drug and cell-therapy engineering, cancer/tumor target engineering, and bone tissue engineering [16,17]. According to PubMed database, there are around 838 publications on gelatin-based electrospinning, among them 33 review articles, one systematic review, one clinical trials study and the others are research articles. Thus, the current review updates the feasible knowledge of electrospun GNFs in drug delivery, wound healing, bone regeneration, and tissue engineering applications.

2. A natural polymer as gelatin

Gelatin is a biodegradable polypeptide produced by partial hydrolysis of natural collagen (Fig. 1). It is a fibrous, water soluble protein primarily found in the body's connective tissues [18]. Collagen is a natural polymer, and has a significant role in the preservation of the integrity of the connective tissues, including bones, corneas, cartilage, tendons, blood vessels, ligaments, and dentin [18]. In the human body there are at least 16 various types of collagen. The most prevalent types are type I, type II, and type III collagens, which constitute around 80 to 90% of all body collagen [19]. Collagen type I is composed of three spiral polypeptide chains, approximately 1.5 nm in diameter and 300 nm in length [19]. The standard triple helix structure of type I collagen is made up of two α -chains and a β -chain [20]. Collagen has limited antigenicity and immunogenicity, resulting from the polypeptide structures of the three spiral chains and the molecules in the central regions. This limited antigenicity has significantly reduced the exploitation of collagen in biological applications, reducing its participation in nanofiber synthesis, unlike gelatin which has a very good biodegradability and non-antigenicity besides the low mechanical strength [20].

Gelatin, however, has many distinct types, based on the collagen structure, which varies according to its source and the hydrolytic treatment processes. For instance, pig and bovine gelatins have been extensively used in regenerative medicine during the last ten years [21]. These gelatins possess similar polypeptide structures as human gelatins. Fish gelatins have a comparatively reduced content of peptide repetitions, or residues of amino acids, in their polypeptide chains as hydroxyprolinamide and prolinamide. Thus, these gelatins have a substantially decreased melting point, poor thermal stability, relatively low gelling temperature and increased viscosity compared with mammalian gelatins [21].

The hydrolytic pathways of collagen I are divided into three groups: chemical, physical, and enzymatic. The complete hydrolytic process from collagen I to gelatin occurs in three phases including the raw material pretreatment stage, a gelatin extraction stage, and purification or drying stage [21]. There are two principal forms of gelatin that can be obtained: type A and type B, depending upon the procedures included in pretreatment of collagen I prior to extraction. The formation of type A gelatin occurs *via* acidic treatment, but the formation of type B gelatin is achieved *via* alkaline or enzymatic treatment [22]. Type A gelatin, which has an isoelectric point of 9.0, is obtained by using hydrochloric or sulfuric acid to perform acidic hydrolysis of collagen I. Molecules of collagen I contain glutamine and asparagine amide groups, which hydrolyze to carboxyl groups, thus forming glutamate and aspartate residues in the final gelatin molecules [22]. The standard triple helix structure of collagen I is partially separated, whereas the hydrolytic mechanisms separate the strands into single-strand polymeric molecules. The molecular weight of gelatin varies from 15,000 to 400,000 Da [21].

Gelatin is a polypeptide mixture in which about 20 amino acids are bound together *via* peptide bonds. The polypeptide gelatins have a molecular weight and length determined by a variety of variables, such as the pretreatment and the parameters of the hydrolytic stage, including temperature, pH and time [21]. Depending on the origin of the gelatins, they are made up of glycine-X-Y peptide triplets, where X and Y could be any amino acid, with the exception of hydroxyproline for the Y- and proline for the X-positions. These are the typical formulations. The composition and sequence of amino acids in the single chains can differ widely according to the gelatin source, which affects its eventual properties [23]. The individual gelatin chains involve strong non-covalent bonds based on hydrogen, hydrophobic and electrostatic interactions and van der Waals forces. Gelatin has weaker immunogenicity than the original collagen I, because of the fragile triple helix structures, and the properties of the propeptides [24].

3. Electrospinning technique

The term electrospinning arises from “electrostatic spinning”, as the fine fibers generated are formed through electrostatic forces [2]. Electrospinning is a valuable method of processing nanofibrous materials using a variety of natural and synthetic polymers, and supramolecular non-polymeric systems. This method can generate a variety of fiber forms and textures, including branched, uniform, porous, beaded, Janus, flat/ribbon, core/shell and hollow forms, at nanoscale dimensions [26]. Electrospinning is an extremely simple and versatile way in which to generate fibers, as it is highly scalable and inexpensive. Electrospinning progress includes not only the machines and their accessories but also the electrospun products, so several companies in the last few years, have introduced electrospun commercial products that could be used for several purposes. For instance, face masks, patches, implants and wound dressings [27]. Gelatin’s superior functionality, possible industrial applications and remarkable potential to form continuous fibers *via* the provided electric forces are valuable [28].

3.1. Types of electrospinning technique

3.1.1. Needleless electrospinning

Most methods of electrospinning are dependent on the use of a syringe to manufacture layers of nanofibers in the range of about 0.1–1 g/h [29]. Needleless electrospinning enables the manufacture of nanofibers on an industrial scale. There are several needleless techniques, including bubble, two layer fluid, splashing, melt differential, and gas assisted melt electrospinning as depicted in Fig. 2 [29].

3.1.2. Nozzle electrospinning

There are two main forms of nozzle electrospinning: horizontal and vertical. The basic apparatus consists of three parts: i) a syringe needle linked to a syringe and syringe pump, containing a droplet of polymer solution placed within the tip, ii) a ground electrode employed as a fiber collector, and iii) a high voltage source that produces a varying electrical potential between the collector and the needle of the syringe [30]. The polymer solution is regularly injected *via* a syringe, producing a droplet in the end of the needle. There are two types of collectors that could be used, stationary and rotary as shown in Fig. 3. The voltage applied to both the feed solution and the collector electrode is usually between 5 and 30 kV [30].

The types of needle-based electrospinning are multi-axial, bi-component, multi-needle, gas assisted/gas jet, magnetic field assisted, conjugate and centrifugal electrospinning (Fig. 2) [29]. Therefore, this review focuses on the types of electrospinning that are involved in the production of gelatin nanofibers.

3.1.2.1. Multi-axial electrospinning. Toward multi-axial electrospinning, modifications have been made to electrospinning systems in order to refine the fiber structures to be porous, core-sheath, tri-axial-channel, or hollow, to be suitable in different applications [31]. Hence, these modifications include coaxial, tri-axial, bi-component, and multi-needle electrospinning.

Coaxial electrospinning is used to synthesize nanofibers with a core-sheath structure. A coaxial spinneret with inner and outer needles is typically used. This method can produce fibers from different pairs of solutions, to produce functional, core-sheath, and

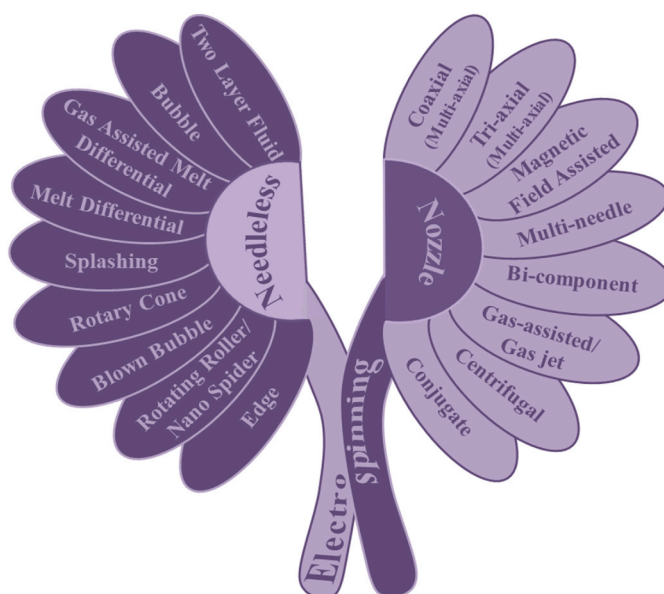


Fig. 2. Various types of gelatin electrospinning techniques.

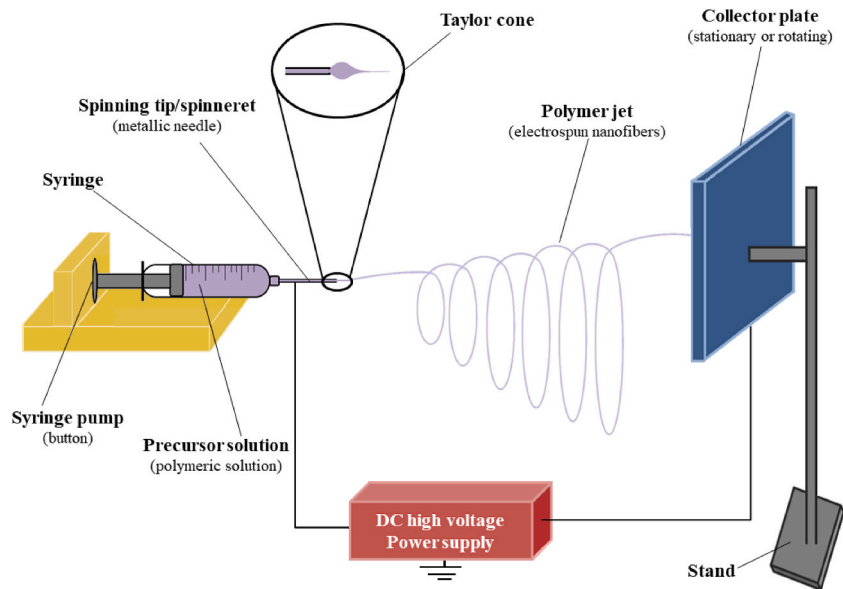


Fig. 3. Electrospinning of gelatin nanofiber based on needle technique [30].

hollow nanofibers, which may include particles [31]. Hollow fibers are produced by coaxial spinning, and generally consist of the core (a temporary material) and the shell (the actual material). In the post-spinning stage, oil is included as a temporary material, since it is simpler than other materials of high molecular weight. In a simple electrospinning system, two syringes supply inter-separated, coaxial inner, and outer fluids to the spinneret [31]. The electrospinning liquid is pulled through a spinneret under high voltage, and produces a compound Taylor cone in a core-shell shape. The core-shell shape can be constructed and preserved by spinning solids in the fibers after the coaxial jet, and gathering them using a collector as represented in Fig. 4a [31].

With tri-axial electrospinning three polymer solutions are provided in the compound Taylor cone by means of the spinneret in tri-axial electrospinning. Tri-axial nanofibers can be produced with different mechanical strengths and hydrophobicities [31].

3.1.2.2. Bi-component electrospinning. In the side by side electrospinning system, two plastic syringes, each of which includes a polymer solution are placed side by side. The flow speed of both polymer solutions is regulated by a typical syringe pump [32]. Each of the solutions includes electrodes which are linked parallel to the high voltage DC source, where the free ends of needles are connected to the syringes. The advantage of bi-component fiber synthesis using a side by side structure is that the individual fibers are capable of reflecting the features of the other fiber component as represented in Fig. 4b [32].

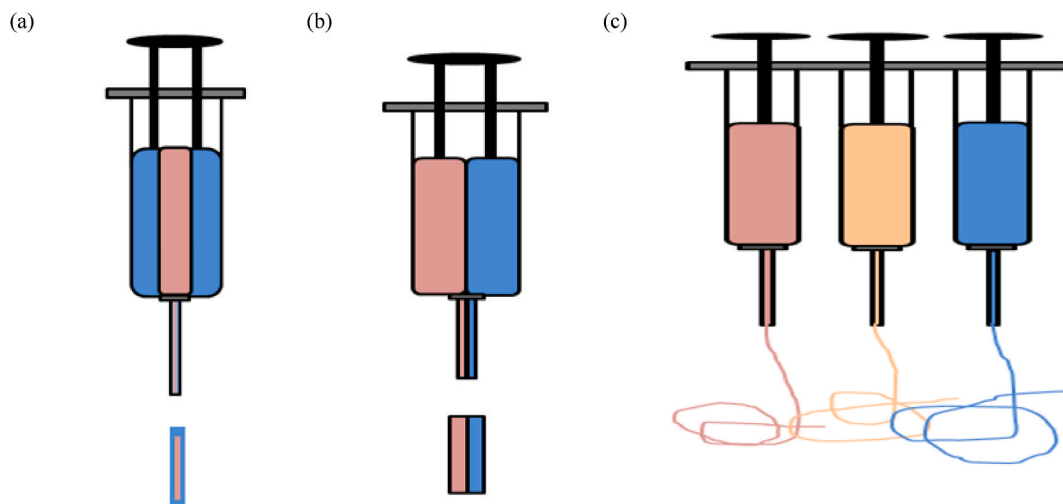


Fig. 4. Various types of electrospinning systems; (a): Coaxial; (b): Side by side; (c): Multi-needle.

3.1.2.3. Multi-needle electrospinning. Adding extra needles, in a process called multi-needle electrospinning, is the most basic method for improving productivity. The polymer solutions are passed out of several needles attached to a high voltage source. These solutions are pumped with the syringe pump into a spinneret. In multiple spinneret process different solutions can be independently injected [31]. Because of the presence of a high mass of spinning solution, high voltage is required to maintain the electrospinning. The limitations of this approach involve clogging at the needle tips, instability in the strength of the electric field, the requirement for the cleaning of multiple needles, and differences in the distributions of fiber sizes. There is also repulsion by adjacent jets, while a high rate of flow in multi-needle systems may be achieved as represented in Fig. 4c [31].

There are distinct electrospinning strategies like generation of electrospun nanofiber yarns and textiles. It is a simple, quick, and cost-effective strategy that is based on combining normal electrospinning method with hand winding and stretching in order to form nanofiber-constructed yarns (NYs). Polycaprolactone (PCL) and silk fibroin (SF) with various mass ratios were treated into NYs [33]. In another example, a combined electrospinning system composed of one nanoyarn-forming unit and one hot stretching unit is combined to form silk fibroin (SF)/poly (L-lactic-acid) (PLLA) nanofibrous yarns (NYs). These techniques possess a potential in the tissue engineering and regenerative medicine applications [34].

3.2. Mechanism of the electrospinning process

During the electrospinning process an electrical potential is introduced between the droplets of polymer solution placed at the tip of the spinneret nozzle and the plate of the grounded collector. Applying an electrical field that exceeds the droplet surface tension leads to ejection of the charged polymer solution jet, which is regulated through the electric field [35]. The emitted jet includes unstable bending, resulting from repulsive forces among the jet charges. The jet is produced in thinner and longer structures until it gathers as fibers on the surface of the collector plate. Electrospun nanofibers have many unique features, including an increase in porosity, a wide surface area per unit mass, good permeability to gas, and small pores [35]. Several microscopic techniques have been utilized to characterize nanofibers, i.e., transmission electron microscope (TEM), scanning electron microscope (SEM), atomic force microscope (AFM), fluorescent microscope (FM), confocal microscope (CM), microcomputed tomography (microCT), scanning pyro- and piezo-electric microscopy, second and third harmonic optical microscopy [36].

3.3. Effects of electrospinning parameters

Several parameters control the electrospinning technique. They are classified into i) solution properties such as viscosity, dielectric features, concentration, surface tension, and electrical conductivity, ii) regulating parameters such as strength of applied voltage, distance between the tip and collector plate, and the solution flow rate, and iii) environmental parameters such as temperature and humidity [35].

3.3.1. Solution properties

The concentration of polymer within a solution indicates whether it could be electrospun into nanofibers, and has a direct influence on the morphology of the fibers. Therefore, a minimum amount of the solution is essential for producing fibers, while an optimum concentration is required to form fibers with no beads [2]. Overall, increasing the concentration of solutions leads to an increase in the diameter and uniformity of the fibers. Increasing weight leads to a decrease in the amount of droplets and beads [2]. Electrospinning involves the transfer of electric charges toward the spinning droplet from the electrode. Thus, minimum electrical conductivity is necessary in nanofiber synthesis. Non-conductive solutions cannot be electrospun to form nanofibers [2,25].

The types of polymers and solvents, concentration of polymers, and temperature have an impact on the electrical conductivity. Whenever the polymers possess ionic properties, the conductivity of the solutions is dependent on the concentration [37]. Surface tension has an important role in electrospinning, which is the main force against the applied voltage in the electrospinning process, and defines the electrospinnability of polymers. Feed solutions with decreased surface tension produce fibers with no beads [37]. However, this does not mean that electrospinning can be used for all solutions with low surface tension. The lowest voltage that can be used to synthesize nanofibers increases with the solution surface tension, but this increase is often nonlinear. The surface tension of polymer solutions alter depending on their chemical compositions, concentrations and temperature [37].

Nanofiber production techniques are affected by rheological properties, particularly viscosity. High-viscosity solutions cannot move out of the spinneret, while low-viscosity solutions cannot generate nanofibers [38]. The viscoelastic force inside a polymer-loaded jet is a primary force that functions against Columbic repulsion. It is the major force that allows the jet to extend from the apex of the Taylor cone. The viscosity of the feed solution influences the size of the fibers, with higher viscosity in general producing wider fibers [38].

3.3.2. Processing parameters

The applied voltage is an essential factor in the electrospinning method since it supplies the electrospinning jet with surface charge and impacts the diameter of nanofibers. Raising the applied voltage typically contributes to a decrease of the nanofibers diameter and elevation of the repulsive forces within the fluid jet [2]. Moreover, extremely high voltage could easily form nanofibers with larger diameters thanks to additional ejection of polymer. Bead formations could also take place at elevated voltages.

The feed solution rate affects the jet speed as well as the solution transfer rate. Decreased rates of feeding are ideal to evaporate solvents and produce solid nanofibers. The feeding rate should preferably be balanced with the removal rate of the solution at the tip [2]. Higher feeding rate causes beaded fibers with large diameter owing to the lack of the correct solvent evaporation duration before

reaching the collector, in contrary, lower rates of feeding could prevent electrospinning [2]. To produce uniform fibers, a particular distance between tip and collector is needed because nanofibers are formed with beads in case of too large or small distances.

3.3.3. Temperature and humidity factors

Electrospun nanofibers are typically smaller in diameter due to the reduced viscosity and solution surface tension with the higher temperature of the electrospinning process. As well, moisture could therefore influence the morphology of fibers. As, high humidity contributes to fibers with circular pores [39].

4. Gelatin electrospun nanofibers

Gelatin has been widely investigated during the last few years due to its biodegradability, biocompatibility, and commercial efficacy. It has a low cost, as it can be conveniently extracted from collagen-rich animal tissues [40]. Once collagen is hydrolyzed to gelatin, its helices can be denatured by hot water. Upon heating, these helices dissociate at a specific temperature based on the composition of the amino acids, the molecular weight, and the amount of plasticizers that are used to reduce the attraction between the polymer chains to make the polymer extra flexible, and the humidity [40]. Several chains re-associate and form triple helix structures when denatured gelatin is cooled. These triple helix structures are heat resistant, and are defined by their transition temperature and enthalpy. Enthalpy is a thermodynamic measure equal to the sum of substance heat content [41]. According to its intended application, gelatin can be produced in several forms, including films, (porous or dense) hydrogels, (nano or micro) particles, and (nano or micro) fibers. Gelatin microfibers are not commonly used as the fibers are weak, but it is recognized as an excellent film-forming material [41].

Once dissolved in cold water, its rapid gelation demonstrates that the molecular weight of gelatin is not greatly decreased, and the production of triple helices is not impacted by the electrospinning technique. The stability of nanofibers in water at slightly increased temperatures is a precondition for most applications [42]. The electrospun gelatin nanofibers are of limited use, particularly in biomedicine, due to their rapid degradation, poor mechanical strength, and complete dissolution. These fibers should be cross-linked, in order to reduce their solubility, making them ideal for several applications. Cross-linking techniques are employed to maintain the water stability of the morphology of nanofibers and enhance their positive properties [42]. This modification could help in promoting water-resistance by reducing solubility and forming chemical bonds among the polymer chains. It also increases the thermo-mechanical efficiency of the processed nanofibers [42]. This results in an increase in mechanical strength, such as the shear modulus and elastic modulus of gelatin-dependent fibrous materials. To withstand and overcome the mechanical forces induced by the cells, electrospun nanofibers are favored as tissue scaffolds.

Cross-linking of gelatin fibers can be achieved using chemical processes, enzymatic and physical pathways, or a mixture thereof. Physical cross-linking occurs through ultraviolet (UV) treatment, dehydrothermal (DHT) treatment, or plasma treatment. Chemical cross-linking uses specific cross-linking agents, including 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC) and glutaraldehyde (GA) [38]. Gelatin could also be thermally cross-linked, even though physical processing typically yields a low level of cross-linking, since the procedure takes place only on the material surface. Chemical processing offers a greater degree of cross-linking, and also affects the material structure [38]. The employment of cross-linking agents beside thermal treatment may improve the characteristics of the material. Many chemical cross-linkers are expensive, rare, or highly toxic, and the safer forms may have undesirable impacts. For example, EDC has been observed to decrease the proliferative potential of fibroblasts [43]. Type A and B gelatin are fiber mats that are cross-linked using DHT and show the most rapid degradation. Other mixtures of DHT and cross-linking chemicals decreased the extent of gelatin fiber mat degradation [38].

Gelatin is a polyelectrolyte in a rigid chain configuration caused by the presence of large amounts of ionizing groups and hydrogen bonds. These characteristics make the electrospinning of gelatin nanofibers more complicated than that of synthetic polymers. The presence of a wide number of functional side groups allows gelatin to hybridize chemically with several natural polymers, synthetic polymers, biopolymers, and minerals [44]. Because of its ability to make gelatin composite/hybrid nanofibers with great efficiency at decreased cost, it offers often excellent biomechanical features. Gelatin is usually paired with synthetic polymers such as polyvinyl alcohol (PVA), polycaprolactone (PCL) and polyhydroxybutyrate (PHB), to enhance the electrospinning potential [44]. The fiber diameter has an effect on the mechanical qualities of gelatin-blend electrospun nanofibers. Whenever the diameter of fibers is reduced, the mechanical strength tends to increase. The diameter of gelatin-blend nanofibers is determined by the composition of the mixture [44]. The diameter of gelatin/PVA nanofibers is decreased with increased the gelatin concentration, leading to improved fiber strength. The addition of biphasic calcium phosphate (BCP) to a gelatin/PVA hybrid enhances the stiffness of the nanofibers, because of the interfacial binding of the BCP with the mixtures [45]. Gelatin/PCL nanofiber hybrids have enhanced flexibility, accompanied by poor strength. Gelatin/PCL hybrid nanofibers have greater elongation and energy tension breakage, double that of pure gelatin nanofiber, though they has low strength in comparison to pure gelatin nanofibers [46]. The strength of gelatin/PHB blend nanofibers can be increased using higher gelatin concentrations. The strength reached to 14.6 MPa when gelatin/PHB nanofibers were cross-linked in glutaraldehyde. The elasticity of cross-linked gelatin nanofibers is increased following cross-linking with glutaraldehyde [47].

Gelatin/PLA blend nanofibers have higher mechanical strength and stiffness upon preparation using 0.7% of glyceraldehyde. The elasticity of gelatin/PLA nanofibers declines when over 0.5% of glyceraldehyde is used. The mechanical characteristics of gelatin electrospun nanofibers can be improved by the manufacture of the nanofiber core-sheath using coaxial electrospinning [48]. It is essential in coaxial electrospinning to choose suitable materials, to limit the combining of materials that are coaxially electrospun. For example, coaxial electrospun gelatin/PVA nanofibers in which gelatin is the sheath and PVA is the core displayed an improvement in

both stiffness and tensile strength, and the plastic deformation was reduced [48]. Furthermore, some minerals are added to gelatin specifically to simulate the natural bone extracellular matrix (ECM). Calcium (Ca) and phosphate (P) combine effectively and individually with electrospun gelatin. The electrospun nanofibers of gelatin minerals have a highly interconnected porous structure, with small pore sizes, of about 377 nm in gelatin-P and 682 nm in gelatin-Ca [49].

The plasma activation method is one of the most common ways to change the surface of polymers and fibers. This approach aimed mainly at increasing the interaction of polymers with various materials and hence has been the subject of numerous scientific studies. For example, gelatin nanofibers that had been electrospun with tannic acid crosslinks were treated in a vacuum chamber with argon and argon-oxygen plasmas [50,51]. Yet, there are not many studies on the enhancement of the resulting nanofibers bioactivity [52,53].

Therefore, gelatin nanofibers can be utilized for instant gelatin yield without the disadvantages of the conventional amorphous instant gelatins. These disadvantages include sensitivity to humidity, poor cold gel modulus, and poor wettability. The electrospun nanofibers of gelatin matrices have different shapes, such as tubular and thick sheet structures, or shapes suitable as a coating agent [8]. These nanofibrous structures have with high porosity and a large specific surface area and have been widely studied for biomedical applications and to a lesser extent for food applications. Their high surface to volume ratio makes it easier for many gelatin types to be penetrated by and dissolved in water so, they are cold water soluble [8]. Submicron and nanometer-scale gelatin fiber mats have large bio-affinity, a biological origin, good biocompatibility, non-immunogenicity, biodegradability, and commercial availability, and they can affect the ECM configuration of human tissues and organs [8]. They also contain space for the growth of cells and tissues, and therefore electrospun gelatin and gelatin-based scaffolds have been designed for many biomedical applications. These include skin, nerve and cardiac tissue engineering, drug delivery, bone regeneration, and tubular scaffolds [8].

5. Applications of gelatin

5.1. Drug delivery

Drug composition affects the ways in which the drug can be administered, because it defines the pharmacological processes and bioavailability, significantly impacting therapeutic effectiveness and clinical responses [54]. Pharmaceutical and pharmacokinetic processes serve an important role in enhancing the bioavailability of drugs and defining the protocol and dose of administration. Considerable research has been dedicated at evolving drugs in various forms and dosages to match different ways of administration [54,55]. Both enteral and parenteral administrations are passages into the systemic circulation, either via the gastrointestinal tract or by other routes [54]. Intramuscular, inhalation, transdermal, intravenous, and subcutaneous pathways are the main important parental pathways, while the enteral paths involve oral, rectal, and duodenal administration. The parenteral administration routes are assumed to have greater bioavailability than other routes, whereas the downsides are local pain, allergic reactions, and infections [54].

5.1.1. Drug delivery systems

Drug delivery systems (DDSs) have been established to manage the delivery of therapeutic agents to specific sites in particular quantities and at appropriate times. The three factors which control and enhance the efficacy and safety of drugs are the rate of drug release, the specificity of the target site, and the time of delivery [56]. The principal purpose of DDSs is to avoid excessive exposure to the therapeutic agent in non-targeted tissue. Side effects resulting from undesirable alterations in drug concentrations can be reduced [56]. Various techniques have been used to produce DDSs such as spray drying, phase separation, and emulsifying procedures, which have been demonstrated to be effective for obtaining site-specific delivery of drugs. Traditional drug encapsulation approaches have several drawbacks, including reduced encapsulation potential for hydrophilic drugs in single emulsion methods, difficulty in mass-production, and polymer aggregation as drops attach to each other before hardening for phase separation [56]. In addition to an excessive quantity of water-soluble particles encapsulated, conventional DDSs require strict temperature regulation, are labor intensive for the double emulsion method, and bead-like structures are shaped in fibers by spray drying. The focus of the latest research is on the employment of electrospinning techniques for establishing efficient DDSs to perfectly regulate the release of drugs [56].

The diameter of nanofibers (NFs) can be modified via this technique through (i) the parameters of the polymer solution, (ii) the distance from the injector to the metal collector, (iii) the strength of the electrical field and (iv) environmental variables [57]. Electrospinning also provides the ability to manufacture hybrid composite NFs using the previously described methods. These composite configurations are formed through integrating different systems of particular qualities. Like metal nanoparticles or CNTs, and magnetic nanoparticles into the polymeric nanofibers [57].

5.1.2. Types of structures of drug-loaded nanofibers

There are three main routes for loading of drugs on nanofibers system; 1) loading particles as nanocarriers within the NFs to increase drug aggregation. Because of their ability to serve as vehicles to transport and distribute various drugs, as well as to increase the amount of chemical therapy molecules, nanocarriers have recently become a focus in drug delivery application. A colloidal system is produced when a dispersive phase consisting of solid particles is dispersed within a liquid. In drug delivery and cancer therapies, the prospect of integrating chemotherapy within these colloidal particles has already been developed [58]. The addition of drugs to certain nanocarriers without incorporating them into NFs typically causes unavoidable bursts of drug release. The introduction of these nanocarriers within polymeric NFs, enhances the delivery of drugs, increases the efficacy of cancer therapy, and resolves the drawback of unavoidable bursts. Nanocarriers which can be used in nanofiber systems include vesicles, micelles [59], silica particles [60], gelatin nanoparticles [58], and stimulus-responsive nanoparticles [61].

- II) Creating polymeric NFs that are capable of being stimulated by external conditions. Stimulus-responsive systems can be affected by external stimuli such as pH, temperature, solvent nature, and ion strength, which have been shown to alter the structures of these systems. Stimulus-responsive nanofibers have two forms of polymers with which to encapsulate drugs: thermo-responsive nanofibers [62] and pH-responsive nanofibers [63].
- III) Producing structures of hybrid systems. These structures involve the inclusion into nanofibers of polymers with gold [64] or magnetic [65] nanoparticles, participating in hyperthermia applicability.

A further important aspect of structured electrospun fiber drug delivery includes layer by layer porous structures for controlled drug release application [66]. Since electrospun nanofibers have a high surface to volume ratio, a suitable amount of drug can be incorporated into them, so they possess an advantage over conventional methods for encapsulation. Nanofibrous mats often permit excellent control of the porosity, and the processed fibers are very similar to extracellular membranes, and thus can be used to produce flexible sheets to pack drugs [66]. This method is significant because it enables the production of nanofibers with characteristics that promote continuous regulated drug release. The conditions of processing allow the maintenance of the bioactivity of drugs and their molecular structures. Accurate adjustments to flow rate that specify the diameter of the produced fibers can generate ultra-thin fibers [66]. Fiber types and approaches to the incorporation of drugs in DDSs are important, as they define the quantity and release patterns of the drugs deposited in nanofibers. An increase in the surface area of electrospun nanofibers, drugs, or both may be required to improve the dissolution rates of drugs.

5.1.3. Main administration routes of electrospun drug-loaded nanofibers

There are various routes for the administration of the electrospun drug loaded nanofibers as illustrated in Fig. 5.

The manufacture of nanofibers with exceptional physico-biochemical properties via electrospinning is an effective multipurpose drug delivery approach [67]. Nanofibers are widely accepted for the delivery of therapeutic agents, such as antibiotics, anticancer, cardiovascular, antihistamines, contraceptives, palliative, gastrointestinal, and nonsteroidal anti-inflammatory drugs (NSAIDs). Similarly, electrospun nanofibers manufactured DDSs have a wide range of uses in cancer therapy, tissue scaffolding, surgical operation, and the development of antibacterial sheets [67].

Nanofibers are intended for the supply of different therapeutic agents, such as Nano medicines and macromolecules, including nucleic acids and proteins. Nanofibers face many technical difficulties including solubility, efficacy of packaging, and the plasma half-life of growth factors, RNA, and DNA [68]. The use of different biodegradable and biocompatible polymers, synthetic or natural or hybrid mixtures are typically preferable in the synthesis of nanofibers and could solve the difficulties associated with the removal of implants via a second surgery.

5.1.4. Gelatin based-nanofibers in drug delivery

Gelatin is biosoluble, biocompatible, non-immunogenic and cheap natural hydrophilic polymer with favorable biological characteristics. Also, this polymer has sequences of arginine-glycine-aspartate (RGD) that empower cell attachment [68]. The sheath of gelatin has shown considerable fibers hydrophilicity properties which are important to increase the biological efficiency of bio-materials during their contact with cells. Thus, it is employed to produce scaffolds with the required biocompatible features for cell

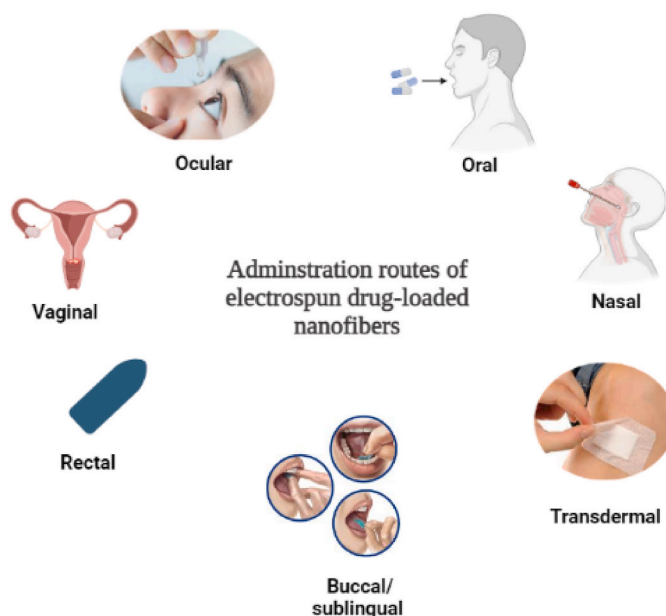


Fig. 5. Common administration routes of drug-loaded gelatin nanofibers.

adhesion as well as cell proliferation especially in polymer-based scaffolds [69]. Gelatin was widely used as biomaterial for both DDSs and tissue engineering owing to its excellent features [70]. Moreover, it already participates in drug composition, one of sealant in prostheses and has many biomedical uses. Therefore it is a successful candidate to combine with other polymers in order to enhance the biological characteristics [71]. Thus, a variety of gelatin-nanofiber-based systems are summarized in Table 1 reflecting the system composition, structure, mode of action and their performance which varied based on system structure and composition.

5.2. Wound dressing

Skin is the largest organ covering the whole body of vertebrates. It has substantial role in the body sensation, temperature regulation, and vitamin D3 synthesis, excretion of water and nitrogenous compounds, and adaptation by changing its structure and composition upon injury, in addition to the ability of protection against mechanical, chemical, thermal effects. Microorganism invasion and UV radiation was proved [80]. The loss of body fluids, nutritional components, and electrolytes are the insults that can trigger the skin damage as it is considered as the first line of defense against environmental factors. The damaged skin provides a suitable environment for bacterial infection that leads to inflammation. Wounds are human skin trauma that involves a great number of cellular reactions and endogenous mediators of the immune system [81].

Skin has the ability to repair itself due to the presence of keratinocytes in the epidermis and fibroblasts that present in dermis layer of skin. Those two cell types play a vital role for tissue growth and regeneration [82]. Full thickness wounds need a long time to repair as the healing process is a complicated process controlled by the patients' physiological conditions, external factors and complications of infections, inflammation and pathogens. The process of wound healing includes many different stages, i.e., hemostasis, inflammation, proliferation and maturation. When deep layers of dermis are injured, fluids escape from injured blood vessels and also lymphatic vessels thus interfere with hemostasis [82]. Biological and physiochemical changes of the affected tissue led to the migration of immune cells and the spread of inflammatory signals. Hemostasis starts within 15 min aiming to form a clot to block the injured area and to prevent any additional bleeding [82]. Platelets begin to collect in the wound bed and secrete proteins like fibrinogen and growth factor like platelet-derived growth factor helping in the clot formation. This clot acts as temporary filling of the matrix in the injured lesion, while the blood and lymphatic vessels undergo vasoconstriction and this phase may take from 1 to 60 min [82].

Then the inflammation phase starts within few hours. Inflammatory cells recruitment is caused by secretion of many platelet chemokines. Macrophages and other immune cells migrate towards the injured area to promote the healing process while the keratinocytes and polymorphonuclear cells (inflammatory cells) release the cytokines [83]. Neutrophils excrete factors to fight bacteria

Table 1
Gelatin nanofibers in drug delivery applications.

Polymer	Nanofibers structure	Assay	Activity of nanofibers	Reference
Poly (vinyl alcohol) (PVA)/chitosan/lidocaine hydrochloride load-ed with gelatin	Spherical, regular shaped particles loaded with smooth surfaces and rather uniform size of nanofibers	<i>in vitro</i>	Anti-bactericidal activity against <i>S. aureus</i> and <i>P. aeruginosa</i> as well as pain reduction in wound area	[72]
Gelatin- rhodamine- Chlorhexidine	Smooth fibers	<i>in vitro</i>	Antibacterial as destroy bacterial biofilms and prevent bacterial growth	[73]
Gelatin/Sodium Bicarbonate and Poly (lactide-co-ε-caprolactone)/Sodium Bicarbonate	Smooth and have relatively uniform diameters	<i>in vitro</i> and <i>in vivo</i>	Antibacterial against <i>E. coli</i> and <i>S. aureus</i> and highly biocompatible with L929 cells	[63]
Gelatin and gelatin-dendrimer	High porosity	<i>in vitro</i>	Antimicrobial activity against <i>S. aureus</i> and <i>P. aeruginosa</i>	[74]
Silk fibroin and gelatin	High porosity	<i>in vitro</i>	Inhibit <i>S. aureus</i> and <i>K. pneumoniae</i>	[75]
Caffeine (CAF) loaded fish gelatin	Smooth surface, with no perceptible CAF crystals	<i>in vitro</i>	Ultra-fast delivery of hydrophilic drugs or active ingredients	[76]
Gelatin/Ciprofloxacin (CIP)/hydroxypropyl-beta-cyclodextrin-inclusion complex	Bead-free morphology with a diameter of ~90 nm	NR	Fast-dissolving character in water and improvement achieved in the solubility of CIP	[58]
Proanthocyanidin-crosslinked gelatin	Smooth and homogeneous in their surface morphology	<i>in vitro</i>	Making L929 fibroblast cells proliferation rate higher	[77]
Arginine-Glycine-Aspartate (RGD) peptide grafted poly (butylene adipate-co-terephthalate)/gelatin electrospun nanofibers loaded with a matrix metalloproteinase	Bead-free thin nanofibers	<i>in vitro</i> and <i>in vivo</i>	Promoting the wound closure within 3 days after treatment initiation and re-epithelialization, collagen deposition, and angiogenesis Antimicrobial against <i>S. aureus</i> and <i>P. aeruginosa</i> .	[78]
Poly (ε-caprolactone) and gelatin blended with metronidazole	Randomly interconnected structure with no beads formed and uniform distribution of nanofibers	<i>in vitro</i> and <i>in vivo</i>	Preventing the anaerobic bacteria colonization and less severe inflammatory response reduce	[79]
Polycaprolactone (PCL) + gelatin (GT) in a mixed acidic solvent containing 5% antibiotics-loaded carboxyl-modified mesoporous silica nanoparticles (CMSNs)	Increased hydrophilicity and degradability, excellent biocompatibility	<i>in vitro</i>	Functional materials against both acute and chronic wounds infections High antibacterial efficiency against <i>P. aeruginosa</i> and <i>S. aureus</i>	[79]

Abbreviations: PVA: Poly (vinyl alcohol); GEL: Gelatin; PHB - Polyhydroxybutyrate; CAF: Caffeine; CIP: Ciprofloxacin; NR: Not reported; RGD: Arginine-Glycine-Aspartate; PCL: Polycaprolactone; CMSNs: carboxyl-modified mesoporous silica nanoparticles.

and remove foreign bodies. Also, macrophage cells help in removing foreign bodies and dead cells as well as enhance the production of collagen that affects the re-epithelialization. Proliferation phase can last from 1 to 10 weeks and starts after wound incident by keratinocytes proliferation and migration across the injured area [83]. Also, fibroblasts are stimulated and differentiated into myofibroblasts that control the growth of other cells and enhance the formation of granulation tissue. The new ECM produced by myofibroblasts degrades provisional ECM. Maturation phase stayed from 1 to 24 months after the wound incident with a change of the granulation tissue into scar tissue where type III collagen and water decreased and type I collagen increased [83]. It is noteworthy to mention that there are several factors like microbial infection, oxygenation, and systemic insults like psychological stress, age and gender, sex hormones, diabetes that affect and delay the process of wound healing [84].

So, it is important to develop a new material to enhance skin repair and shorten the healing process. The history of wound dressings goes back to ancient Egyptians and Sumerians who used natural products like mud, honey milk, plants and animal fat to dress injuries to avoid microbial infection and dehydration [85]. A perfect wound dressing should provide optimum conditions for skin repairing such as to preserve a moist wound environment, allow gas exchange, remove exudates from wound, protect the wound against microorganism invasion, supply mechanical protection and enhance the formation of connective tissue with no allergic or toxic reactions and with high cytocompatibility [85]. Wound dressing can be categorized into four groups: passive, interactive, advanced and bioactive dressings. Passive products like gauze, can enhance the healing process, but cause pain and usually traumatic to remove [86]. Interactive dressings like semi-permeable foams and amorphous hydrogels can supply the wound with effective barrier against bacteria and other microorganisms but semi-permeable foams aren't recommended for light exuding wounds and amorphous hydrogels aren't recommended for moderate to heavily exuding wounds. Advanced dressings like alginates and hydrocolloids can maintain a moist environment for the wound but hydrocolloids aren't suitable for heavily exuding wounds. Bioactive dressings act as significant step in the effective repair of wounds and involve biological dressing, skin substitutes and drug delivery dressings [86].

Wound dressings made of electrospun nanofibers exhibit significant properties for wound healing as the porous structure of the nanofibers matrices not only remove the wound exudates but also prevent bacterial infection and allow nutrients and gas exchange [87]. Electrospun nanofibers provide cell adhesion and proliferation as they mimic the structure of the skin extracellular matrix. In fact, the large surface area to volume ratio of the nanofibers can promote the hemostasis in the wound site, enhance skin growth, and reduce scar development. Electrospun nanofibers can provide bioactive molecules and medications to promote wound healing [87] as represented in Fig. 6.

Variety of nanofibrous scaffolds were made of various natural polymer including gelatin that promotes wound healing due to its availability, biodegradability, biocompatibility and adaptability, additionally it is non-toxic and easy to use. During the electrospinning process, it does not denature under the applied voltage effect [88]. Also, gelatin extracted from fish was proved useful in promoting human keratinocytes growth and adhesion, providing oxidative protection, facilitating wound healing [89]. Needleless nanotechnology gelatin (Gel) and Poly- ϵ -caprolactone (PCL) were suggested and their biocompatibility and therapeutic activities were identified using *in vitro* cell cultures in an experimental rat model. Cell proliferation and adhesion of keratinocytes, human dermal fibroblast and mesenchyme stem cells that were seeded on nanofibrous surfaces were detected by the actin and phalloidin staining prior to fluorescent microscopy imaging [90]. Gelatin promoted the cell proliferation, showed a faster wound closure as well

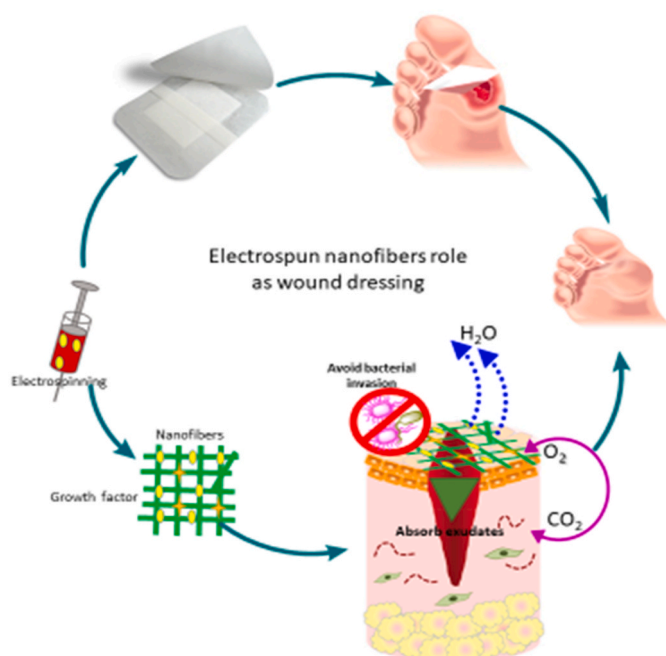


Fig. 6. Schematic illustrating of sputtering gelatin electrospun nanofibers for wound dressing engineering application.

accelerated skin regeneration compared to PCL and the control group treated with gauze solely [90]. Furthermore, an effective antibiotic namely cephalexin (CEX) was loaded by electrospinning into PCL-gelatin meth acryloyl nanofibers (GeLMA). The nanofibrous surface was continuous, uniform and smooth with a diameter of 280 to 330 nm. CEX was released from the surface of PCL/GeLMA [90]. However, upon CEX concentrations increase, the swelling ratios decreased and hydrophilic characteristics were improved. The electrospun nanofibers surface could absorb water up to 400 to 600%. PCL/GeLMA-CEX showed a significant protective effect against both *Staphylococcus aureus* and *Escherichia coli*. Collagen deposition and re-epithelialization were also observed when utilizing hematoxylin-eosin and Masson's trichome staining [90].

Taken together, PCL/GeLMA-CEX were effective polymers with antibacterial impact when used as wound dressing [91]. Recently, gelatin and *Centella asiatica* (CA) plant were used. CA is a medicinal plant used to promote wound healing via enhancing fibroblast proliferation, synthesizing of collagen and evidential antibacterial activity. Gelatin nanofiber and extract of CA were synthesized via electrospinning. High rate of recovery was noticed in electrospun gelatin membranes containing *C. asiatica* (EGC) [71]. EGC showed more deposition of collagen and a greater number of capillaries in the wound area. Moreover, EGC prevented wound adhesion to the dressing, aided absorbing and removing exudates from wound, in addition to a continuous secretion of CA [71]. These benefits help in reducing discomfort and decrease the frequency of dressing changes. Hence, EGC is considered to be more promising material for skin wounds treatment [71]. Thus, recently, PCL/gelatin nanofibers of diameter 3.7 mm were utilized for skin tissue engineering and was successfully applied within *in vitro* assay [92]. An additional example is the fabrication of methacrylated gelatin (MeGel)/poly (L-lactic acid) (PLLA) hybrid nanofibers with an extracellular matrix (ECM) presenting nanofibrous structure and hydrogel-like characters

Table 2
Gelatin nanofibers in wound dressing applications.

Polymer	Fiber diameter (nm)	Structure/properties	Assay	Activity of nanofibers	Reference
Polyhydroxybutyrate (PHB) + Gelatin (GEL)	80	Smooth, uniform and non-woven fine architecture with very good interconnectivity	<i>in vivo</i> and <i>in vitro</i>	Enhance healing process as degradation rate is about 71.8% during 12 h	[95]
Gelatin + polymethyl vinyl etheraltmaleic anhydride (PMVE/MA) + nano zinc oxide	500–700	Randomly oriented uniform nanofibers with interconnected pores	<i>in vivo</i>	Wound healing is about 99% during 10 days	[96]
Gelatin/Oleoyl Chitosan (OC)	150–400	Revealed mechanical strength, moderate surface wettability, and suitable degradation rate	<i>in vitro</i>	The swelling is around 380%, Enhance collagen synthesis, re-epithelialization and epithelial cells stratification	[97]
Polycaprolactone/gelatin-methacryloyl/cephalexin	280–300	Uniform, smooth, and continuous	<i>in vivo</i> and <i>in vitro</i>	Inhibit <i>S. aureus</i> (+ve gram bacteria) <i>E. coli</i> (-ve gram bacteria) and the swelling is around 400–600%	[91]
Cellulose acetate/gelatin/nanohydro-xyapatite	115–316	Spherical morphology with smooth surface and uniform morphology	<i>in vivo</i> and <i>in vitro</i>	Promote cell proliferation of L929 cells with Highest wound closure is around 93.5 ± 1.6% during 7 days	[98]
Gelatin/keratin	160.4	Uniform morphology and bead-free structure	<i>in vivo</i> and <i>in vitro</i>	A great reduction in wound area at 4 days and a wound repair at 14 days with a thicker epidermis	[99]
Gelatin nanofibres containing <i>C. asiatica</i>	150–350	Smooth and homogeneous membranes	<i>in vivo</i> and <i>in vitro</i>	Highest recovery rate. Promote cell proliferation. Antibacterial activity against <i>S. aureus</i> and <i>E. coli</i>	[71]
Silk fibroin/gelatin	NR	High porosity	<i>in vivo</i> and <i>in vitro</i>	High wound closure rate	[100]
Cellulose acetate/gelatin nanofibrous containing berberine	502 ± 150	Uniform, straight without any beads and deformities	<i>in vivo</i> and <i>in vitro</i>	Antibacterial activity against gram-positive and gram-negative bacteria. Promote wound healing in wound ulcer foot	[101]
Poly (lactic-co-glycolic acid) + gelatin + Lira through (PLGA/Gel/Lira)	NR	Increased pore size, hydrophilicity, elasticity and degradation properties	<i>in vitro</i>	Improve the healing efficiency of diabetic dermal wounds characterized by shortened wound closure time, increased blood vessel density, and elevated collagen deposition and alignment	[102]
Sponge + Gelatin (Ultralight 3D gelatin)	2–3 µm	Good cytocompatibility, high cell permeability, low hemolysis ratio	<i>in vivo</i>	Absorbable hemostatic agent for rapid hemostasis, promote the formation of blood vessels tissue	[103]
Curcumin + gelatin	147 (147 ± 34)	Low diameters large surface area volume ratio and enough film porosity as well as improved mechanical strength	<i>in vivo</i>	Sustained release of curcumin and oxygen to wounds during healing	[104]

Abbreviations: PHB: Polyhydroxybutyrate; GEL: Gelatin; PMVE/MA: polymethyl vinyl etheraltmaleic anhydride; OC: Oleoyl Chitosan; PLGA: Poly (lactic-co-glycolic acid); NR: Not reported.

suitable for wound dressing [93]. Similarly, new bi-layered and multifunctional dressing patches formed by one layer of electrospun methacrylated gelatin (MeGel)/poly (L-lactic acid) (PLLA) radially-oriented nanofiber mats (RNMs) and one layer of *Salvia miltiorrhiza* Bunge-*Radix Puerariae* herbal compound (SRHC)-loaded MeGel hydrogel were prepared to enhance the closure and healing of diabetic wounds [94]. Thus, gelatin containing multi-component fibers developed using different synthesis approach and of different diameters are more promising systems for wound dressing applications as illustrated in different systems indicated in Table 2.

5.3. Bone regeneration

Bone is a complex hard connective tissue, providing shape, protection to different internal organs as well supplying the body with structural integrity and facilitating the movement. Also, it contributes to homeostasis, mineral storage and regulation of blood PH [105]. Bone remodeling is a lifelong process that depends on the equilibrium on bone resorption by osteoclasts and bone deposition by osteoblasts that are really important in bone regeneration, healing and provide the structural integrity to the tissue [105]. Bone is well-known for repairing and healing itself in mild fractures without surgical intervention. But patient with a large bone defect don't have the potency for self-healing because lack of orchestrated regeneration in bone [106]. Bone trauma, tumors, diabetes, abnormalities and aging are the most popular bone defects that affect the length and quality of human life. Indeed, annually more than twenty million people worldwide are influenced by bone tissue loss caused by disease or trauma [106]. Importantly to note that, the complex fracture and repairing bone defects are believed to be the most challenging and urgent problems in bone surgery.

Metal implants, auto/alloy bone grafts and materials of bone substitute transplantation are the surgical alternative of bone; however, metallic implants can cause inflammation, osteolysis and bone resorption. Auto grafting is considered as a promising standard in the bone defects treatment [107]. Nevertheless, there are various problems related with donor site injury and morbidity, pain associated to morbidity, high costs, limited quantity and tissue availability. Allografts carries hazards of disease transmission, donor shortage, higher immune rejection, high cost, and limited bioactivity [107]. Therefore, the seeking for new bone regeneration materials to replace bone grafting is urgent to improve the bone regeneration process. Bone tissue itself could be a promising candidate of bone regeneration and repairing bone defects. Thanks to bone tissue engineering which interconnects traditional techniques and engineering materials with life science for development of a suitable scaffold to repair and regenerate the damaged bone tissue [108]. Thus, most of bone tissue engineering techniques depend on cell substitutes to replace nonfunctional cells while maintaining their functions and avoiding immunological rejection [108]. Therefore, the design and development of a suitable scaffold should improve bone healing, mechanical support and thus provides help in the new bone formation, mimicking the extracellular matrix (ECM), supporting the adhesion, and promoting the proliferation and differentiation of cells [109]. A perfect scaffold should include bio-materials that are having unique features such as biocompatible, biodegradable, controllable and porous. Hence, nanofibers scaffolds are found to exhibit small pore size, large surface area to volume ratio and high porosity [109].

Thanks to electrospinning technique that can provide functionally and structurally similar ECM for bone implant process as ECM is very important for cells and tissues. Additionally, the nanofibers scaffolds have aided the mechanical and biological contexts by matching the real matrix of bone tissue [8]. Nanofibers scaffolds have the potency to resemble the native ECM architecture at the scale of nanometer to help the organization, migration and survival of cells in bone regeneration [8]. Moreover, the unusually high porosity

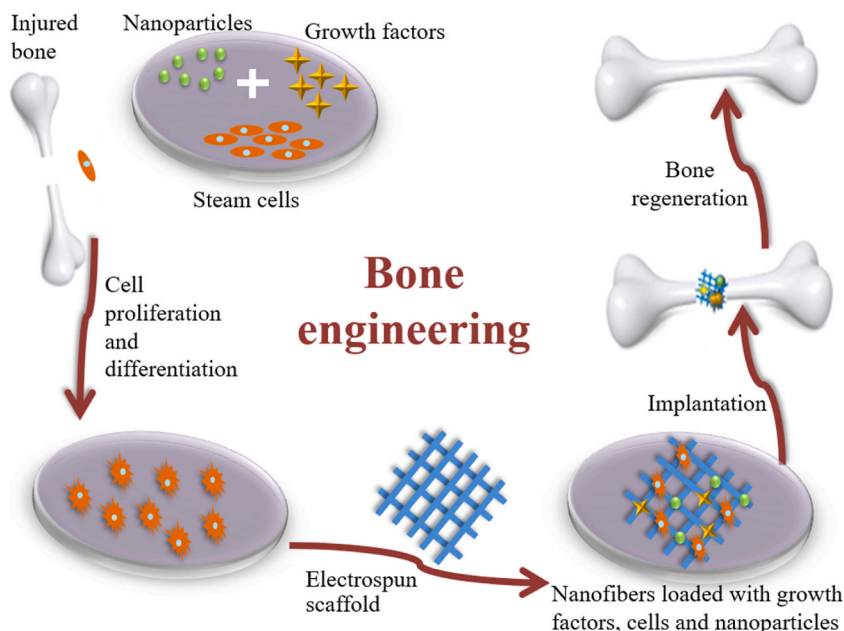


Fig. 7. Schematic illustrating of sputtering gelatin electrospun nanofibers for bone engineering application.

of nanofibers scaffolds improves cell adhesion, diffusion and differentiation and achieves the preferable cell growth [73].

Osteoconduction (electrospun scaffolds), osteoinduction (bioactive molecules), and osteogenesis (stem cells) are the main three major elements that promote the process of bone formation in bone tissue engineering. Osteoconduction is the ability to permit the bone tissue to grow and rearrange on the implanted nanomaterials surface. These materials help in the osteoinduction and osteogenesis [110].

Interestingly, the osteoconductive scaffolds in the absence of bioactive molecules and stem cells loss the ability to form a new bone, however, osteoinduction signals growth factor and molecules help in bone formation. Electrospun scaffolds with morphogenic proteins (osteoinductive agent) can attract the mesenchymal stem cells [110]. Then the stem cells develop to preosteoblasts for ultimate bone formation. Osteogenesis also can produce new tissue by osteoblasts cells. Bone engineering can be summarized in these steps to enhance bone formation *i.e.* cells taken from injured bone upon the nanofibers scaffold and growth factor stimulate the cell proliferation and differentiation. The scaffold implanted in the injured part of bone and lead finally formation of new tissues [110] as shown in Fig. 7.

Gelatin is one of the most suitable materials to build a bone scaffold. Gelatin has a very good biodegradability and non-antigenicity besides the low mechanical strength. Interestingly, the guided bone regeneration (GBR) showed a great role in bone tissue engineering. GBR is effective technique that widely used in alveolar bone deformities augmentation [111]. GBR could improve cell adhesion and proliferation that is necessary in the bone regeneration. GBR showed high mechanical properties, and known to be biodegradable, and biocompatible. Nanofibrous GBR membranes are more suitable to improve the tissue reconstruction. PCL and Gel nanofibrous membranes were fabricated via electrospinning with different weight ratios. Acetic acid was successfully providing homogenous PCL/Gel hybrid nanofibers [111]. Nanofibers showed smooth and uniform structures with a diameter of 200 to 600 nm. Gelatin incorporation could improve the cell viability and wettability that support cell attachment and proliferation. PCL/Gel hybrid membranes could increase the osteogenesis capability. This indicated that the nanofibers enhance bone formation [111].

New scaffolds were developed based on inclusion of gelatin with PCL and bioactive glass (BG) where the scaffolds were prepared by PCL/Gel/BG via electrospinning technique with ratios of 50/50, 25/75, and 75/25 for the polymers with 5 wt % of BG. The diameter decreases by a range of 557 to 167 nm as a result of adding gelatin to PCL and BG [112]. High mechanical properties and bone cell response improvement were showed in PCL/Gel (50/50)/BG Nano composite. The result showed that the degradation rate increases by addition of gelatin and bioactive glass, thus the degradation time showed a major similarity with remodeling time [112]. Current efforts focus on producing electrospun scaffolds with controlled hierarchical structures [113]. The use of gelatin as a scaffold for bone tissue engineering is directly related to hydrophilicity, high biocompatibility and bioactivity associated with specific peptide sequences. Gelatins containing multi-component fibers are more promising methods than gelatin itself, due to the mixture of electrospinnable solutions and structural, mechanical and chemical properties that mimic the natural ECM features. Therefore, several systems were summarized in Table 3 [109] reflecting the usage of gelatin electrospun nanofibers in bone regeneration applications.

5.4. Tissue engineering

In the early 1990s Langer and Vacanti define tissue engineering as “an interdisciplinary field in which life sciences and the principles of engineering are applied to develop biological substitutes that repair, maintain and restore tissue function”. In tissue engineering, fabrication of scaffolds by electrospinning methods is of a great interest [117]. Electrospun nanofiber scaffold properties

Table 3
Gelatin nanofibers in bone generation applications.

Polymer	Fiber diameter (nm)	Cells loaded on nanofibers	Assay	Nanofibers structure	Activity of nanofibers	Reference
PCL/Gel	200–600	MC3T3-e1 cell	<i>in vitro</i>	Relative uniform and smooth	High osteogenesis ability, Promote bone formation and effective GBR	[111]
PCL/Gel (50/50)/BG	557–167	MG-63 osteoblast cell	<i>in vitro</i>	High porosity with slightly decrease in size	High degradation rate and degradation time similar to remodeling time	[112]
HA-PLGA/Gel	783 ± 91	Mesenchymal stem cells	<i>in vitro</i>	Smooth and random morphology	Enhance osteogenic differentiation of stem cells	[113]
PCL/Gel/chitosan/ β -TCP	200–500	MG-63	<i>in vitro</i>	Smooth and bead-free fibers	High degradation time, swelling and mechanical properties that enhance bone formation and effective GBR	[115]
Gelatin/siloxane (GS)	NR	Bone marrow-derived mesenchymal stem cells (BMSCs)	<i>in vitro</i>	Uniform fibers mat with random orientation	High stem cell proliferation and differentiation and promoting bone regeneration	[114]
Poly (vinyl alcohol)/gelatin (PVA/Gel)	150 ± 10	MG-63 cells	<i>in vitro</i>	Large surface area, adequate pore size, uniformly distributed and interconnected porous structures	Good Osteoblasts proliferation that enhance bone regeneration	[116]

β -TCP: β -tricalcium phosphate; PCL/Gel: Polycaprolactone/Gelatin; HA-PLGA/Gel: hydroxyapatite-coated hybrid poly(lactic-co-glycolic acid)/gelatin; GS: Gelatin/siloxane; PVA/GEL: Poly (vinyl alcohol)/gelatin.

can mimic those properties of nanoscale of native ECM and also can supply a specific response for regeneration of tissue. Besides that, they have high ratio of surface area-to-volume with highly porous characters [118]. Scaffolds with (3D) structure promote migration, proliferation and adhesion of cells, thus the hybrid composite nanofibers production with desired features are more promising systems in tissue engineering application. Blending polymer architecture in nanofibers with natural and biocompatible materials give scaffolds with bio-functional properties [119]. There is a great interest in biodegradable scaffolds production with biopolymers that extracted from plant and animal origin [8].

Biopolymers offer several advantages like hydrophilicity, chemical cues, and biocompatibility and degradation properties making them a major player in cell behavior modulation. Natural polymers i.e. collagen, fibrinogen, gelatin, elastin and laminin are used in tissue engineering due to their properties. [120], serving different applications of tissue engineering of gelatin-based electrospun scaffolds. Furthermore, recently various reported results were discussed in other point of view [121].

5.4.1. Nerve engineering

Nervous system is one of the body important systems and it is controlling the sensory and motor functions. The nervous system is an extremely complex organization where signals from and to different body parts are sent all the day around to regulate actions as well as sensory information [122]. The central and peripheral nervous system are two main parts of nervous system. The central nervous system (CNS) consists of the brain and spinal cord, on the other hand the peripheral nervous system (PNS) is mainly formed of nerves that are collected in bundles of long fibers that bind CNS with every part of body [122]. Despite the delicate function and structure of the nervous system, it has a limited ability of regeneration especially CNS.

Thus, repairing the damaged nervous system and restoring its function have been a challenge. Several strategies have been proposed including the direct, end-to-end surgical reconnection for treating nerve injuries in the small injury gap of PNS [123]. Also autograft and allograft are used but they are facing many technical problems such as donor site morbidity, multiple surgeries and scarring besides that an allograft patient needs to adopt to an indefinite immunosuppression after surgery to overcome the rejection problems [123]. Then appear the challenge of restoring the function of the damaged nerve cells. Recently, tissue engineering has been introduced as a new method to overcome the current methods ineffectiveness and as an alternative to the traditional implantation methods using polymeric biomaterials.

To minimize immune responses occurrence, biocompatible and biodegradable polymers are used. Depending on the applications, chemical and physical properties of artificial grafts can be adapted [124]. To enhance tissue regeneration, architecture of scaffolds besides the suitable biochemical and topographical signals of scaffolds components are adjusted. Also modification of artificial scaffolds can supply axons with permissive substrate to attract the regrowth of nerve ends [124]. Mimicking the ECM is a common tissue engineering approach as ECM has a vital role in controlling cell behavior [125]. The choice of materials plays also an important role in the success of neural tissue engineering strategies. Nanofiber polymers are a promising choice as per its high surface area-to-volume helping in the sustained release of proteins, nucleic acids and drugs [126]. Besides that, the large surface of nanofibers promotes the contact between cells and fibers. In this context, some of studies were performed with gelatin-based electrospun scaffolds to evaluate its effect on the behavior of neural cell. According to Alvarez-Perez et al., Poly ϵ -caprolactone (PCL) electrospun membranes promoted with gelatin showed positive response on PC-12 nerve cells [126].

Membranes hydrophilic impact was owned to the gelatin content as validated by the contact angle measurements [126]. Gelatin cues have a vital role in promoting the biocompatibility of this scaffold and have a vital role improving affinity of cells as compared to PCL synthetic polyester fiber. Gelatin cues enhance neural differentiation and gene expression as well as the neurite outgrowth in *in vitro* as the incorporation of gelatin improves all the essential biological events [126]. According to Binan et al. poly L-lactic and gelatin coaxial electrospun nanofibers were developed where NSLCs stem cells were seeded and examined for differentiation into motor neurons via retinoic acid and purmorphamine controlled release [127]. Neural stem cells offer an optional therapeutic cell

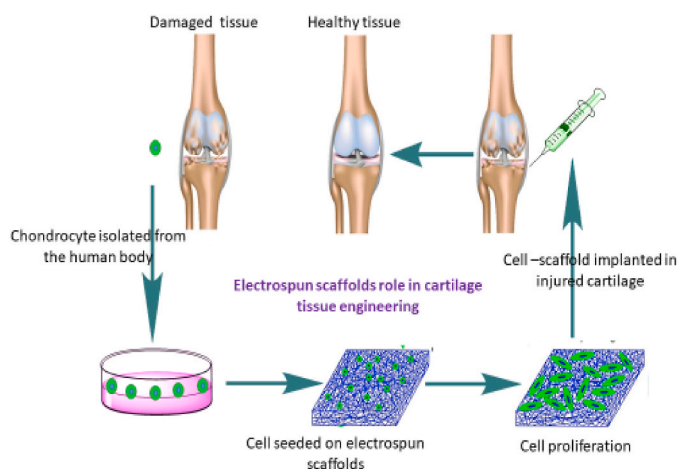


Fig. 8. Gelatin electrospun nanofibers in cartilage tissue engineering.

replacement strategy in spinal cord injuries. This study was a starting point to fabricate a tissue regeneration material with instructive cues, where a sufficient mechanical properties were able to guide and instruct the neural growth [127].

5.4.2. Cartilage

Cartilage is a connective tissue that possess a limitation of self-regeneration ability because it is free from lymphatic system and blood vessels and cellular density is low [128]. Therefore, any damage to this tissue is remaining for many years and this may cause degenerative diseases. Damages of cartilage occur in rheumatoid arthritis, osteoarthritis, and various traumas. Scaffolds of three-dimensional play an important role in cartilage regeneration [129]. Cartilage consists of chondrocytes attached to ECM via binding proteins of cell surface which are really important for compressive and tensile strength. In tissue engineering of articular cartilage, collagen which is considered main compound of the cartilage matrix has a great role in protecting the organization of chondrocytic phenotype and supporting chondrogenesis as shown in Fig. 8. Collagen fibers maintain tissue integrity that is necessary for mechanical properties [129].

Torricelli et al. used poly (L-lactic acid) (PLLA) and gelatin (GEL) fabricated via co-electrospinning. GEL and PLLA combination influence the tensile mechanical properties. Chondrocytes are seeded on scaffolds display good proliferation rate and viability [130]. PLLA70GEL30 (Poly (L-lactic acid)/gelatin weight ratio: 70/30) and PLLA50GEL50 (Poly (L-lactic acid)/gelatin weight ratio: 50/50) scaffolds enhance differentiation of Chondrocytes. The results demonstrated that scaffolds are suitable for cartilage tissue engineering. GEL/PLA electrospun fibrous membranes were fabricated via electrospinning. After that an ear-shaped titanium alloy mold helps in building an ear-shaped cartilage in a sandwich model [130]. Then chondrocytes in a sandwich model are grown on scaffolds. The ear-shaped cartilage retained the original shape to a large extent. This result demonstrated that the engineered tissue exhibits a good mechanical strength and elasticity. GEL/PLA has a great potency to be used in cartilage tissue engineering [130,131].

5.4.3. Corneal tissue engineering

Corneal disease is believed to be the second reason of loss of vision. Approximately ten millions of people all over the world loss vision because of corneal lesion [132]. To overcome corneal disease donor corneal grafts are used, but there are many problems because there are few eligible donors with corneal tissue. And also, it is very difficult to get high-quality graft material because of endothelial decompensation and immunological rejection [133]. Therefore, substitutes of corneal tissue engineering are needed to be developed. Gelatin hydrogel is one of the more suitable material for corneal tissue engineering because of biocompatibility, low antigenicity, transparency, and cell attachment [133]. Tonsomboon et al. used gelatin nanofiber-reinforced alginate that was prepared by electrospinning, and this method promotes alginate hydrogels mechanical properties. Gelatin nanofibers crosslinking was very important to stop a rapid degradation after implantation into the body. The result demonstrated that these nanofibers can be used as scaffolds for corneal tissue engineering because of their optical transparency (Fig. 9) [134].

5.4.4. Retinal tissue engineering

Cells of retinal pigment epithelium are a monolayer of cells in the outer layer of retina which play an important role in maintaining the vision, neural retinal communication and supplying fluid, ions, nutrients via metabolite transport, and enhance homeostasis between the choroid and the photoreceptor [135]. Ocular disorders result from retinal pigment epithelium alternation. Dysfunction of retinal pigment epithelium (RPE) is one of the main pathological changes causing retinal degenerative diseases like retinitis pigmentosa and age-related macular degeneration that are considered the main causes of blindness.

Unluckily, there is no revolutionary treatment to relieve the development of these diseases or to restore the lost vision. Tissue engineering supplies a chance to develop cell-based RPE treatment. The basic concept is to employ scaffold and RPE implant to enhance functional monolayer maturation under the retina [136]. In the last years, electrospun nanofibers have attracted a great attention in the field of retinal tissue engineering. Noorani et al. developed nanofibrous scaffolds of gelatin/chitosan using electrospinning, then RPE was implanted on gelatin/chitosan scaffolds and this scaffold enhanced the cells adhesion. The seeded RPE showed no change and these scaffolds have no toxicity, but further clinical trials are needed to ensure these scaffolds feasibility for retinal replacement [137].

Xiang et al. developed porous and ultrathin nanofibrous membrane that was fabricated using regenerated wild Antheraea pernyi silk fibroin (RWSF), GEL and PCL. Then human RPE was implanted on membranes of RWSF/PCL/GEL scaffolds and transplanted into chinchilla rabbit's eye prior to its evaluation (after 12 weeks) [138]. RWSF/PCL/Gelatin membranes promote cell proliferation and cell growth. Besides that, they enhance RPE cell functionalization without seeing any inflammatory reaction. These electrospun RWSF/PCL/Gelatin showed good biocompatibility *in vivo* and *in vitro* for the use of prosthetic Bruch membrane for RPE implantation [138]. Still the mechanism of nanofibers scaffolds containing gelatin needs deep investigation and clinical trials. Also, gelatin crosslinking with another material has a superior effect than gelatin, but the challenges also remain to better understand the mechanisms by which nanofiber scaffolds affect cell behavior and tissue regeneration processes.

6. Conclusions

Research awareness towards gelatin electrospun nanofibers (GNFs) has tremendously increased. No doubt, gelatin nanofibers' features, synthetic routes, applications, economic feasibility, and limitations are diverse insults that affect the involvement of gelatin nanofibers in various research disciplines. Recently, there are a great focus on the employment of GNFs in a wide spectrum of medical applications such as bone regeneration, drug delivery, wound dressing and tissue engineering. However, gelatin nanofibers have a plethora of advantages such as biodegradability, biocompatibility, and low toxicity, they show a few limitations including poor water-

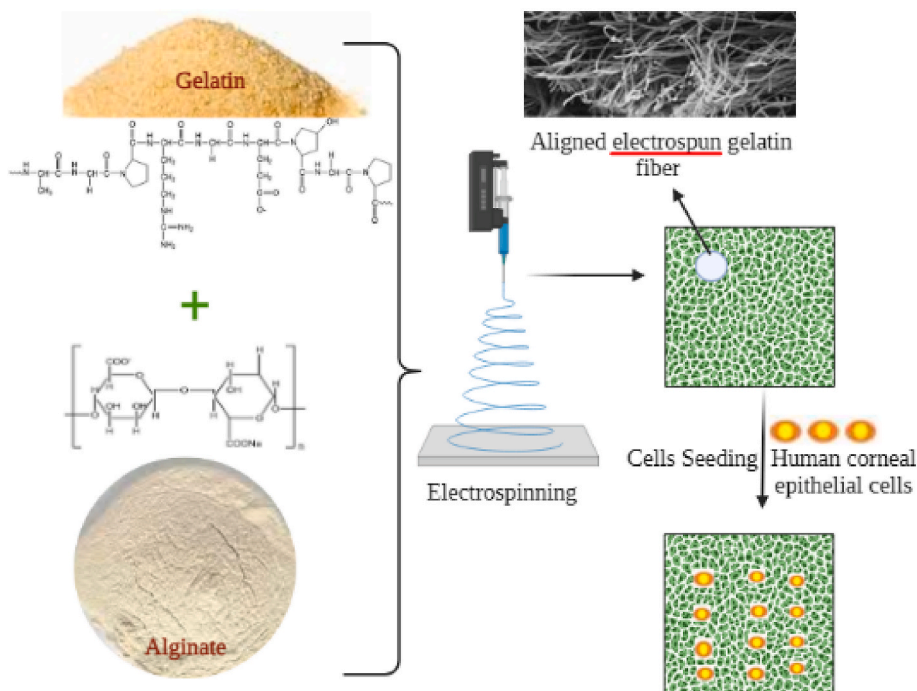


Fig. 9. Gelatin electrospun nanofibers in corneal tissue engineering.

resistance and weak mechanical stability, sensitivity to humidity, poor cold gel modulus, and poor wettability. Thus, the structural alteration of GNFs to improve their interaction with the living body is one of the potential future tactics that will greatly boost their implications. Further novel approaches are warranted for the future perspectives in developing and incorporating polymers nanofibers electrospun into the appropriate therapeutics' applications, to hold a promise not only for the preclinical studies but also to the clinical trials.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

The data that has been used is confidential.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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