Collagen-Based Scaffolds for Bone Regeneration

Subjects: Engineering, Biomedical

Contributor: Yashan Feng , Yanhong Shi , Yafang Tian , Yongxin Yang , Jun Wang , Haiwei Guo , Seyedeh Nooshin Banitaba , Sanaz Khademolqorani , Jing'an Li

Orthopedics has been identified as a major clinical medicine branch since the 18th century for musculoskeletal disease diagnosis and therapeutics. Along with technological progress, the surgical treatment of bone disorders became available in the 19th century, while its growth faced several obstacles due to a lack of proper biocompatible material and alternative structures. Therefore, tissue engineering has emerged as a key building block to overcome these challenges, providing the capability for bone growth, and fabricating scaffolds with enriched desirable cellular compatibility as well as mechanical properties. Among various structures, the electrospun layer has implied high porosity and fine pore sizes, and succeeded in cell growth and proliferation. Collagen nanofibers have represented a wide potential for mineralization, bone regeneration, and forming processes. Despite this, such scaffolds have accosted bone remodeling limitations due to inadequate osteoinductivity and mechanical strength. Hence, the tendency to fabricate efficient collagen-based nanofibrous layers enriched with organic and inorganic materials has been extensively declared. Embedding these materials leads to engineering a membrane with appropriate physical, degradability, and mechanical properties, as well as proper mineralization and biological activity required for better replicating the bone organ's natural microenvironment.

collagen bone regeneration electrospun collagen fibers electrospinning inorganic additives organic materials

1. Introduction

Collagen is assumed as a major constituent of the extracellular matrix (ECM) in both hard and soft tissues, forming 25 to 33% of the body's protein mass in mammals. Generally, 28 collagen types have been recognized so far, disseminating in various tissues, such as bone, teeth, tendon, skin, and so forth. Collagens could be synthesized through different body cells based on their localizations. As an example, in the connective tissues, fibroblast cells, as well as bone osteoblast cells support collagen production. Along with the structural characteristics of collagen, proper hemostatic capacity, low iminium genicity, and appropriate dimensional stability are considered their unique features [1][2][3][4].

According to the literature, a different hierarchical conformation of collagen has been identified, including amino acid-triplet, alfa-helix, triple-helix, and collagen fibrils. Moreover, three types of collagens are the most common

among the collagen family, differing in the size and length of the helix. Type I collagen is recognized as the most vital family member, which could be found in skin, tendons, and bone. Additionally, collagen type II is commonly extracted from cartilage, while type III is frequent in the vascular system [5][6][7][8]. The above-mentioned characteristics of collagen and its great compatibility with the body have resulted in promising applications in biomedical and tissue engineering fields, containing cosmetic surgeries, corneal bandages, contact lenses, implants, sutures, dental composites, and drug delivery systems [9][10].

As a critical organ, bone provides fate-bearing sustainment, motion assistance, and physical protection of the brain and heart. Meanwhile, the crucial roles of bones could be adversely influenced by injuries, fractures, diseases, accidents, and functional disorders. Correspondingly, various treatment strategies have been introduced, in which employing implants is considered a major great solution for bone tissue function [11]. In this era, material selection, matrix fabrication, and biological interactions are three factors affecting the final efficiency of engineered implants [12]. Regarding the material parameter, extracted biopolymers from natural resources, such as collagen, chitosan, alginate, silk, cellulose, chitin, agar, starch, and lignin, as well as their derivatives (e.g., gelatin) are the most applicable substances through revealing proper biocompatibility, biodegradability, and great cell response in bone tissue engineering.

2. Bone Regeneration Techniques

The treatment method for bone defects is divided into major bone grafting and transplant strategies, in which transplants include autograft, allograft, and xenograft [13]. Bone graft is known as an implanted material in the body, resulting in osteogenesis, osteoinduction, and osteoconduction [14]. In the autograft method, an iliac or fibular bone graft from the patient's body is used, causing several disadvantages, such as essential secondary surgery, donor limitation, high cost, infection, and pain [13][15]. Allograft also applies fresh or transplanted tissue from another person, possibly facing some difficulties, including the rejection or transmission of diseases, infections (e.g., HIV), and high cost [13]. Meanwhile, xenograft is a bone graft from the body of other organisms (e.g., animals), suffering from the transmission of common diseases between animals and humans, immune response, and poor outcomes [14]. As a result, many efforts are looking for suitable alternatives to fix defects related to bone. Material selection, matrix design, and biological interactions are key factors toward approaching an ideal structure for bone regeneration. Inorganic and organic biomaterials are the main substances used for mimicking the structural characteristics of natural bone tissues.

Tissue engineering knowledge is aimed at providing an appropriate scaffold that can mimic the natural bone environment to respond to bone damage. In this case, the designed scaffolds are expected to have sufficient mechanical strength and porous architecture. These features ameliorate cell migration and differentiation to scaffolds [16]. Various biomaterials have been utilized as scaffolds for bone tissue engineering. Among them, the combination of type I collagen and different types of bioceramics, such as hydroxyapatite (HA), are frequently suggested. This could be linked with the point that natural bones are mostly assembled from type I collagen with a tiny amount of type V to form a network with nanosized bioceramics crystals [2][17]. The HA bioceramic has received extensive attention in bone tissue engineering due to its excellent features, such as biocompatibility,

osteoinductivity, and osteoconductivity. Therefore, in the design of suitable networks for bone reconstruction, benefiting from the synergistic effect of collagen along with additives could be a valuable strategy [17].

Besides material type and synthesis process in the determination of biomaterial characteristics, fabrication methods have a key role in morphological, physiochemical, mechanical, and biological features. In 1988, Dahlin et al. used a physical substrate for bone tissue regeneration for the first time [18][19]. Then, different configurations, including hydrogels, films, sponges, micro- and nano-spheres, and electrospun layers were declared as other promising approaches for bone tissue engineering. Among the mentioned fabrication techniques, cross-linking, 3D printing, and electric-field techniques have caught many interests, leading to the formation of hydrogels, proper membranes, and nanofibrous structures, respectively.

The cross-linking procedure could successfully generate various hydrogel configurations. Hydrogels are hydrophilic polymeric materials, capable of holding large amounts of water in their three-dimensional structure. In other words, they are formed by the interaction of one or more monomers that produce a cross-linked network structure with the ability to swell while not being dissolved in water [20]. Collagen could be prepared in hydrogel forms for use in bone tissue engineering. Sotome et al. [21][22] produced a hydrogel composed of collagen/HA and alginate that is automatically cross-linked by the simultaneous titration coprecipitation method in 30 min. The proposed hydrogel was also employed as a carrier of recombinant human bone morphogenetic protein 2 (rh-BMP2), resulting in successful implantation after 5 weeks without structural deformation. Wang et al. [23] developed chitosan and collagen hydrogels with different ratios using beta-glycerophosphate as an initiator, loaded by adult human bone marrow-derived stem cells. The presence of collagen in the hydrogel increased cell expansion and proliferation, the hydrogel density, and stiffness.

Another technique used to fabricate collagen structures is 3D printing. For the first time in 1980, 3D printer technology was used to produce segments of different sizes, shapes, and materials. It is also able to synthesize porous scaffolds with layer-by-layer techniques, which are suitable for tissue applications, especially for bone tissue engineering [17]. Kim et al. [18] designed a 3D-printed polycaprolactone (PCL) scaffold reinforced with different percentages of carbonated HA coated with marine atelocollagen (MC). The obtained results of the culturing of MC3T3-E1 cells on the 10% CHA/MC/PCL scaffold had a 1044% higher osteogenic differentiation than the pure PCL. Diogo et al. [14] also designed and fabricated a 3D printable cell-laden hydrogel, consisting of blue shark (Prionace glauca (PG))/Collagen/HA/alginate. The presence of collagen in the scaffold made the fibroblast cell line viable during and after successful printing.

Electrospinning is a fabrication technique, in which a polymer solution or melt is stretched in an electrostatic field, resulting in the assembling of fibers with distinct diameters onto a conductive collector. This simple procedure could mimic the porous structure of natural ECM due to the production of a large surface area and highly porous substrate with multi-scale fibers [19][20]. Electrospraying is another method that has been investigated for bone tissue engineering, in which droplets are created instead of fibers [21]. This route is similar to electrospinning, and so can be adjusted by manipulating the electrospinning conditions, such as voltage, polymer concentration, and syringe-to-collector distance. Based on the literature, bioceramic nanoparticles are able to coat a layer over the

electrospun membranes via the electrospray procedure. Benefiting from the electrospinning procedure, Chen et al. [15] depicted that the electrospun PCL/collagen fibers incorporating 5% cerium/HA could result in uniform fibers with an average diameter of 396 nm, as well as a 30-times-greater corrosion rate than the bare Mg alloy substrate.

Electrophoretic deposition is supposed to be another fabrication technique in the electric field category, which is utilized for thick-film coating. Accordingly, charged particles (metal, polymer, ceramic, and glass) that are dispersed in a liquid phase migrate to the opposite electrode under the electric field. Unlike other solutions, the combination of organic and inorganic materials deposits to create different coatings in this technique. Deposition time, the electrical field, and concentration can influence the morphology of the produced coating. Collagen (type I), known as a natural material with a net-positive charge can move over the electrical field and deposit onto the cathode. Also, it can combine with HA or other nanoparticles (e.g., calcium phosphate) to cover the surface. In addition, chitosan as another biomaterial could co-deposit with collagen to form a film by modifying the deposition properties. Rosei et al. [16] co-deposited phosphate glass doped with copper via collagen–chitosan over a stainless-steel surface during the electrophoretic deposition method.

Overall, in comparison with different available fabrication methods, electrospinning has illustrated outstanding advantages, due to versatility, simplicity, cost-effectiveness, high loading capacity, and applicability at room temperature. Also, multi-scaled fibers produced via electrospinning have illustrated unique configurations with desirable surface area, porous structure, tiny and interconnected pores, pore tortuosity, diameter, and the ability to adjust the morphological features. Furthermore, a wide variety of polymer solutions can be used in this process [20]. These benefits make the electrospinning process reliable in abundant research areas, specifically in the design and engineering of versatile bone tissues. Therefore, the collagen resources, suitable conditions for its electrospinning procedure, as well as their characterizations as bone tissue scaffolds are comprehensively reviewed in the following sections.

3. Collagen Resources and Electrospun Fiber Formations

Collagen is a fibrillar protein in animals and comprises more than 25% of the total protein content of the mammalian body [22]. Collagen maintains structural integrity by forming molecular lines that reinforce tendons using large elastic sheets [23][24]. Being known as a viscoelastic material, collagen possesses high tensile strength with low extensibility. In the form of an ECM, collagen provides structural and mechanical support to connective tissues, such as skin, joints, and bones [22]. Collagen fibers have a similar structure to the fibers that make up the ECM. The triple-helix construction allows collagen molecules to form fibrils that provide mechanical strength and elasticity to tissues. It also supports the skin by providing elasticity and strength, as well as protecting it from pathogens and toxins. In addition, collagen is a key building block in cell biological functions, such as proliferation and differentiation, and contributes to the healing of damaged bones and vessels, resulting from the presence of specific sites for integrin receptors on cell surfaces, which attest to the cell's adherence and interaction with the ECM [25]. Moreover, collagen can act as a signaling molecule through binding to specific receptors on cell surfaces and activating intracellular signaling pathways, which can regulate bioactivity behavior. Furthermore, its biodegradability and biocompatibility are vital means, enabling it to be well tolerated by the body and be broken

down through natural procedures. Bones and teeth are the components, where collagen could be found in association with mineral crystals, especially HA [23].

The collagen family have consisted of polypeptidic chains with a triple-helix structure. The diameter of the three polypeptide fibrils is in the range of 10–500 nm with an estimated length of 1400 amino acids and a molecular weight of 285 kDa [25][26]. A repeating sequence of three amino acids consisting of glycine, proline, and hydroxyproline forms the primary structure [27]. In each individual chain, the atoms are linked by covalent bonds. In contrast, a triple-helical structure of three chains is generated by weaker bonds (dipole–dipole bonds, hydrogen bonds, van der Waals interactions, and ionic bonds) [28]. The stabilization of collagen within a fibril is caused by various intramolecular and intermolecular forces, while the hydrogen bonds are critical in stabilizing the triple helix of collagen. The charged groups in the collagen molecules lead to electrostatic interactions that contribute to the intramolecular structure. Thus, each collagen molecule creates a strong molecular bond with neighboring molecules [27][28].

Three types of collagens, including types I, II, and III, are the most common in the collagen family, differing in the size and length of the helix. Type I collagen, which is the most important member of the family is obtained from skin, tendons, and bone. Type II collagen is extracted from cartilage and type III from the vascular system. It is worth mentioning that type I collagen involves about 90% of the body's collagen and is the most commonly employed type for various applications [29][30].

Collagen can be extracted from different resources. Bovine, porcine, and marine collagen are the most common bases [19]. Bovine collagen, obtained from the skin and bones, is one of the major resources utilized in various industries. The bovine Achilles tendon is used to obtain type I collagen, while type II is derived from articular or nasal cartilage. The suitable biocompatibility of bovine collagen makes it a proper candidate for various applications. Generally, it is used to cover burns and extra-oral wounds of the body. The major concern about bovine collagen is the prevalence of diseases, such as bovine spongiform encephalopathy, transmissible spongiform encephalopathy, and foot and mouth disease, which are considered a threat to humans. Moreover, about 3% of people are allergic to bovine collagen, motivating researchers to seek a safer alternative for collagen sources [31].

In contrast, another widely used industrial source of collagen is porcine collagen, obtained from pigs' skin and bones. Since porcine collagen is very similar to human collagen, it causes fewer allergic reactions. Collagen matrices made from porcine collagen have been applied for soft tissue grafting. It has been shown that porcine collagen materials can be a biocompatible alternative for autogenous transplant; however, there is still a risk of zoonosis. Moreover, religious constraints limit this collagen source in some regions and cases [32].

Given the concern about the outbreak of various diseases in terrestrial animals, marine sources have been considered for obtaining collagen. This collagen source can be considered the safest collagen base and has been classified by the FDA as GRAS (generally recognized as safe). The numerous advantages of marine collagen over

land collagen could be no zoonotic risk, higher collagen content, lower molecular weight, better absorption, less inflammatory and immune responses, as well as fewer religious and ethical restrictions [19].

Considering several disadvantages compared to animal-derived collagen, including batch-to-batch inconsistency and the risk of inflammation and disease transmission, synthetic collagen has recently been investigated. In this type of collagen, the amino acids self-arrange into a triple-helix structure that mimics natural collagen [25]. Synthetic collagens could also be obtained using recombinant technology, in which insect, yeast, mammalian, and plant cell cultures are employed as precursors. Recombinant collagen has been successfully utilized for several medical applications in the form of sponges, gels, and fibers. Results have revealed the superior performance of the recombinant collagen than that of the collagen extracted from animals in terms of processing ability and therapeutic efficiency [33]. Despite the mentioned advantages of synthetic collagens, some shortcomings, such as low profitability and high cost, have limited their applications. In addition, the lack of enzymes essential for bioactive collagen formation leads to a decrease in demand for this product. Therefore, animal-based collagen remains the first choice for the field of research and clinical applications [24].

Reconstructive medicine is a brilliant instance where collagen-based materials have been successfully utilized and have resulted in approaching top results. Collagen matrices (especially type I) are usually used as an ECM substitute for tissue regeneration or repair [34]. Collagen-based three-dimensional scaffolds for tissue engineering have been fabricated using various methods, such as gas-forming foam [35], thermally induced phase separation [36], freeze-drying [37], 3D printing [38], and electrospinning [39].

As discussed earlier, electrospinning is a robust and potential technique for the fabrication of matrices with nanoscale and/or microscale fibers that mimic the fibrous nature of native ECM [40][41]. Electrospun fibers have a fibrous structure that is similar to the fibrous network of the ECM, providing robust mechanical support and a proper scaffold for cells to attach and grow. Additionally, they have a high surface area to volume ratio, providing more surface for cell attachment and growth, which is in line with the ECM architecture. Electrospun fibers could be fabricated using biocompatible and biodegradable substances, as well. Moreover, the properties of tissue engineering scaffolds fabricated with electrospinning can be tuned by changing the spinning parameters. The diameter and orientation of the fibers, as well as the porosity of the electrospun scaffold, can be altered during the spinning process, resulting in a wide range of scaffold architectures with the ability to mimic the ECM [42]. Moreover, various crosslinking techniques could be employed to improve the mechanical properties of collagen-based electrospun scaffolds and increase their degradation resistance [43]. For instance, UV radiation [44] and dehydrothermal treatment (DHT) [45] are physical crosslinking methods, while EDC/NHS is a common chemical crosslinking system in this field [46]. It is declared that the crosslinked samples with EDC/NHS have higher compressive modulus than the DHT-treated ones. Additionally, EDC/NHS enhances the resistance of the scaffold to degradation against collagenase [47].

Huang et al. [48] first studied the electrospinning of collagen in 2001. They employed 10 mM HCl as the solvent system and reported that no fibers could be obtained with pure collagen at a concentration of less than 2 wt.%. This could correspond to the low solubility and high viscosity of collagen in general organic solvents. So, mixing

collagen with synthetic and frequent polymers, such as PCL, polyglycolide acid (PGA), polyethylene oxide (PEO), etc., has been declared as an influential strategy. For example, the addition of PEO to the solution led to uniform fiber formation with diameters ranging from 100 to 150 nm. Another way to overcome the challenging electrospinning process of collagen is by using organic versatile solvents. In 2002, Matthews et al. [49] described the prosperous electrospinning of pure collagen (type I and type III) using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as a solvent. Li et al. [50] investigated the influence of polymer concentration on the resulting electrospun collagen nanofibers. They concluded that increasing the concentration contributes to the rise in the diameter, and uniform fibers could be yielded above the concentration of 5%. Furthermore, the tensile modulus of collagen nanofibers was reported to be 262 ± 18 MPa. The discovery of HFIP as a strong solvent suitable for the electrospinning of numerous natural and synthetic biopolymers led to performing several attempts for various applications [51][52][53]. Meanwhile, HFIP is a highly volatile and corrosive solvent that poses serious risks to humans. There are critical concerns about the use of HFIP due to the danger to laboratory personnel and the environmental hazards of its use. Furthermore, some studies have claimed that HFIP can denature collagen into gelatin during the electrospinning process [49]. Another beneficial solution, specifically in the case of medical applications, is applying non-toxic aqueous systems. Accordingly, Dong et al. [54] described the electrospinning of type I collagen using ethanol and phosphate-buffered saline. The diameter of the resulting fibers was reported to be in the range of collagen nanofibers obtained from the HFIP solvent system. In another study, Liu et al. [55] published a paper on the electrospinning of type I collagen using 40% acetic acid. They compared the degradation of collagen in HFIP and a 40% acetic acid solvent system for electrospinning and reported greater denaturation of the scaffolds obtained from HFIP.

In addition, collagen is a suitable biopolymer for blending with other biomaterials. Therefore, the downsides of the collagen fibrous structures could be modified through fabricating collagen-based composites. Several electrospun collagen nanofiber composite systems have been extensively investigated for application in bone tissue engineering. In such cases, the poor mechanical strength of the collagen could be tackled with the presence of other substances. Considering this, Balasubramanian et al. [56] prepared the electrospun collagen/PCL nanofibers using HFIP as the solvent and a subsequent dip-coating step in 45S5 bioglass. The results demonstrated suitable osteoblast differentiation in vitro. According to tremendous studies carried out to design bone tissue composites based on the collagen polymer, the electrospun collagen-based fibrous structures could be promising as the matrix of many cell types. In the following section, the techniques used for the fabrication of nanofibrous bone tissues, as well as their performances are highlighted.

4. Collagen-Based Nanofibrous Bone Scaffolds

Considering the downsides of bone-therapeutic techniques, such as transplants and implants, the ability of bone for regeneration has received wide attention. In this era, the formation of collagens was focused more on other substances, due to their biocompatibility, biodegradability, non-toxicity, non-antigenicity, mimicking of native bone ECM, proper topology, biological renewability, ability to be cross-linked, and so forth. Collagen nanofibers have illustrated superior pros compared with other polymers because they could closely imitate the native tissues' ultra-

architectures. Additionally, collagen-based nanostructures do not activate the host iminium response in the body. Also, the cells are compatible with nano collagen structures, since collagen is present in most body parts. Among various collagen types, electrospun fibrous structures of collagen types I, II, III, and IV are more applied in bone tissue engineering. These structures could be feasibly obtained in the shapes of aligned and random nanoarrays. It is widely shown that the aligned configurations could result in better mechanical properties and more appropriate cell activity.

Meanwhile, several drawbacks have been declared for the collagen-based bone tissues, such as the negative effect of sterilization, low melting point, poor mechanical stability, challenging processes, and difficult degradability adjusting. Therefore, collagen-based structures are commonly combined with other organic materials, inorganic compounds, or both organic and inorganic structures to address the mentioned obstacles.

Table 1 summarizes the attempts reported toward enriching the collagen electrospun bone tissues with organic materials, such as alginate, polyvinyl alcohol (PVA), PCL, PLGA, silk, and so on, and inorganic materials, including HA, metal alloys, bio-active glass, etc.

Table 1. Summary of collagen-based electrospun-synthesized with organic and inorganic materials.

Route	Inorganic Element	Organic Element	Main Results	Ref.
Collagen/PVA nanofibers grafted on titanium alloy	Ti-6Al-4V	PVA	The effects of fiber alignment and density on osteoblast mineralization were investigated. In the first week of cell culturing, the collagen-aligned fibers could induce osteoblasts to elongate along the fiber direction. Meanwhile, it could not be observed in randomly aligned collagen fibers. Also, the cell growth, found on the high-density aligned collagen fibers showed more calcium than it on both high- and low-density collagen random fibers.	Lin and Peng [57]

Route	Inorganic Element	Organic Element	Main Results	Ref.
Electrospun PVA/collagen/HA nanofibers	HA	PVA	The designed filled nanofibrous membrane showed in vitro degradability, better mechanical properties, and hydrolytic resistance. Also, excellent adhesion and proliferation of the MC3T3 cells were obtained on the designed scaffold, proposing the capability for an orthopedic prosthetic surface.	Song et al. [58]
Electrospinning of Collagen/PCL nanofibers on titanium	Titanium	PCL	The cytocompatibility of titanium was improved through the addition of electrospun Collagen/PCL nanofibers, resulting from the increase in the titanium's surface roughness. It also influenced the shear strength of titanium implants by facilitating the connective tissue growing on them.	Khandaker et al. [59]
Mineralization of n-HA through Ca–P treatment on PLGA/collagen nanofibrous layer	HA	PLGA	Bone-like apatite was shaped on the collagen/PLGA nanofibrous layer and showed the presence of collagen-boosted n-HA nucleation. More n-HA affected the osteoblasts' attachment and proliferation.	Ngiam et al. [60]

Route	Inorganic Element	Organic Element	Main Results	Ref.
Electrospun Collagen/Silk fibroin/bioactive glass composite	bioactive glass	Silk fibroin	Collagen/Silk fibroin/CaO-SiO_2 composite nanofibers were fabricated and the result of the MTT assay corroborated the Saos-2 cell proliferation with no negative effects of a glass substrate.	Wu et al. [61]
Electrospinning of PLLA/collagen/HA composite scaffold	HA	PLLA	The osteoblasts MC3T3-E1 cell culturing on PLLA/collagen/HA electrospun scaffold showed enhanced spreading, proliferation, and differentiation as well as mineralization.	Zhou et al. [62]
Bi-layered bioactive glass and fibrous layers o Fcollagen/PCL	bioactive glass	PCL	The advantages of collagen composite led to the flattening and attachment of chondrocytes as well as HA formation.	Balasubramanian et al. [56]
Melt-plotted PCL/β-tricalcium phosphate composite scaffolds combined with collagen nanofibers	β- tricalcium phosphate	PCL	According to SEM images and MTT assay of osteoblast-like cells with (MG63)-seeded scaffolds, the 2.2-times-higher initial attachment in composite–collagen scaffolds was obtained on the composite scaffold, compared to pure collagen nanofibers. Also, the synergistic effects of the	Yeo et al. [63]

References

1. Parenteau-Bareil, R.; Gauvin, R.; Berthod, F. Collagen-based biomaterials for tissue engineering applications. Materials 2010, 3, 1863–1887.

Route	Inorganic Element	Organic Element	Main Results	Ref.
			collagen nanofibers and β-TCP particles in the scaffold were observed on cell activity.	
Embedding the electrospun collagen filled with the antimicrobial agents into an alginate film	Ag425K antimicrobial agent	Chitosan/Alginate	In vitro, L929 murine fibroblasts cell assay confirmed a proper cytocompatibility for the collagen–alginate scaffold with antibacterial agents.	Matei et al. [64]

devices: Versatile platform for energy, environment, and health monitoring. Mater. Horiz. 2022, 9, 2914–2948.

9. Rezvani Ghomi, E.; Nourbakhsh, N.; Akbari Kenari, M.; Zare, M.; Ramakrishna, S. Collagen-based biomaterials for biomedical applications. J. Biomed. Mater. Res. Part B Appl. Biomater. 2021, 109, 1986–1999.
10. Neela, S.; Bandigari, P.; Mayuri, K. Collagen—A Review. YMER 2022, 21, 100–110.
11. Nasari, M.; Poursharifi, N.; Fakhrali, A.; Banitaba, S.N.; Mohammadi, S.; Semnani, D. Fabrication of novel PCL/PGS fibrous scaffold containing HA and GO through simultaneous electrospinning-electrospray technique. Int. J. Polym. Mater. Polym. Biomater. 2022, 1–17.
12. Khademolqorani, S.; Tavanai, H.; Chronakis, I.; Boisen, A.; Ajalloueian, F. The determinant role of fabrication technique in final characteristics of scaffolds for tissue engineering applications: A focus on silk fibroin-based scaffolds. Mater. Sci. Eng. C 2021, 122, 111867.
13. Wang, L.; Stegemann, J.P. Thermogelling chitosan and collagen composite hydrogels initiated with β-glycerophosphate for bone tissue engineering. Biomaterials 2010, 31, 3976–3985.
14. Diogo, G.S.; Marques, C.F.; Sotelo, C.G.; Pérez-Martín, R.I.; Pirraco, R.P.; Reis, R.L.; Silva, T.H. Cell-laden biomimetically mineralized shark-skin-collagen-based 3D printed hydrogels for the engineering of hard tissues. ACS Biomater. Sci. Eng. 2020, 6, 3664–3672.
15. Chen, Z.; Zhang, Z.; Ouyang, Y.; Chen, Y.; Yin, X.; Liu, Y.; Ying, H.; Yang, W. Electrospinning polycaprolactone/collagen fiber coatings for enhancing the corrosion resistance and biocompatibility of AZ31 Mg alloys. Colloids Surf. A Physicochem. Eng. Asp. 2023, 662, 131041.

16. Deen, I.; Selopal, G.S.; Wang, Z.M.; Rosei, F. Electrophoretic deposition of collagen/chitosan films with copper-doped phosphate glasses for orthopaedic implants. J. Colloid Interface Sci. 2022, 607, 869–880.
17. Haleem, A.; Javaid, M.; Khan, R.H.; Suman, R. 3D printing applications in bone tissue engineering. J. Clin. Orthop. Trauma 2020, 11, S118–S124.
18. Kim, S.-C.; Heo, S.-Y.; Oh, G.-W.; Yi, M.; Jung, W.-K. A 3D-Printed Polycaprolactone/Marine Collagen Scaffold Reinforced with Carbonated Hydroxyapatite from Fish Bones for Bone Regeneration. Mar. Drugs 2022, 20, 344.
19. Liu, S.; Lau, C.-S.; Liang, K.; Wen, F.; Teoh, S.H. Marine collagen scaffolds in tissue engineering. Curr. Opin. Biotechnol. 2022, 74, 92–103.
20. Dhand, C.; Ong, S.T.; Dwivedi, N.; Diaz, S.M.; Venugopal, J.R.; Navaneethan, B.; Fazil, M.H.; Liu, S.; Seitz, V.; Wintermantel, E. Bio-inspired in situ crosslinking and mineralization of electrospun collagen scaffolds for bone tissue engineering. Biomaterials 2016, 104, 323–338.
21. Khademolqorani, S.; Banitaba, N. Application of Electrosprayed Nanoparticles as Targeted Drug Delivery Systems: A Mini Review. J. Appl. Sci. Nanotechnol. 2022, 2, 1–7.
22. Blackstone, B.N.; Gallentine, S.C.; Powell, H.M. Collagen-based electrospun materials for tissue engineering: A systematic review. Bioengineering 2021, 8, 39.
23. Sionkowska, A.; Skrzyński, S.; Śmiechowski, K.; Kołodziejczak, A. The review of versatile application of collagen. Polym. Adv. Technol. 2017, 28, 4–9.
24. Bazrafshan, Z.; Stylios, G.K. Spinnability of collagen as a biomimetic material: A review. Int. J. Biol. Macromol. 2019, 129, 693–705.
25. Avila Rodríguez, M.I.; Rodríguez Barroso, L.G.; Sánchez, M.L. Collagen: A review on its sources and potential cosmetic applications. J. Cosmet. Dermatol. 2018, 17, 20–26.
26. Woodley, D.T.; Keene, D.R.; Atha, T.; Huang, Y.; Lipman, K.; Li, W.; Chen, M. Injection of recombinant human type VII collagen restores collagen function in dystrophic epidermolysis bullosa. Nat. Med. 2004, 10, 693–695.
27. Goh, K.L.; Hiller, J.; Haston, J.L.; Holmes, D.F.; Kadler, K.E.; Murdoch, A.; Meakin, J.R.; Wess, T.J. Analysis of collagen fibril diameter distribution in connective tissues using small-angle X-ray scattering. Biochim. Biophys. Acta (BBA)-Gen. Subj. 2005, 1722, 183–188.
28. Bella, J. A new method for describing the helical conformation of collagen: Dependence of the triple helical twist on amino acid sequence. J. Struct. Biol. 2010, 170, 377–391.
29. Miller, A. Collagen: The organic matrix of bone. Philosophical Transactions of the Royal Society of London. B Biol. Sci. 1984, 304, 455–477.

30. Silvipriya, K.; Kumar, K.K.; Bhat, A.; Kumar, B.D.; John, A. Collagen: Animal sources and biomedical application. J. Appl. Pharm. Sci. 2015, 5, 123–127.
31. Ellingsworth, L.; DeLustro, F.; Brennan, J.; Sawamura, S.; McPherson, J. The human immune response to reconstituted bovine collagen. J. Immunol. 1986, 136, 877–882.
32. Herford, A.S.; Akin, L.; Cicciu, M.; Maiorana, C.; Boyne, P.J. Use of a porcine collagen matrix as an alternative to autogenous tissue for grafting oral soft tissue defects. J. Oral Maxillofac. Surg. 2010, 68, 1463–1470.
33. Wang, T.; Lew, J.; Premkumar, J.; Poh, C.L.; Win Naing, M. Production of recombinant collagen: State of the art and challenges. Eng. Biol. 2017, 1, 18–23.
34. Shekhter, A.B.; Fayzullin, A.L.; Vukolova, M.N.; Rudenko, T.G.; Osipycheva, V.D.; Litvitsky, P.F. Medical applications of collagen and collagen-based materials. Curr. Med. Chem. 2019, 26, 506–516.
35. Lv, Q.; Feng, Q.; Hu, K.; Cui, F. Three-dimensional fibroin/collagen scaffolds derived from aqueous solution and the use for HepG2 culture. Polymer 2005, 46, 12662–12669.
36. Martínez-Pérez, C.A.; Olivas-Armendariz, I.; Castro-Carmona, J.S.; García-Casillas, P.E. Scaffolds for tissue engineering via thermally induced phase separation. Adv. Regen. Med. 2011, 35, 275–294.
37. Lowe, C.J.; Reucroft, I.M.; Grota, M.C.; Shreiber, D.I. Production of highly aligned collagen scaffolds by freeze-drying of self-assembled, fibrillar collagen gels. ACS Biomater. Sci. Eng. 2016, 2, 643–651.
38. Inzana, J.A.; Olvera, D.; Fuller, S.M.; Kelly, J.P.; Graeve, O.A.; Schwarz, E.M.; Kates, S.L.; Awad, H.A. 3D printing of composite calcium phosphate and collagen scaffolds for bone regeneration. Biomaterials 2014, 35, 4026–4034.
39. Boland, E.D.; Matthews, J.A.; Pawlowski, K.J.; Simpson, D.G.; Wnek, G.E.; Bowlin, G.L. Electrospinning collagen and elastin: Preliminary vascular tissue engineering. Front. Biosci.-Landmark 2004, 9, 1422–1432.
40. Meamar, R.; Ghasemi-Mobarakeh, L.; Norouzi, M.-R.; Siavash, M.; Hamblin, M.R.; Fesharaki, M. Improved wound healing of diabetic foot ulcers using human placenta-derived mesenchymal stem cells in gelatin electrospun nanofibrous scaffolds plus a platelet-rich plasma gel: A randomized clinical trial. Int. Immunopharmacol. 2021, 101, 108282.
41. Norouzi, M.-R.; Ghasemi-Mobarakeh, L.; Itel, F.; Schoeller, J.; Fashandi, H.; Borzi, A.; Neels, A.; Fortunato, G.; Rossi, R.M. Emulsion electrospinning of sodium alginate/poly (ε-caprolactone) core/shell nanofibers for biomedical applications. Nanoscale Adv. 2022, 4, 2929–2941.

42. Khosravi, A.; Ghasemi-Mobarakeh, L.; Mollahosseini, H.; Ajalloueian, F.; Masoudi Rad, M.; Norouzi, M.R.; Sami Jokandan, M.; Khoddami, A.; Chronakis, I.S. Immobilization of silk fibroin on the surface of PCL nanofibrous scaffolds for tissue engineering applications. J. Appl. Polym. Sci. 2018, 135, 46684.
43. Tierney, C.M.; Haugh, M.G.; Liedl, J.; Mulcahy, F.; Hayes, B.; O'Brien, F.J. The effects of collagen concentration and crosslink density on the biological, structural and mechanical properties of collagen-GAG scaffolds for bone tissue engineering. J. Mech. Behav. Biomed. Mater. 2009, 2, 202–209.
44. Gaspar, A.; Moldovan, L.; Constantin, D.; Stanciuc, A.; Boeti, P.S.; Efrimescu, I. Collagen–based scaffolds for skin tissue engineering. J. Med. Life 2011, 4, 172.
45. Haugh, M.G.; Jaasma, M.J.; O'Brien, F.J. The effect of dehydrothermal treatment on the mechanical and structural properties of collagen-GAG scaffolds. J. Biomed. Mater. Res. Part A Off. J. Soc. Biomater. Jpn. Soc. Biomater. Aust. Soc. Biomater. Korean Soc. Biomater. 2009, 89, 363–369.
46. Nong, L.-M.; Zhou, D.; Zheng, D.; Jiang, Y.-Q.; Xu, N.-W.; Zhao, G.-Y.; Wei, H.; Zhou, S.-Y.; Han, H.; Han, L. The effect of different cross-linking conditions of EDC/NHS on type II collagen scaffolds: An in vitro evaluation. Cell Tissue Bank. 2019, 20, 557–568.
47. Kozłowska, J.; Sionkowska, A. Effects of different crosslinking methods on the properties of collagen–calcium phosphate composite materials. Int. J. Biol. Macromol. 2015, 74, 397–403.
48. Huang, L.; Nagapudi, K.; Apkarian, R.P.; Chaikof, E.L. Engineered collagen–PEO nanofibers and fabrics. J. Biomater. Sci. Polym. Ed. 2001, 12, 979–993.
49. Matthews, J.A.; Wnek, G.E.; Simpson, D.G.; Bowlin, G.L. Electrospinning of collagen nanofibers. Biomacromolecules 2002, 3, 232–238.
50. Li, M.; Mondrinos, M.J.; Gandhi, M.R.; Ko, F.K.; Weiss, A.S.; Lelkes, P.I. Electrospun protein fibers as matrices for tissue engineering. Biomaterials 2005, 26, 5999–6008.
51. Wakuda, Y.; Nishimoto, S.; Suye, S.-I.; Fujita, S. Native collagen hydrogel nanofibres with anisotropic structure using core-shell electrospinning. Sci. Rep. 2018, 8, 6248.
52. Rho, K.S.; Jeong, L.; Lee, G.; Seo, B.-M.; Park, Y.J.; Hong, S.-D.; Roh, S.; Cho, J.J.; Park, W.H.; Min, B.-M. Electrospinning of collagen nanofibers: Effects on the behavior of normal human keratinocytes and early-stage wound healing. Biomaterials 2006, 27, 1452–1461.
53. Dulnik, J.; Denis, P.; Sajkiewicz, P.; Kołbuk, D.; Choińska, E. Biodegradation of bicomponent PCL/gelatin and PCL/collagen nanofibers electrospun from alternative solvent system. Polym. Degrad. Stab. 2016, 130, 10–21.

54. Dong, B.; Arnoult, O.; Smith, M.E.; Wnek, G.E. Electrospinning of collagen nanofiber scaffolds from benign solvents. Macromol. Rapid Commun. 2009, 30, 539–542.
55. Liu, T.; Teng, W.K.; Chan, B.P.; Chew, S.Y. Photochemical crosslinked electrospun collagen nanofibers: Synthesis, characterization and neural stem cell interactions. J. Biomed. Mater. Res. Part A 2010, 95, 276–282.
56. Balasubramanian, P.; Roether, J.A.; Schubert, D.W.; Beier, J.P.; Boccaccini, A.R. Bi-layered porous constructs of PCL-coated 45S5 bioactive glass and electrospun collagen-PCL fibers. J. Porous Mater. 2015, 22, 1215–1226.
57. Lin, H.-Y.; Peng, Z.-X. Nanofibers grafted on titanium alloy: The effects of fiber alignment and density on osteoblast mineralization. J. Mater. Sci. Mater. Med. 2017, 28, 149.
58. Song, W.; Markel, D.C.; Wang, S.; Shi, T.; Mao, G.; Ren, W. Electrospun polyvinyl alcohol–collagen–hydroxyapatite nanofibers: A biomimetic extracellular matrix for osteoblastic cells. Nanotechnology 2012, 23, 115101.
59. Khandaker, M.; Riahinezhad, S.; Sultana, F.; Morris, T.; Wolf, R.; Vaughan, M. Effect of collagen-polycaprolactone nanofibers matrix coating on the in vitro cytocompatibility and in vivo bone responses of titanium. J. Med. Biol. Eng. 2018, 38, 197–210.
60. Ngiam, M.; Liao, S.; Patil, A.J.; Cheng, Z.; Chan, C.K.; Ramakrishna, S. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. Bone 2009, 45, 4–16.
61. Wu, J.; Wang, S.; Zheng, Z.; Li, J. Fabrication of Biologically Inspired Electrospun Collagen/Silk fibroin/bioactive glass composited nanofibrous scaffold to accelerate the treatment efficiency of bone repair. Regen. Ther. 2022, 21, 122–138.
62. Zhou, G.; Liu, S.; Ma, Y.; Xu, W.; Meng, W.; Lin, X.; Wang, W.; Wang, S.; Zhang, J. Innovative biodegradable poly(L-lactide)/collagen/hydroxyapatite composite fibrous scaffolds promote osteoblastic proliferation and differentiation. Int. J. Nanomed. 2017, 12, 7577.
63. Yeo, M. Polycaprolactone, β-tricalcium phosphate, and collagen nanofibers: Fabrication, physical properties, and in vitro cell activity for bone tissue regeneration. Biomacromolecules 2011, 12, 502–510.
64. Matei, E.; Gaidau, C.; Râpă, M.; Stefan, L.M.; Ditu, L.-M.; Predescu, A.M.; Stanca, M.; Pantilimon, M.C.; Berechet, M.D.; Predescu, C. Sustainable Coated Nanostructures Based on Alginate and Electrospun Collagen Loaded with Antimicrobial Agents. Coatings 2021, 11, 121.