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Design of Collagen and Gelatin-based Electrospun Fibers for Biomedical Purposes: An Overview

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Abstract

Collagen and gelatin are two of the cardinal natural biopolymers used in the field of biomaterials and tissue engineering because of their excellent physico-chemical and biocompatibility properties. They can be used either in combination with other biomacromolecules or particles or even exclusively for the enhancement of bone regeneration or for the development of biomimetic scaffolds.

The use of the electrospinning technology can convert collagen or gelatin derivatives into nanofibrous materials that exhibit porous micro- or nano-structures with good mechanical properties and excellent biocompatibility features. Specific attention was recently paid to electrospun mats of such biopolymers, because of their high surface area-to-volume ratio, their biocompatibility, degradability and low immunogenic property. The resulting fiber mats with submicro- and nano-meter scale can simulate extracellular matrix structure of the human tissues, organs and can widely be used in the tissue engineering field, due to their excellent bio-affinity. The drawbacks may include rapid degradation, and complete dissolution in aqueous media, which seriously limits the use of gelatin/collagen electrospun nanofibers in this form for biomedicine. Therefore, these fibers need to be cross-linked for controlling their aqueous solubility. One such modification induces an improvement of the biological properties of the fibers, which made them highly suitable candidates for numerous biomedical applications.

This review aims to present the important research pertaining to electrospinning of collagen and gelatin and their biomedical applications. The structure and performance of the electrospinning fiber mats which were manufactured from different solvents, electrospinning processes, and cross-linking methods are highlighted. Judiciously selected examples from literature will be presented to demonstrate major advantages of such biofibers. Current advances and challenges on this research field are also discussed.

Keywords: gelatin, fiber, collagen, electrospun scaffolds, biomedical applications

1) Introduction

Tissue engineering involves three core components: signaling molecules, cells, and biomaterials, which are commonly known as the tissue engineering triad ¹. This field usually requires the fabrication of engineered scaffolds to aid in the repair and regeneration of damaged tissue. The extracellular matrix (ECM) (Figure 1) is composed of polysaccharides and proteins, for the most part hyaluronic acid, elastin, proteoglycans, glycosaminoglycans, and collagen.

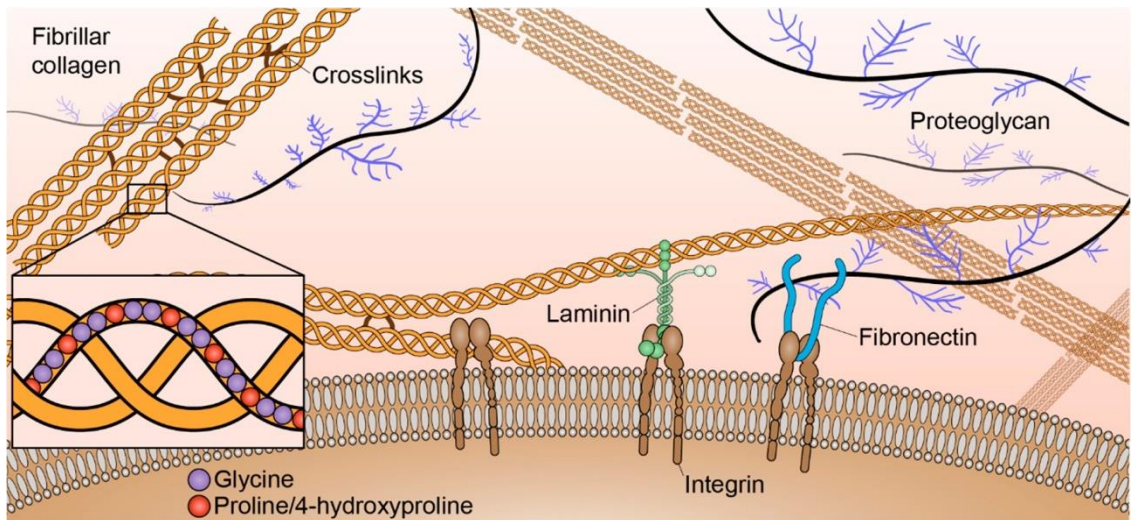


Figure 1. Extracellular matrix components of tumor microenvironment. Reproduced with permission from ². Copyright [2021] [Bioengineering, Open access].

Collagen (Figure 2) is one of the major extracellular matrix proteins and is a major constituent of numerous tissues and organs (specifically type I), including tendon, skin, blood, bone, vessels, and cardiac tissue ³. Collagen has several functional characteristics (i) properties associated with gelling behavior, i.e. gel formation, thickening, texturizing, and water binding capacity and ii) properties related to their surface behavior, including emulsion and foam formation and stabilization, protective colloid function...) that are beneficial for cell and tissue growth, and thus it has been used widely as a biomaterial for biotechnological and medical purposes ⁴.

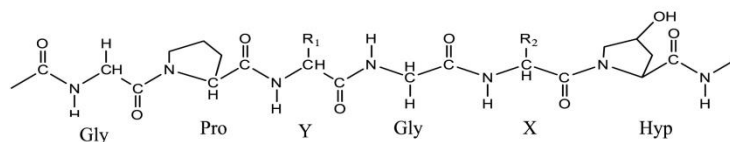
Gelatin is a denatured protein that is a result of acid, alkaline, and enzyme processing of collagen. It can present very similar physical and biological properties to those of collagen ⁵. It deserves noting that gelatin properties strongly depend on the source and type of collagen, and type of

conversion of collagen to gelatin (acidic versus basic hydrolysis). Gelatin plays an important role in tissue remodeling ^{5b}, and it is known to express biological activities, having anti-osteoporosis, antioxidant, and anti-photoaging properties⁶.

Due to their excellent biodegradability, biocompatibility, and immunogenicity profiles, gelatin and collagen are the most commonly used biopolymers for making biomaterials for tissue engineering ⁷.

In tissue engineering, nanofibers are widely used as scaffolds in nerves, bones, cartilage, dermal tissue and blood vessels ⁸. More precisely the preparation of nanofiber scaffolds that structurally mimic the ECM of body tissue is of major interest in tissue engineering. Several methods have been developed to produce nanofibers, including self-assembly ⁹, nanolithography ¹⁰ and electrospinning ¹¹. Among these methods, electrospinning is the most simple and efficient method and will be highlighted in this review ¹². Given the fibrous nature of the ECM, electrospinning, a technique that creates polymeric matrices comprised of nanometric or micron-sized fibers, is frequently used to create scaffolds for tissue engineering ^{8, 12a, 13}. Indeed, this technique allows the production of fibers from almost all of the soluble natural polymers with sufficient molecular weight ¹⁴. The characteristics of the fabricated fibers can be tuned by manipulating the spinning parameters (polymer, solvent, solution concentration, needle-ground distance, applied voltage), leading to a wide variety of scaffold architectures that can be manufactured to mimic the ECM structure ¹⁵. The resulting electrospun fibers often possess extremely high surface areas, tunable pore structures, high porosities, and superior mechanical properties ¹⁶. Additionally, mechanical properties and resistance to degradation can be controlled via a cross-linking step and/or through the incorporation of higher strength components in the spinning process ¹⁷. In this sense, it has been shown that electrospinning is a useful technique for transforming collagen or gelatin into nanostructured fibrous materials ^{4a}.

(a) Primary amino acid sequence of collagen I



(b) Triple-helix structure of collagen I

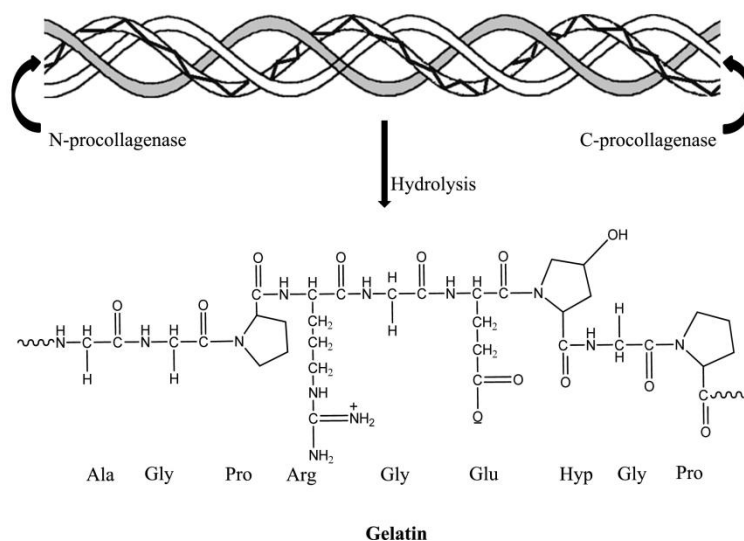


Figure 2. Illustration of hydrolysis mechanism that converts collagen (a) into gelatin (b). Reproduced with permission from ¹⁸. Open access [2017] [Polymers].

Nonetheless, for tissue engineering applications, collagen and gelatin fibers are usually cross-linked or blended with synthetic polymers or particles to enhance their physical properties. Different chemical and physical methods have been developed to achieve this and will be discussed herein.

In this context, this review aims to discuss the important available literature on electrospinning of collagen and gelatin, the challenges and applications in the biomedical field, and to provide insights regarding the way forward. Benefits and disadvantages of biomaterials based on these biomacromolecules are reported in Table 1.

Table 1: Benefits and disadvantages of biomaterials based on collagen or gelatin

Biopolymer considered	Benefits	Disadvantages
Collagen	<ul style="list-style-type: none">- High biocompatibility- Low antigenicity response- Low cytotoxic response	<ul style="list-style-type: none">- Poor mechanical properties- Poor structural integrity in materials
Gelatin	<ul style="list-style-type: none">- Good biocompatibility- Low antigenicity response	<ul style="list-style-type: none">- Poor mechanical properties

It deserves noting that both synthetic (*e.g.*, poly(lactic acid) or poly(butylene succinate)) and natural polymers (*e.g.*, chitosan, collagen, gelatin, and cellulose) can be used for biomedical applications. However, the synthetic ones are more suitable for superficial wounds, presenting deficiencies of some important properties of wound dressings, such as low absorption, adherence, and permeability¹⁹. Natural polymers are extensively employed in skin tissue engineering, due to their biodegradability, biocompatibility, easy resorption, and capacity to repair or regenerate the damaged tissue. Collagen is the main component of the extracellular matrix implied in scar formation, considered as a secondary process of healing, after wound contraction²⁰. Mixtures of synthetic/natural polymers are mainly proposed to prolong the degradation time, up to that of the healing process, to enhance the mechanical properties, maintaining in the same time the gas and water vapor permeability of the biomaterial as well as the biocompatibility.

2) Brief presentation of the electrospinning basics

Electrospinning (also called electrostatic spinning) is an electrohydrodynamic technique for processing solutions of polymer or melts to generate micro- or nanofibrous scaffolds. Briefly, when a polymer solution or melt is treated with a high voltage, the pending drop surface held by its own surface tension is electrostatically charged (Figure 3). Once the electric field established between a grounded collector and a spinneret tip ends up at a certain threshold value, the electrostatic forces of the surface tension of the examined solution prevail and generate a microjet from the pendant drop. Before arriving at the collector, the jet of liquid undergoes stretching and whipping and during the process the solvent evaporates (process presented in Figure 3).

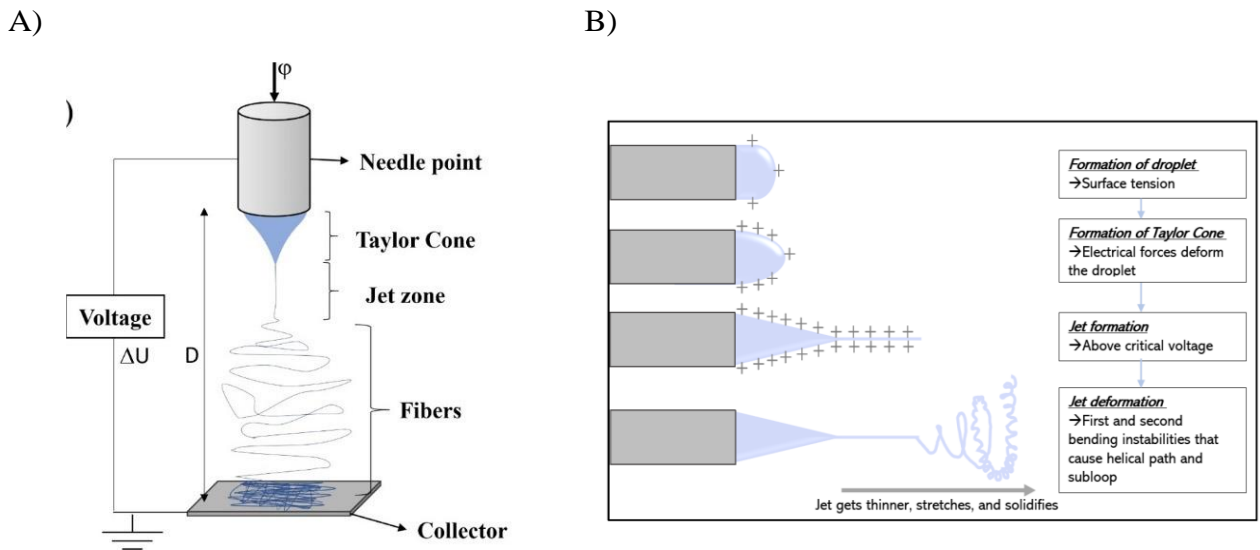


Figure 3. A) Electrospinning process to fabricate fibers ²¹. Adapted with permission from [21], B) Different stages of the electrospinning process ²² Adapted with permission from [22]

The fibers fabricated by electrospinning lead to the generation of non-woven mats that contain either randomly-oriented or aligned fibers ²³. Despite the fact that many authors have presented electrospinning as a simple technique, the process is in reality complex. The characteristics of the resulting mats are strongly influenced by various parameters. These include intrinsic solution properties such as the structure of the polymer, average molar mass, concentration, nature of the solvent (including any co-solvents), surface tension, viscosity, and electrical conductivity. Processing parameters also play a critical role, including electric voltage, flow rate, spinning distance, position, collector geometry, and type, as well as collector polarity. Additionally, ambient parameters like temperature and moisture significantly affect the outcomes, as detailed in Table 2.

For more insight into the parameters impacting the electrospinning process, the reader may refer to details published elsewhere. ^{21, 23-24}

Table 2. Impact of polymer solution, process parameters, and ambient parameters on the resulting fibers during the electrospinning process ^{8, 23}

Parameters	Impact on the electrospun fiber morphology
<u>Solution parameter</u> ^{8, 23} - polymer concentration/solution viscosity - conductivity - solvent	- increase in fiber diameter with higher concentration or viscosity (within optimum concentration/viscosity range) - decrease in fiber diameter with increase in solution conductivity - i) solvent with a high-solubility parameter does not necessarily produce a solution suitable for electrospinning, ii) if the volatility is too low, the fibers will still be wet when they are deposited in the collector, leading to the formation of beaded nanofibers
<u>Processing parameter</u> ^{8, 21, 23-24} - applied voltage - distance between needle tip to collector - feed rate - flow rate	- no definite relationship between fiber diameter and voltage - a higher than threshold voltage induces a higher probability of polymer bead formation - decreasing fiber diameter with increasing distance (within an optimal range of distance) - a higher rate (above a threshold rate) induces a higher fiber diameter and an increasing probability of polymer bead formation - increasing in the flow rate induces larger fiber diameter and fiber with beads when flow rate too high
<u>Ambient parameters</u> ^{8, 21} - Temperature - Humidity	- decreasing fiber diameter with increasing temperature (i.e., above room temperature) - higher humidity induces the fusion of fibers with circular porous morphology

Therefore, to generate defect-free continuous fibers with reproducible fiber morphology, diameter distribution and orientation, the above cited parameters must be finely controlled. For a deeper insight into how these parameters may affect the electrospinning process, the reader should refer to detailed literature.^{21, 23-24}

Some advantages and limitations of the electrospinning process are briefly presented in Table 3.

Table 3: Advantages and limitations of electrospinning process

Advantages	Limitations
<ul style="list-style-type: none">- rather inexpensive- easy to handle- high porosity (>80%) and high surface area to volume ratio	<ul style="list-style-type: none">- often requires toxic solvent- low-volume scaffolds- limited control of the structure of the pore- smaller average pore size

3) Collagen and collagen-based fibers

3.1) Brief definition of collagen

Collagen is the most abundant protein in the human body. This protein is a key element of the ECM, and imparts structural integrity and tensile strength to tissues. Note that there are numerous classes of collagenous structures in the ECM, including networks, fibrils, and transmembrane collagenous domains ²⁵. In forming fibrillar structures, the collagens most involved include types I, II, III, V and XI ²⁶. For instance, in the human body, at least 16 various types of collagen exist. The most prevalent types are type I, type II, and type III collagens, which compose above 80 to 90% of all body collagen ²⁷. Collagen type I is constituted of three spiral polypeptide chains, approximately 300 nm in length and 1.5 nm in diameter ²⁷. The standard triple helix structure of type I collagen is composed of two α -chains and a β -chain ²⁷.

3.2) Electrospinning of pure collagen

Collagen can be extracted from several tissues and a wide variety of organisms, e.g. mammals, fish, amphibians, and birds ²⁸. For electrospinning, collagen from calf dermis and bovine dominates the literature, followed by collagen of rat origin. To use electrospinning to design collagen scaffolds, generally, some level of denaturation of the collagen is needed to solubilize the collagen and yield a solution able of being electrospun ²⁹. The process of

solubilization is often thought to denature the protein to the extent that the D-banding seen with native collagen type I is lost. This banding is observed at the quaternary structure level, where collagen type I fibrils are obtained by monomers assembled end to end and aligned in a parallel and staggered fashion. This structure is of utmost importance for the mechanical properties of native collagen³⁰. This D-banding was mainly observed via TEM for collagen from Calfskin³¹.

1,1,1,3,3,3-hexafluoropropan-2-ol (HFP) is a widely employed solvent for the electrospinning of collagen fibers³², along with 2,2,2-trifluoroethanol (TFE)^{29, 33}. For instance, solutions of HFP ranging in concentration from 0.03 to 0.10 g/ml produced scaffolds composed of collagen fibers measuring 100 nm to 5 μ m in diameter³⁴ (Figure 4). Some electrospinning conditions are listed in Table 2, which presents the parameters that are important in the maintenance of ultrastructure.

Nevertheless, it has been highlighted that Fluor alcohols can induce a conformational change in native proteins³⁵. Although in early publications electrospinning fibers were reported to have the 67 nm banding typical of native collagen³⁴, some recent studies have shown that most of the triple-helical collagen was apparently lost when electrospun with the discussed Fluor alcohols^{29, 33, 35-36}. Matthews et al. and co-workers optimized the voltage input parameters for type I collagen electrospinning³⁴. When setting the collagen concentration at 0.083 g mL⁻¹ in HFP and varying voltages from 15 to 30 kV, they showed that the most prominent formation of fibers happened at 25 kV with an optimal air gap distance of nearly 125 mm³⁴. Another research group found that an applied voltage of 10 kV, distance of 15 cm and flow rate of 5 mL min⁻¹ is an appropriate condition for pure collagen electrospinning³⁷. Furthermore, the use of Fluor alcohols as the solvent for the electrospinning of collagen has been reported to yield collagen nanofibers that do not swell in an aqueous environment³⁸, but are readily soluble in water, blood or tissue fluids^{32,39}.

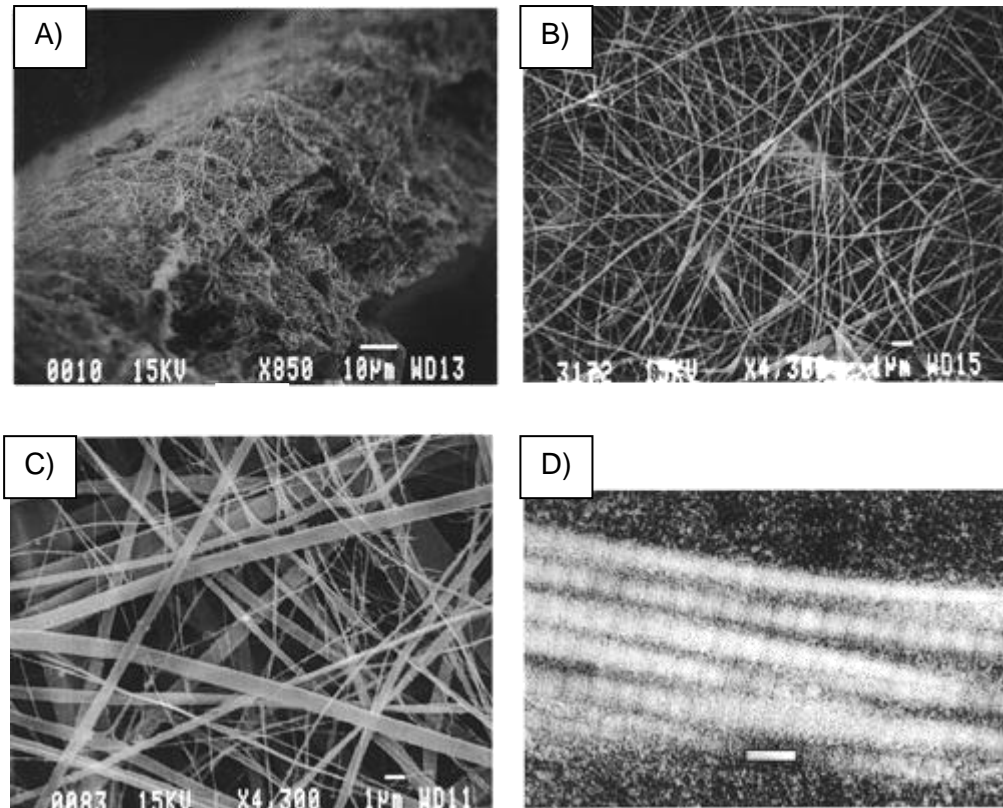


Figure 4. (A) Scanning electron micrograph (SEM) of calfskin type I collagen electrospun onto a static, cylindrical mandrel. Cut edges of the matrix illustrate the porous, three-dimensional nature of the scaffold (magnification 850 \times). (B). Detail SEM of electrospun calfskin type I collagen. Under optimal conditions electroprocessed collagen collects on the ground target mandrel as a complex, non woven matrix of fibrils. Average filament diameter in this type of matrix is 100 ± 40 nm. Our extensive surveys of this material have not revealed evidence of free ends, this observation is consistent with current views that electrospinning can produce a single, continuous polymer fiber¹² (magnification 4300 \times). (C) SEM of electrospun type I collagen isolated from human placenta. The source of collagen used in electrospinning dictates the structural properties of the deposited filaments. Collagen fibers electrospun from human placental type I collagen produce filaments that are less uniform in diameter than the filaments produced with calf skin collagen. Filaments range from 100 to 730 nm in diameter (magnification 4300 \times). (D). TEM of the electrospun type I calfskin collagen. Electroprocessed fibers exhibit the 67 nm banding typical of native collagen (inserted scale bar = 100 nm) Adapted with permission from ³⁴. Copyright [2002] [Biomacromolecules].

Other solvents have been examined as a potential solvent of collagen with the aim of maintaining the original structure. It has been reported that continuous fibers cannot be

spun from pure acidic aqueous solutions of pure collagen; the addition of sodium chloride to the solution may promote the formation of continuous fibers, probably due to the increase in solution conductivity ⁴⁰. Fish-derived collagen type I was solubilized in a 93/7 ratio of glacial acetic acid/dimethyl sulfoxide at 10% weight/volume ⁴¹. At a flow rate 0.60 mL/h, fibers ranging in diameter from 200 nm to 1100 nm were obtained and displayed D-banding ⁴¹. Improvements in structure were achieved by changing the aqueous solvent; nevertheless, some drawbacks were seen. The slower evaporation rate of acidic solutions, in contrast to fluoroalcohols, requires much slower flow rates, which limits the size of the fibers and their collection over time. Furthermore, decreasing porosity and alterations in mechanical behavior can be seen.

Nevertheless, it is important to emphasize the shift towards the use of aqueous collagen solutions for electrospinning. In particular, studies have highlighted the preservation of collagen's higher-order structure using acetic acid-based solutions: this shift from polar solvents such as HFIP to aqueous systems marks an important research direction ⁴².

A few approaches addressing the improvement of the orderliness of electrospun collagen fibers have been reported. For instance, Matthews et al. ³⁴ employed a rotating mandrel as a ground target to collect fibers of collagen. By controlling the mandrel rotation speed, the authors obtained collagen fibers aligned along the axis of rotation. Katta et al. ⁴³ used a macroscopic copper wire-framed rotating drum as the collector, and the electrospun fibers collected on the drum as it rotated were parallel to each other. These approaches allow the fabrication of more or less aligned fibers.

To summarize this section, electrospun pure collagen can be designed to provide a basic matrix for *in vitro* cell culture. Nonetheless, electrospun nanofibers based on pure collagen protein still face many issues, including low stability in water media, poor resistance to collagenase environments, a rapid degradation rate, and potential immunogenic properties ⁴⁴. Furthermore, pure electrospun collagen fibers are denatured easily during the electrospinning process ^{29, 36}.

An alternative approach involves electrospinning blends of collagen and synthetic polymers. Cross-linked fibers and composite or hybrid nanofibers with different compositions (e.g., organic/organic, organic/inorganic) have been developed as

promising collagen-based fibers.

It is worth noting that another alternative involves the coating of collagen on electrospun nanofibers. In this process, the natural structure and function of collagen is generally maintained. Coating with collagen leads to good biocompatibility of the nanofibers and better meets the requirements of tissue engineering.

3.3) Cross-linking of collagen-based electrospun fibers

Covalent cross-linking has been proven to enhance the mechanical, thermal, and biological stability of collagen biomaterials ⁴⁵.

Chemical cross-linking methods use agents such as carbodiimide, glutaraldehyde, genipin, 1,4-butanediol diglycidyl ether and transglutaminase. Glutaraldehyde is widely employed as a chemical cross-linker for collagen-containing electrospun materials because it rapidly generates covalent bonding between lysine residues in the collagen macromolecules. Immersion in dilute glutaraldehyde solutions (0.25%) was enough to induce stability for four weeks in *in vitro* culture followed by implantation for three weeks ^{31b}. Indeed, glutaraldehyde has been shown to introduce a high degree of cross-linking and water-resistance in electrospun collagen fibers ³². However glutaraldehyde can cause toxicity ⁴⁶, which arises from residual and reversible fixation ⁴⁷. 1,6-diisocyanatohexane (HMDI) is another cross-linking material that has been utilized in electrospun protein fibers ⁴⁸. In contrast, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) covalently binds collagen macromolecules but is not incorporated into the collagen structure ⁴⁶ and then can easily be rinsed out, resulting in enhancement of the improvements in scaffold stability while retaining cytocompatibility. Cross-linking with EDC alone or in combination with N-hydroxysuccinimide (NHS) was used in 28% and has been proven to maintain collagen fiber stability *in vitro* ⁴⁹. EDC is a carbodiimide with relatively low cytotoxicity compared to glutaraldehyde; it favors the formation of amide bonds between amino and carboxylic groups on the collagen macromolecules with the benefit of not being part of the resulting linkage ⁵⁰. Comparison of the cross-linking of electrospun collagen involving glutaraldehyde immersion (25% for 24 h) and EDC-NHS (20 mM EDC and 10 mM NHS in 90% ethanol for 24 h) revealed a large increase in scaffold hydration with EDC-NHS cross-linking; nevertheless, all cross-linked scaffolds were able to prevent rapid degradation

of the scaffold and support cell adhesion and proliferation ^{31a}.

To tackle the problems associated with partial dissolution of the scaffolds while immersed in the cross-linking solution, vapor-based and physical cross-linking methods have been used. Glutaraldehyde vapor is widely used as a cross-linker and has been shown to lead to an increase in collagen fiber biostability in in vitro culture for up to four weeks ⁵¹. However, concerns about unreleased aldehydes continue to exist.

Natural cross-linking agents present benefits because of their low cytotoxicity e.g. genipin ⁵², D,L-glyceraldehyde ⁵³ or even transglutaminases ^{45b, 54}. Physical cross-linking approaches, such as microwave and ultraviolet irradiation (UV) [20,54,71], argon laser irradiation ⁵⁵, photo-oxidation ⁵⁶ and dehydrothermal treatment (DHT) ⁵⁷ are effective ways to improve scaffold stability and mechanics without exposure to potentially cytotoxic agents. Such cross-linking is often considered to be advantageous because it does not require that the fibers come into contact with solvents and, thus, can be effective under solid-state conditions. For instance, UV treatment of electrospun collagen fibers was found to impart stability in in vitro cell culture for up to 28 days ⁵⁸. In another study, UV treatment was found to slightly improve cross-linking and thermal stability compared to no treatment ⁴⁷. As another example, dehydrothermal treatment of collagen fibers for 24 h has shown excellent preservation of fiber diameter after hydration, and increased its resistance to degradation in an aqueous medium and under collagenase load in comparison with no cross-linking ⁵⁷. While it did not achieve the level of crosslinking achieved by chemical cross-linkers, it seems that physical cross-linking results in better-preserved fiber morphology and porosity ⁴⁷. Note that physical cross-linkers can be combined with chemical cross-linkers, which results in significant scaffold improvements ⁵⁷. For instance, while the separate use of DHT and EDC cross-linkers significantly decreased electrospun collagen scaffold degradation in an aqueous medium and enhanced its mechanics, the combined treatment of these two cross-linkers further improved resistance to degradation and ultimate tensile strength ⁵⁷.

3.4) Electrospinning of collagen with synthetic polymer blends

Collagen fibers may lose its physiochemical properties from the hierarchical structure passing through two stages: (1) in isolation and extraction and (ii) in processing ⁵⁹. Therefore, collagen fibers

basically represent poor mechanical properties and thermal and water instability. Accordingly, polymer/nanofiller additives have been evidenced to be important in enhancing the physicochemical properties of collagen fibers ⁶⁰. Typically, the presence of additives in the collagen-based polymer matrix serves to reinforce the mechanical behavior of the composite nanofibers. PCL, poly(3-hydroxybutyrate-co-3-hydroxy valerate) and Poly(glycolic acid) (PGA) are examples of synthetic polymers that have been incorporated with collagen fibers as a reinforcing agent for tissue engineering ⁶¹.

Another approach for enhancing the mechanical stability of an electrospun collagen fiber involves incorporating biodegradable polymers into the structure to impart strength to the fiber while maintaining a high level of bioactivity. Note that electrospinning of a blended collagen mixture is much simpler because it prevents the slow mass transfer process and also implies lesser amounts of chemical reagents.

Several collagen blends such as poly(lactide-*co*-glycolide) acid (PLGA)/collagen ⁶², polycaprolactone (PCL)/collagen ⁶³, poly-(L-lactide) (PLLA)/collagen ³⁷, collagen/PEO ⁴⁰ or polydioxanone (PDO)/collagen ⁶⁴ have been fabricated by electrospinning and used for cell culture. The benefits of the resulting blends are evident in the improvement of resistance to water and collagenase, and their biocompatibility is the same as that of pure collagen fibers.

For instance, Venugopal *et al.* ⁶³ used PCL/collagen to create nanofibers with a diameter of 210–225 nm. The tensile modulus of the resulting structures was 18 MPa, with a tensile strength of 7.79 MPa, which is highly appropriate for a blood vessel conduit. Another example is that electrospun scaffolds of PDO/collagen ⁶⁴ have been successfully designed and when hydrated, exhibited mean peak stresses between 4.6 and 6.7 MPa, mean tangential moduli between 7.6 and 18.0 MPa, and mean strain to failure values between 56.5% and 186.4%.

A variety of other additives than polymers have also been proposed for increasing the physicochemical properties of collagen fibers including clays, synthetic silicate nanoparticles, hydroxyapatite and carbon nanofillers ⁶⁵. In general, composite fibers can be prepared mostly through three processes including blending ⁶⁶, coaxial spinning⁶⁷, and fiber mixing and multi-layering⁶⁸.

For instance, Ghorbani *et al.* fabricated electrospun collagen/PCL/Zein (a protein) hybrid nanofibers

co-loaded with aloe vera and zinc oxide nanoparticles for wound dressing purpose ⁶⁹. The mechanical performance of the designed nanofibers includes increased tensile strength. The in vitro biodegradation studies demonstrated weight loss of over 30% of the initial weight for the Zein/PCL nanofibers with a ratio of 90:10, 42% for Zein/PCL nanofibers with a ratio of 80:20, and 54% for the Zein/PCL nanofibers with a ratio of 70:30, showing that the high amount of PCL increased the rate of biodegradation. The loading of bioactive agents (aloe vera and zinc oxide nanoparticles) improved biocompatibility and antibacterial activity making these scaffolds ideal for wound dressings. The collagen-based nanofibers loaded with Ag particles were formulated by Rath *et al.* and the nanofibers demonstrated excellent antibacterial activity against *S. aureus* and *Pseudomonas aeruginosa*, known wound pathogens ⁷⁰.

3.5) Biomedical applications of electrospun collagen

Collagen fibers have unique structural properties of major interest for tissue engineering because they transmit forces, dissipate energy, avoid premature mechanical failure and lead to biological signals to adjacent cells. Additionally, collagen-based fibers are resorbable, present high water affinity, low antigenicity, good cell compatibility and the ability to enhance tissue regeneration ⁷¹. All the cited criteria make collagen-based fibers ideal biopolymers for tissue engineering applications.

For instance, Shih *et al.* ⁷² reported that cells grown on type I collagen fibers had significantly higher cell viability than a tissue culture on the polystyrene control.

Other examples of biomedical applications of collagen-based fibers are presented in **Table 4**.

Table 4: Examples of biomedical applications of collagen electrospun nanofibers

Composition	Solvent for the ES	Diameter of fiber (nm)	Biomedical applications
Collagen/PLGA	HFP	50–500	Bone tissue scaffolds ⁷³
Collagen/PHBV	HIFP	300–600	Scaffold for tissue engineering ⁷⁴
Collagen/PCL	HFP	210–225	Vascular tissue engineering ⁶³
Collagen/PLLA	HFIP	1290–1560	Tissue engineering ⁷⁵
Collagen/TPU	HFP	700–800	Tissue engineering and functional biomaterials ⁷⁶

Collagen/alginate/chitosan/hydroxyapatite	Ethanol/Glycerol	300–800	Scaffold for regenerating bone tissue ⁷⁷
Collagen/PHBV/GO	TFE	400–500	Wound coverage material ⁷⁸
Collagen/chitosan	HFP	434–691	Vascular and nerve tissue engineering ⁷⁹
Collagen/elastin	HFP	110–1120	Cardiovascular tissue engineering ⁵¹
Collagen/PEO	Aqueous	100–150	Wound dressings and tissue engineering ⁴⁰
Collagen/PLLA-CL	HFP	100–200	Vascular tissue engineering ⁸⁰

ES: electrospinning; PLGA: poly(lactic-co-glycolic acid); PHBV: poly(3-hydroxybutyrate-co-3-hydroxyvalerate); PCL: poly(ϵ -caprolactone); PLLA: poly(L-lactic acid); TPU: thermoplastic polyurethane; GO: graphene oxide; PEO: poly(ethylene oxide); PLLA-CL: poly(L-lactic acid-co- ϵ -caprolactone)

Another possible alternative to collagen fiber is the electrospinning of nanofibers employing gelatin directly. Despite the fact that gelatin is a degradation material of collagen, electrospun gelatin fibers present the same biocompatibility as does collagen. Moreover, collagen presents limited immunogenicity and antigenicity, resulting from the structures of polypeptide of the three spiral chains and the molecules in the central regions. This limited antigenicity has significantly decreased the exploitation of collagen in biological fields, reducing its use in nanofiber design, unlike gelatin, which presents good biodegradability and non-antigenicity besides its low mechanical strength.

4) Gelatin and gelatin-based fibers

4.1) Brief definition of gelatin

Gelatin is a derivative of collagen, obtained by denaturing the triple-helix structure of collagen²⁶. More precisely, gelatin is a fibrous protein comprised of a sequence of amino acids obtained from collagen via hydrolysis or denaturing of collagen that induces it to lose the original α -triple helix conformation by breaking intermolecular bonds. Two types of gelatin exist and are extracted from collagenous tissue: type A, treated by an acidic pretreatment, and type B, treated by an alkaline pretreatment. Gelatin is thus a polypeptide

mixture in which about 20 amino acids are bound together through peptide bonds. The polypeptide gelatins have a molecular weight and thus length conditioned by a variety of variables, as the pretreatment and the parameters of the hydrolytic stage, e.g. temperature, pH and time ⁸¹. Gelatin is thus a highly water-soluble biopolymer and is a polyelectrolyte polymer that has many ionizable groups. Gelatin presents the advantages of being low-cost compared to collagen, exhibits good biocompatibility and biodegradability, and is non-immunogenic.

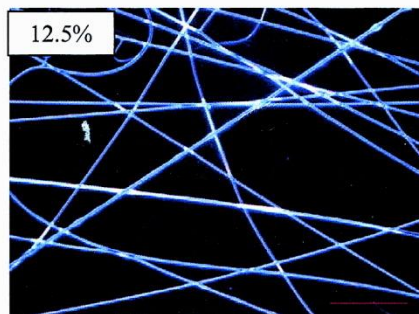
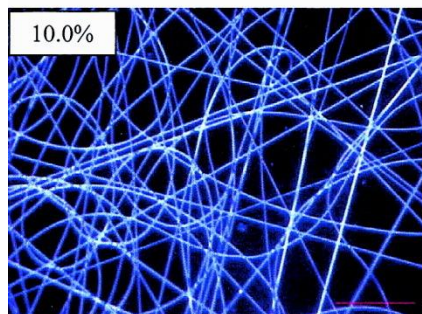
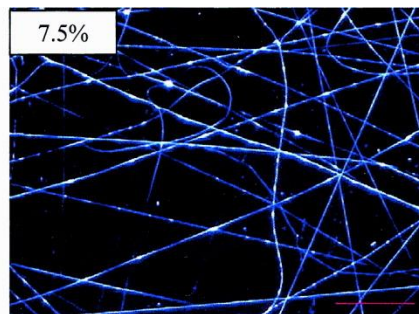
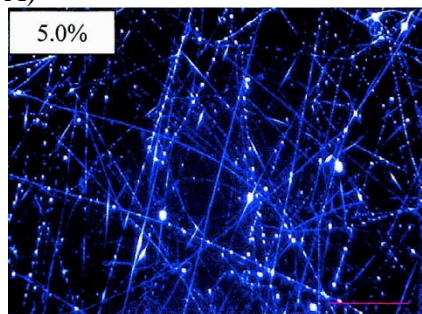
4.2) Electrospinning of pure gelatin

Gelatin is electrospinnable only from solutions in which this macromolecule has a random coil conformation. Gelation occurs in aqueous solutions below 30 °C, making it impossible to electrospin at room temperature. Furthermore, the high surface tension of aqueous solutions complicates electrospinning because of the destabilization of polymer jets and droplet formation. Water has a high boiling temperature, which leads to an additional problem, i.e. incomplete water evaporation before reaching the collector. This results in the fusion of fibers and heterogeneity. For instance, Lu et al. utilized water as a solvent to electrospin a pure gelatin solution. They found that at low concentrations of gelatin, the viscosity was insufficient to generate continuous nanofibers, resulting in the formation of unwanted microbeads. However, at a high concentration of gelatin (>25%), the spinning solution became too viscous, which seriously inhibited efficient electrospinning, leading fewer nanofibers ⁸².

In this sense, the use of organic solvents is crucial. Aside from toxic organic solvents (i.e. 2,2,2-trifluoroethanol (TFE), trifluoroacetic acid (TFA) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)), an effective way to obtain gelatin electrospinning solutions (Figure 5) is to use acidic solvents such as aqueous solutions of acetic acid or formic acid ⁸³. For instance, pure gelatin scaffolds (8% w/v) were produced with fiber diameters of 800 nm, tensile strength of 5.7 MPa, modulus of 499 MPa, and an elongation of 96% ⁸⁴. Songchotikunpan *et al.* ⁸⁵ fabricated gelatin fibers from gelatin extracted from Nile tilapia; these fibers were electrospun from an acetic acid solvent or a formic acid solvent. The resulting materials present fiber diameters ranging from 161 to 761 nm for acetic acid and 109 to 302 nm for formic acid. Mechanical properties of the gelatin fiber mats included a tensile

strength of 2.4–4.2 MPa, Young's modulus of 118–194 MPa, and an elongation of 3.3–37.6%.

A)



B)

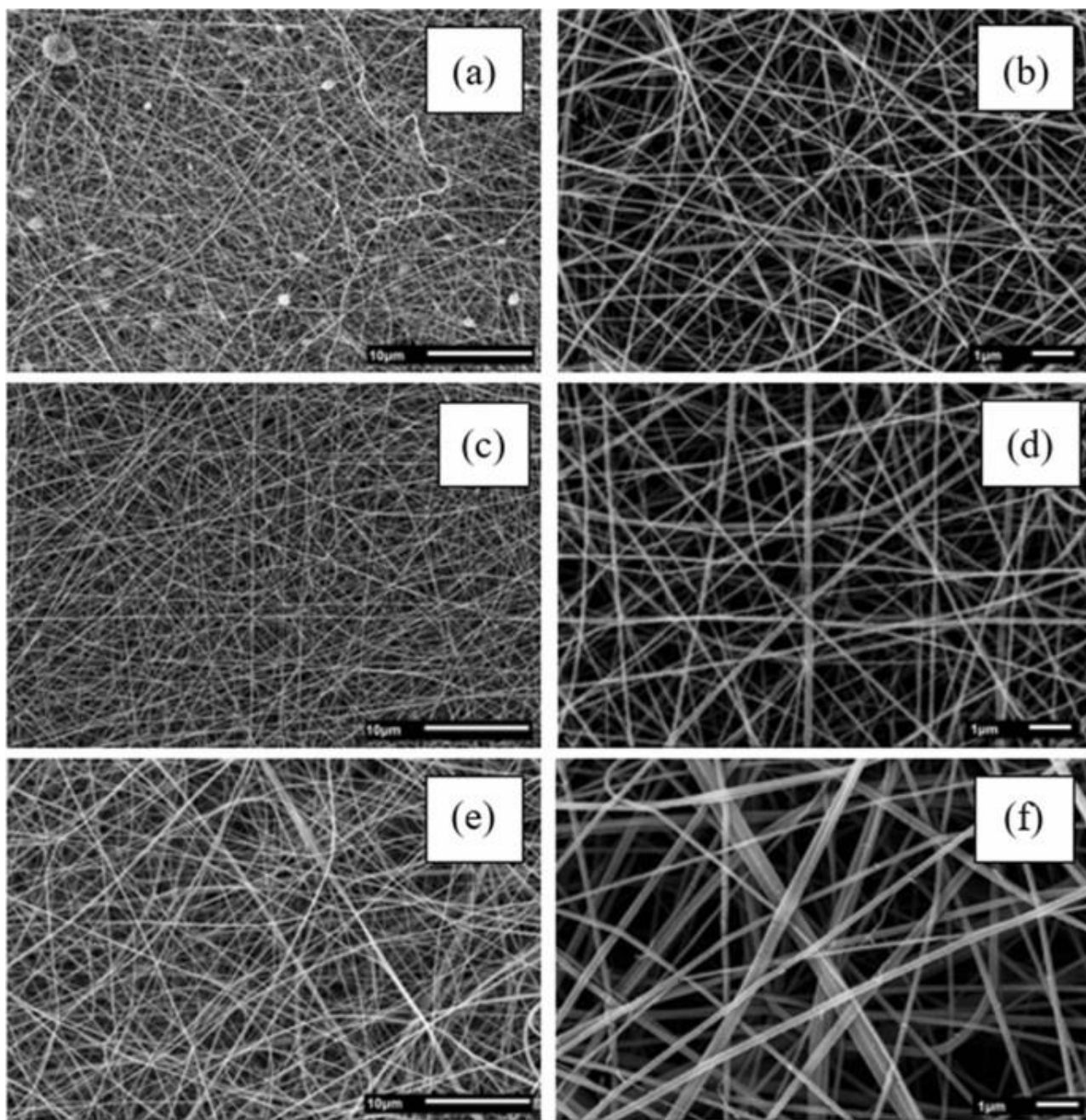


Figure 5. A) Optical microscope images of electrospun gelatin at different concentrations ⁸⁶. Reproduced with permission from ⁸⁶. Copyright [2005] [Wiley Periodicals, Inc.]. B) Scanning electron microscope images of bovine gelatin fibers: 20% [(a) $\times 2500$; (b) $\times 10,000$]; 25% [(c) $\times 2500$; (d) $\times 10,000$]; 30% [(e) $\times 2500$; (f) $\times 10,000$] Reproduced with permission from ⁸⁷ Copyright [2023] [Sci Rep]

4.3) Cross-linking of gelatin-based electrospun fibers

For biomedicine purposes, electrospun gelatin nanofibers are of limited use because of their rapid degradation, poor mechanical strength, and complete dissolution in aqueous media. Thus, these fibers need to be cross-linked to reduce their water solubility, to enhance the thermo-mechanical efficiency of the processed nanofibers and to maintain the integrity of the fabricated scaffolds in aqueous media⁸⁸.

Cross-linking of gelatin fibers can be done via either chemical processes or an enzymatic approach (Figures 6, 7).

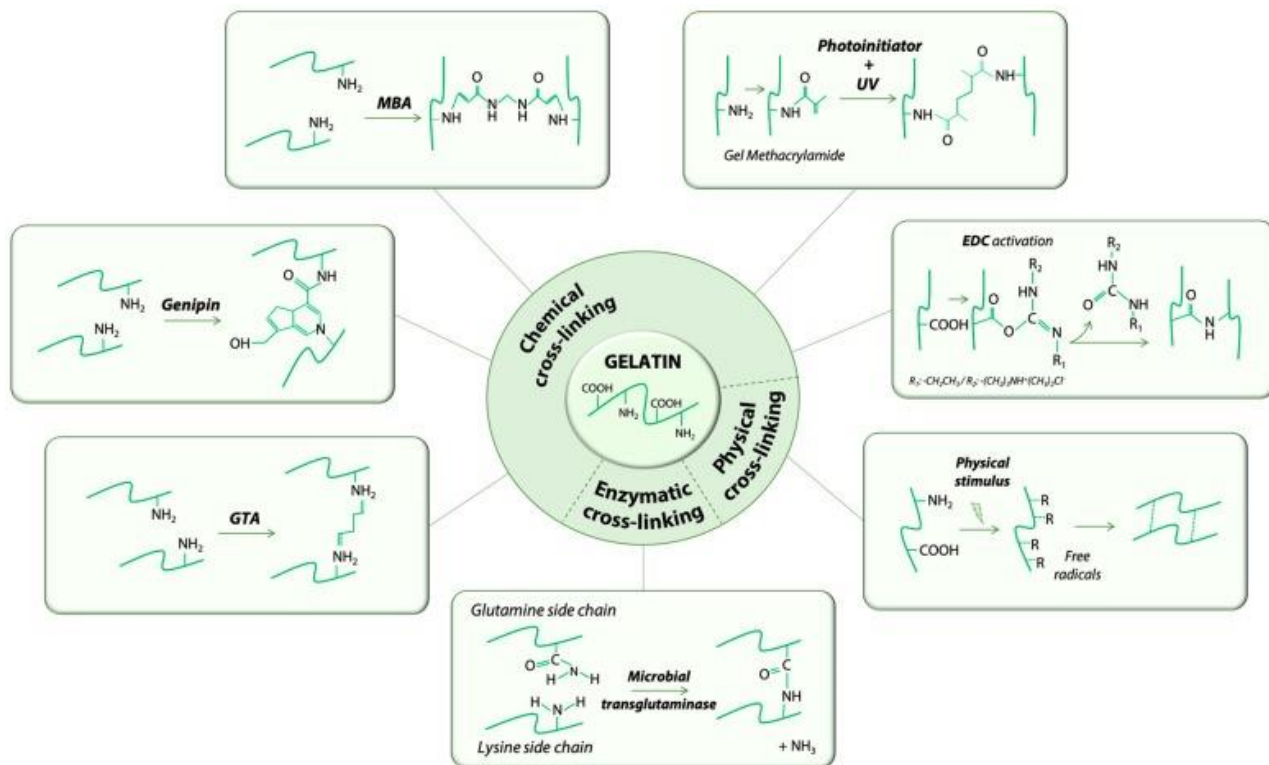


Figure 6. Different cross-linking processes for gelatin scaffolds⁸⁹ Reproduced with permission from⁸⁹. Copyright [2019] [Materials (Basel)].

Chemical cross-linking involves specific cross-linking agents, e.g. 1-ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride or glutaraldehyde⁹⁰.

For example, Zhang et al. showed that the treatment of electrospun pristine gelatin nanofibers with saturated GTA vapor for 2 h considerably increased the mechanical properties of the pristine

gelatin nanofiber matrix ⁸⁸. Electrospun gelatin nanofibers were immersed in 0.2 L of ethanol/water [90/10 (v/v)] containing an aqueous solution of EDC/NHS (5:2) for 24 h at 4 °C ⁹¹. Improved mechanical properties were observed, with an increase in tensile strength from 1.11 MPa to 2.44 MPa and tensile modulus from 9 MPa to 156 MPa, and reduced degradation rate. In another study, Lu *et al.* ⁹² prepared gelatin fibers via the electrospinning process and then cross-linked the resulting fibers using liquid and vapor phase GTA. They reported that the tensile strength of gelatin nanofibers increased from 1.33 to 2.6 MPa after GTA vapor cross-linking and from 1.33 to 5.08 MPa after liquid phase cross-linking. Research suggests that gelatin cross-linked fibers with glyceraldehyde are relatively well tolerated in vivo ⁵³.

Natural cross-linkers such as genipin or enzymes can also be used. For example, after cross-linking with genipin [0.5% (w/w)], gelatin electrospun fiber had better biochemical properties and better biocompatibility ⁹³. It was demonstrated that genipin cross-linked gelatin is around 10 000 times less cytotoxic than glutaraldehyde cross-linked gelatin ⁹⁴. As another example, gelatin scaffolds treated with microbial transglutaminases as the cross-linker have been reported to present improvements in cell adhesion and proliferation. ^{45b}

It is noteworthy that the general issue of chemical cross-linking of gelatin electrospun fibers is related to variation in the original morphology and toxicity due to the presence of unreacted cross-linkers. In this sense, it could be interesting to treat the gelatin fibers with “physical” cross-linking, including ultraviolet (UV) treatment, dehydrothermal (DHT) treatment, or plasma treatment. Such physical processes often yield a low level of cross-linking, as the procedure mainly takes place on the fiber surface. For example, Zheng *et al.* ⁹⁵ reported that irradiation of gelatin-based fiber with UV had no significant influence on its physicochemical or mechanical properties. It deserves noting that plasma treatment also provides an interest in improving surface hydrophilicity, biomolecules absorption (e.g. proteins), and cell attraction to porous electrospun scaffold. Yet, there are not many studies on the increase in the resulting bioactivity of nanofibers. ⁹⁶

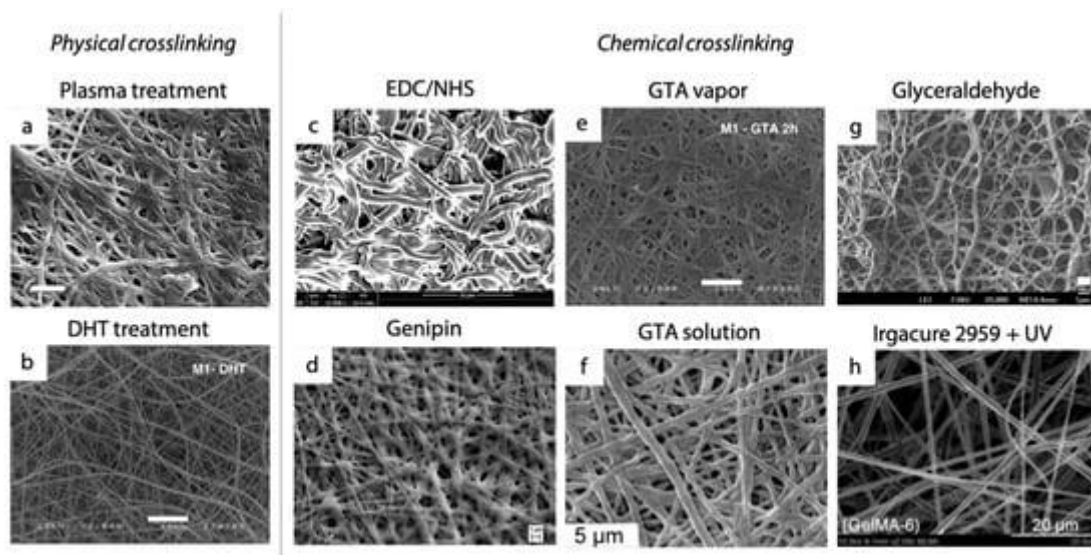


Figure 7. Morphology of electrospun gelatin nanofibers cross-linked with different strategies: (a) Plasma treatment (scale bar: 5 μm) (b) dehydrothermal treatment (scale bar: 10 μm), (c) EDC/NHS (scale bar = 10 μm), (d) genipin (scale bar: 1 μm), (e) glutaraldehyde vapor (scale bar: 10 μm), (f) glutaraldehyde solution (scale bar: 5 μm), (g) glyceraldehyde (scale bar: 1 μm), and (h) Irgacure 2959 with UV treatment (scale bar: 5 μm).⁸⁹ Reproduced with permission from⁸⁹. Copyright [2019] [Materials (Basel)].

4.4) Electrospinning of gelatin with synthetic polymer blends

Gelatin can be paired with synthetic polymers, such as poly(vinyl alcohol) (PVA), poly(ϵ -caprolactone) (PCL) and poly(3-hydroxybutyrate) (PHB), to improve the electrospinning potential⁹⁷. The diameter of gelatin-blend nanofibers is conditioned by the composition of the mixture^{97a}. The diameter of gelatin/PVA fibers is decreased by increasing the gelatin concentration, resulting in an improvement in fiber strength⁹⁸. Gelatin/PCL nanofibers present greater flexibility but with poor strength. These nanofibers have greater elongation and energy tension breakage than that of pure gelatin fibers⁸⁶. Gelatin (5%, 7% and 10% w/v) electrospun with PCL (1%, 7%, 5%, and 10% w/v) generates scaffolds that do not need post-fabrication cross-linking. Fiber sizes had diameters ranging from 640 nm to 880 nm depending on the amount of gelatin and PCL (i.e. an increase in gelatin and PCL content resulted in higher fiber diameters).

Except for the mixing of gelatin and single biopolymers, a few studies have employed two, or more than two, additives to combination with gelatin to generate gelatin-based composite nanofibers *via* electrospinning. Goudarzi *et al.* incorporated acetylated cellulose nanofibers (ACNFs) into electrospun gelatin-based nanofibers (mix of gelatin and PCL) and evidenced that the ACNFs loaded gelatin-based nanofiber mats presented enhanced ultimate strength than gelatin-based nanofiber mats (4.3 ± 0.1 MPa vs. 2.5 ± 0.1 MPa)⁹⁹. Massoumi *et al.* loaded copper or zinc ions into halloysite nanotubes, which were then loaded into gelatin/sericin nanofibers by blend electrospinning¹⁰⁰. The resulting fiber exhibited good bactericidal activity. In the work from Cai *et al.*, Fe₃O₄ nanoparticles were loaded into electrospun gelatin/chitosan nanofibers¹⁰¹. The nanoparticle-loaded gelatin/chitosan nanofiber mats were shown to present 155% increase in Young's modulus and 128% augment of ultimate strength compared to the gelatin/CS nanofiber mats. The antibacterial activity was also notably increased due to the encapsulation of Fe₃O₄ nanoparticles. The existing research also reported the design and development of electrospun ternary nanofiber mats with different multifunctional performances for wound dressing applications. Most particle¹⁰²s are introduced into the gelatin solution and then electrospun such as copper oxide nanoparticle/gelatin/PCL nanofiber mats¹⁰³, ferric oxide/gelatin/glycerol nanofiber mats¹⁰⁴, cerium oxide nanoparticle/gelatin/PCL nanofiber mats¹⁰⁵, tellurium nanoparticle/gelatin/PCL nanofiber mats¹⁰⁶, silver nanoparticle/gelatin/PVA nanofiber mats¹⁰⁷, and halloysite nanotube/gelatin/PCL nanofiber mats¹⁰⁸.

4.5) Biomedical applications of electrospun gelatin

In tissue engineering, gelatin-based fibers can also successfully be fabricated as scaffolds by electrospinning methods¹⁰⁹. A few examples are listed in Table 5 below.

Table 5. Examples of biomedical application of gelatin electrospun nanofibers

Composition	Solvent for the ES	Diameter of fiber (nm)	Biomedical applications
Gelatin/PCL	TFE	2790–4630	Tissue engineering ¹¹⁰
Gelatin/zein	Acetic acid	380.3–695.5	Bioactive delivery in food industry ¹¹¹
Gelatin/chitosan	Acetic acid	202 ± 13.4	Drug release ¹¹²
Gelatin/PANi	HFP	61 ± 13–803 ± 121	Biocompatible scaffolds for tissue engineering ^{50b}
Gelatin/PLLA	Aqueous acetic acid solution	86–148	Wound dressing ¹¹³
Gelatin/PCL	TFE	160–232	Neural tissue engineering ¹¹⁴
Gelatin/PCL	HFP TFE	640–880 50–1000	Cardiovascular tissue engineering ^{51, 86}

ES: electrospinning; PCL: poly(ϵ -caprolactone); PANi: polyaniline; PLLA: poly(L-lactic acid)

Note that targeted mechanical properties for scaffolds strongly depends on the tissue targeted, e.g. myocardial tissue is characterized by left ventricle stiffness ranging from 20–50 N/cm² (200–500 kPa) during systole and 1–2 N/cm² (10–20 kPa) during diastole ¹¹⁵, optimal heart patches should have a stiffness ranging from several tens of kPa (starting from 1 N/cm²) to near 1 MPa (up to 100 N/cm²) ¹¹⁵.

5) Comparison of collagen-based fibers vs. gelatin-based fibers in biomedical applications

In this section, we compare the performance of collagen versus gelatin fibers in biomedical applications. Telemeco *et al.* ¹¹⁶ showed differences in several morphological characteristics between fibers of electrospun collagen and gelatin. First of all, the pore size of electrospun gelatin material appears to be smaller (around 2000–6000 nm²) than the average pore size of electrospun collagen (around 1500–4000 nm²). Secondly, electrophoretic analysis has demonstrated that electrospun gelatin material is constituted of fragments of type I collagen.

Compared with electrospun collagen fibers, it appears to be constituted of intact monomers. The *in vivo* performance of the two structures (i.e. collagen vs. gelatin) can also differ. Electrospun collagen type I and gelatin were implanted into rat stomach and after 7 days were recovered for microscopic observation. The electrospun collagen had i) fully infiltrated the interstitial cells and was well integrated with the surrounding muscle tissue and ii) showed no fibrotic encapsulation, with a smooth continuum of cells from the host tissue into the electrospun collagen. In contrast, electrospun gelatin was found to delaminate from the host tissue after 7 days. Microscopic observation demonstrated that these implants i) developed fibrotic capsules, ii) were infiltrated poorly with the interstitial cells, and iii) were infiltrated by lymphocytes¹¹⁶.

6) Conclusion and Outlook

Collagen and gelatin are natural macromolecules that present high biocompatibility, biodegradability and low immunogenicity. In this sense, developing the processes needed to produce materials based on these biopolymers is of major interest. The rapid evolution of electrospinning techniques is well suited to meet this need, as this technique enables fabricating advanced functional nanofibrous scaffolds as discussed in this review. The resulting fibers present three-dimensional pore structures, which can imitate the microstructure of the extracellular matrix.

The organic solvent utilized for the electrospinning process results in potential toxicity issues, so the development of strategies that allow electrospinning in aqueous media is highly desirable. As discussed in this review, current problems with collagen- or gelatin-based fibers have mainly been resolved; these include low efficiency and low mechanical strength.

From our point of view for maintaining proper support and stability during the healing process, the degeneration rate must be balanced. Moreover, collagen or gelatin derived from animal collagen may induce immunological reactions in a certain group of individuals. We believe that developing collagen- or gelatin-based products with lower immunogenicity or examining alternate gelatin/collagen sources can help alleviate this issue. Furthermore, we are convinced that improving some characteristics of collagen- or gelatin-based nanocomposites as bioactive

and osteoconductive can greatly help the implant integrate with the surrounding bone tissue, in other word to enhance cell adhesion and proliferation. Finally, we think that large-scale manufacture of collagen- or gelatin-based nanocomposites at a reasonable cost is needed for their broad use in tissue engineering. It is critical to make significant effort to develop cost-effective manufacturing procedures and optimize production scalability based on electrospinning process. In future, efforts should be made i) to enhance the reproducibility of spinning, ii) improve spinneret design, iii) optimize the collecting device, and iv) improve solution delivery methods.

Challenges also remain in terms of improving our understanding the mechanisms by which nanofiber materials impact cell behavior and processes of tissue regeneration. Physiological and mechano-transductory signals need to be examined in more detail to optimize electrospun scaffold material in terms of composition and structure.

If these issues can be tackled, it is anticipated that collagen- or gelatin-derived fibrous materials will have a major place in biomedical and biotechnological applications.

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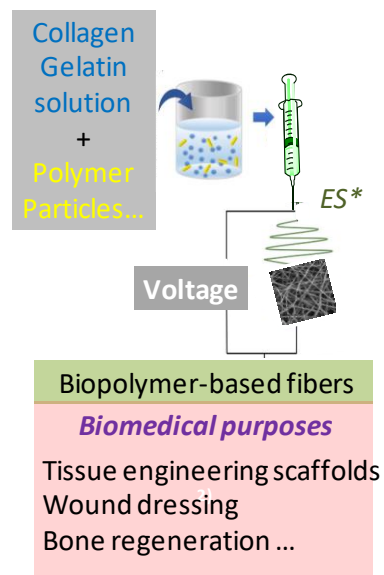
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TOC Graphic



* ES = electrospinning process