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Nanofiber Technology for Regenerative Engineering

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Abstract

Regenerative engineering is powerfully emerging as a successful strategy for the regeneration of complex tissues and biological organs using a convergent approach that integrates several fields of expertise. This innovative and disruptive approach has spurred the demands for more choice of biomaterials with distinctive biological recognition properties. An ideal biomaterial is one that closely mimics the hierarchical architecture and features of the extracellular matrices (ECM) of native tissues. Nanofabrication technology presents an excellent springboard for the development of nanofiber scaffolds that can have positive interactions in the immediate cellular environment and stimulate specific regenerative cascades at the molecular level to yield healthy tissues. This paper systematically reviews the electrospinning process technology and its utility in matrix-based regenerative engineering, focusing mainly on musculoskeletal tissues. It briefly outlines the electrospinning/3D-printing system duality and concludes with a discussion on the technology outlook and future directions of nanofiber matrices.

Graphical Abstract

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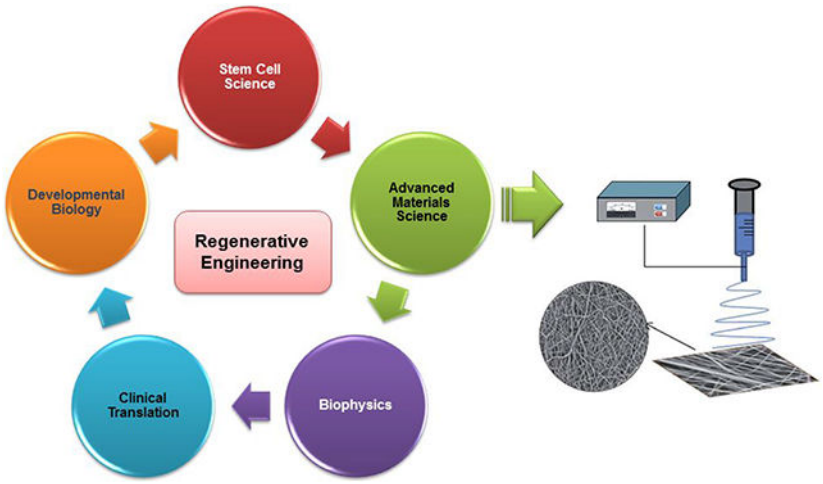

Keywords

electrospinning; electrospun nanofiber; tissue regeneration; drug delivery; biodegradable polymer; stem cells; 3D-printed scaffold; dual-scale matrix; biomaterial-cell interactions; sustained release

The approach of regenerative engineering has witnessed tremendous growth and advancements in the last decade.[1–3] These advances (including sophisticated material design, development of induced pluripotent stem cells, fresh insights in cell biomechanics, *etc.*) are stimulated by the desire to salvage limbs after major bone loss through technologies based on nanomaterials.[3] In light of the urgent needs for a more effective treatment option for bone injury and deformities, regenerative engineering presents a multidisciplinary framework that interconnects and merges expertise from different fields such as advanced materials science, biophysics, stem cell science, developmental biology, and clinical translation for the active regeneration of complex tissues and organs.[4–6] This innovative and paradigm methodology is fascinating, and it circumvents most of the drawbacks associated with current treatment options.[1] Regenerative engineering employs bioengineered materials as a structural fabrics to stimulate body's self-healing mechanism and maximize functional tissue development.[7] Despite the rapid progress attained in this field, challenges still exist in our ability to device instructive cues that can induce favorable cellular responses and direct tissue formations, which are ultimate goals for regenerative engineering.[7–9]

Enormous effort has been focused on the design of appropriate biomaterials that can be employed to fabricate scaffolds with topographical, biological, and mechanical features needed to stimulate tissue regeneration.[8, 10] Advanced materials science has enabled the development of nanotechnology, which is a valuable and resourceful tool for regenerative engineering.[4, 10] The use of nanotechnology can offer the dynamism to control the biochemical and mechanical microenvironment for the successful delivery of cells and the regeneration of tissues.[11, 12] Therefore, nanomaterials encompass features with size in the nanoscale (1-100 nm), and this nanostructured hierarchical arrangement mimics the anatomical features of the native extracellular matrix (ECM) of the musculoskeletal tissues (Figure 1 & 2a).[12–14] Owing to the exceptional physicochemical properties of nanomaterials

such as high surface-to-volume ratio, high reactivity, enhanced specificity, and small size, they present an ideal platform for use in a variety of regenerative applications.[12, 15] Nanofibers represent an attractive class of nanomaterials for the development of scaffolding matrices due to their ease of fabrication and their ability to be generated from different polymers.[16] Currently, a number of techniques are utilized to make nanofibers, including self-assembly, temperature-induced phase separation, template synthesis, drawing, melt blowing, melt spinning, centrifugal spinning, and electrospinning.[12, 17] Amongst these techniques, electrospinning stands out as the most widely used and straightforward process that has demonstrated the most favorable results in regenerative applications.[12] Some of the advantages of electrospinning technique over the other major nanofiber production methods (such as phase separation and self-assembly) include the efficiency and simplicity of the protocol, inexpensive setup, tunability of properties, such as fiber diameter, orientation, and composition, and the ability to utilize a wide range of polymers. The last one is critical as phase separation, and self-assembly techniques can only be carried out with a limited number of polymers, making it difficult for mass production and commercialization. Nevertheless, the demerits of the electrospinning process include the utilization of organic solvents and uncontrollable pore structures.[18]

The scope for the development of nanofiber-based scaffolds (notably, the ones produced *via* electrospinning) has been broadened by a wide spectrum of natural and synthetic biomaterials at our disposal.[19, 20] The 3D nanofibrous scaffolds not only serve as a supportive template, but also regulate cellular activities such as cell adhesion, proliferation, and differentiation.[17, 20] Nanofiber materials used for bone scaffolds can be characterized by properties such as high and interconnected porosity, light weight, high axial strength, high surface area per unit mass, structural integrity, degradability, biocompatibility, osteoconductivity, and osteoinductivity.[12, 15] These attributes provide an effective means of controlling the material-cell interactions (including adsorption of plasma proteins and ECM components) and biomaterial integration with the host tissues.[10, 21] This paper reviews the development of nanofiber technology and its scope of applications in regenerative engineering. Also, we will discuss the various components of regenerative engineering and the current progress on the integration of nanotechnology with this convergent approach for the design of the next generation of bone grafts.

MATRIX-BASED STRATEGIES FOR TISSUE REGENERATION

Current biomedical approaches for bone defect repair and regeneration are presented with challenging issues, and an innovative alternative has become the Holy Grail[1, 26]. Regenerative engineering is a nascent and increasingly important strategy that harmonizes the fields of advanced materials science, stem cell science, biophysics, developmental biology, and clinical translation for the efficient regeneration of complex tissues and organs.[1, 5, 22]

Advanced Materials Science

Matrix-based regenerative engineering classically employs a biomimetic scaffold that provides a supportive template and also regulates the activities of cells for the functional

development of tissues[22]. Hence, the success of this strategy will depend significantly on biomaterials, especially biodegradable polymers.[5] Advances in materials science have enabled a means to design biomaterial-based scaffolds with the ideal characteristics. A suitable scaffold would present sufficient initial mechanical strength and have a degradation rate that tallies with the process of regeneration with resorbable degradation products.[23]

Developmental Biology and Stem Cell Technology

Developmental biology presents a reservoir of knowledge in the process of bone healing and limb regeneration, which familiarizes us with several morphogenetic progressions.[4, 22] The understanding obtained from this biological process, along with the control of cell growth and cellular differentiation, can be leveraged and integrated into the modern regenerative concept.[8, 24] Additionally, the utilization of growth factors and small bioactive molecules to regulate the physiological process is a spin-off from developmental biology as these biochemical cues can serve as potent osteoinductive factors.[1, 5] Stem cells undergo inductive effects as they are induced to mitogenesis, which involves cell division and differentiation of mesenchymal stem cells (MSCs) and other osteoprogenitor cells towards osteoblasts.[25] Stem cell science and technology are an integral part of regenerative engineering as they continue to play a pivotal role in the delivery of cell therapy for the repair and regeneration of damaged tissues.[2, 6]

Biophysics

Biophysics has added a valuable and exquisite tool that helps in understanding the mechanics of cells and how they respond to external stimuli, such as force and stress, within a bioengineered construct.[22, 26] This biomechanical stimulation can control and regulate the morphogenetic actions of the cells by generating, sustaining, and interacting with the mechanical forces within the microenvironment, which are transformed into biochemical signals that modulate a variety of cellular responses (Figure 2b).[5, 6]

Clinical Translation

Clinical translation of this technology, as the last component of regenerative engineering, encompasses the pragmatic development of modern diagnostics and treatments for diseased and damaged tissues of patients. This also helps in navigating the regulatory hurdles to ensure the safety and success of the technology in translation.[2, 6, 27] So when a bioengineered tissue construct is being designed, conglomerate insights and expertise from the various facets of regenerative engineering are of paramount importance and provide a powerful and gamechanging platform for positive outcome.

As tissue defects and diseases present complicated and hierarchical tissue architecture, sophistication in scaffold design becomes imperative and necessary for matching this complexity.[1, 6] The focus of recent investigations on regenerative engineering has been to develop three-dimensional porous structures (scaffold) that are architecturally and morphologically similar to ECM and can induce biological response on the molecular level.[5, 12, 15] In other words, the biomimetic scaffold would promote and encourage cell growth and organization, leading to the successful integration of materials into the native ECM.[20, 28] The ECM is composed of fibrous protein mesh (collagen type II and proteoglycans) whose

design and morphology are meant to provide efficient structural support for the physiological functions.[20, 29] This forms the basis for the exploration of different methods, including the electrospinning process, for the development of desirable biomaterials that mimic the ECM. Inspirations from this consideration led to the development of electrospun nanofibers for biomedical purposes, which are meant to create an artificial microenvironment that resembles the native ECM, providing signals that facilitate the regeneration of tissues. The development of nanofiber technology represents a bright future for regenerative engineering.

ELECTROSPINNING TECHNOLOGY AND PROCESS

Electrospinning is a multifunctional method that is utilized to produce continuous polymer nanofibers with diameters in the range of nanometers to micrometers.[12, 15] A typical apparatus for electrospinning is cost-effective and straightforward, and it consists of a high voltage supplier (positive and negative electric field), a capillary tube with a small diameter needle or pipette, and a grounded fiber collecting screen (Figure 3).

This process is operated at room temperature and can be set up vertically or horizontally.[30] A syringe pump, gravitational forces, or pressurized gas are typically utilized to force the polymer solution through the capillary forming a dangling droplet at the tip.[19, 30] When an electric field is applied between the polymer solution (the end of the capillary tube with the solution) and the collector, the solution is subjected to tensile forces, elongating the pendant droplet at the capillary tip.[31] As the intensity of the electric field increases, the hemispherical droplet at the tip of the capillary tube changes to a conical shape, popularly known as Taylor cone.[12, 31] At this point, equilibrium exists between the repulsive electrostatic force and the surface tension of the liquid. At a further increase of the electric field beyond this critical point, the electrostatic force overcomes the surface tension, allowing the ejection of fiber jet from the tip of the Taylor cone onto the collecting screen.[31] The fiber jet experiences a chaotic bending instability while being accelerated toward the collector and this increases the travel path and allows the smooth fiber elongation and thinning as well as solvent evaporation process.[12, 17] The evaporation of the solvent due to the bending instability aids in solidifying the polymer fibers.

The nanofiber collected can be randomly-oriented or highly aligned, depending on the collector type. Collectors like the rotating drum or a two-parallel system can yield fibers with specific axial alignment (*i.e.*, uniaxially aligned or radially aligned), whereas the regular collector results in nanofibers with random orientation (Figure 3).[19, 32] The diameter and morphology of the electrospun nanofibers can be modulated and optimized using operating/process parameters (such as feed rate, applied voltage, and the nozzle-collector distance) and system parameters or solution characteristics (such as polymer concentration, molecular weight, surface tension, conductivity, and dielectric constant).[33, 34]

The customization of the fiber composition can be achieved by the employment of different polymers, composite materials, or encapsulation during electrospinning.[12] This flexibility has led to the utilization of over 100 types of polymers, including natural and synthetic polymers, to produce nanofibers for wide-ranging applications.[19, 32] Also, the fiber

composition can further be subjected to post-treatment, such as surface functionalization and thermal treatment, thereby expanding their multifunctional possibilities and enhancing its physicochemical properties.[19, 35]

The use of spinnerets with special design configurations holds even more enormous potential for obtaining different secondary structures such as porous, core-sheath (Figure 4a–c), hollow (Figure 4d), and multicompartment (Figure 4E).[36, 37] Additionally, the ability to adjust and control the concentration of the solution and the molecular weight of the polymers allows researchers to have a firm handle on the generation of various fiber shapes (*i.e.*, beaded and beltshaped fibers). As mentioned earlier, a wide range of nanofiber assemblies and scaffolds can be obtained by using a specially configured collector (Figure 4). These nanofiber assemblies, including random fiber mat, uniaxially aligned fiber mat, random-to-aligned fiber mat, radially aligned fiber mat, and fiber yarns/bundles, offer unmatched versatility for the potential range of biomedical applications.[37, 38]

Furthermore, one shortcoming associated with electrospun mats obtained from traditional electrospinning is its low porosity due to the tight packing of nanofiber layers.[12, 15, 39] This inevitable feature hampers oxygen diffusion, nutrient transport, and the penetration of cells throughout the nanofibrous scaffolds.[16, 30, 31] However, an advanced fabrication strategy offers a means of controlling the pore size of the nanofiber scaffolds by adjusting the fiber diameter or disbanding the sacrificial fibers.[37, 40, 41] In general, the process of electrospinning is an essential technology that presents a powerful platform for the transformation of a variety of polymers into nanofibers. The electrospun nanofiber meshes bear a striking resemblance to the hierarchical and structural organization of the ECM of the native tissues (Figure 2). As a result of this morphological similarity, the electrospinning process is regarded to have the edge over any other techniques as it presents a more excellent avenue of simulating the characteristics of ECM (*i.e.*, fiber diameter, porosity, and mechanical strength).[12, 39, 42]

ELECTRONSPUN FIBER PROPERTIES AND PERFORMANCE

Suitability for Cell Regulation

Since porosity is highly desirable for enhanced cell-material interactions and vascular network formation, nanofibers hold great importance as scaffolding constructs for biomedical applications due to their high porosity and large surface-area-to-volume ratio.[19, 39, 43–45] Cell-material interactions play a critical role in tissue regeneration as biomaterials are designed to regulate and mediate the activities of the cells. The ease with which compositions, size, order, alignment, mechanical properties, and release patterns can be controlled makes electrospun nanofibers feasible substrates used in manipulating cellular responses, including morphologies, proliferation, differentiation, ECM synthesis, and gene expression (Figure 2b). Studies have shown that the morphology of cells and tissues can be modulated using electrospun nanofiber with topographic cues. In one of such studies, Xie *et al.,*[46] demonstrated that rat tendon fibroblast cells could exhibit different morphologies when seeded on different nanofiber surfaces with different orientations (Figure 5a–d). PLGA-based nanofibers with “aligned-to-random” orientation were seeded with the fibroblast cells, and it was found that cells on the aligned surface exhibited an elongated

morphology longitudinal to direction of the fiber alignment. In contrast, the cells on the surface with random orientation showed an irregularly shaped morphology. Correspondingly, the aligned fibrous portions exhibited higher tensile properties (ultimate strength and modulus) than the fibrous portions with random orientation (Figure 5e–f). The composition of a nanofiber can influence cellular responses. This was illustrated in a study by Theisen *et al.*[43], where they explored the effects of nanofiber scaffolds on the gene expression and matrix deposition of human tendon derived fibroblasts (TDF's). It was observed that the PLLA nanofibers exhibited growth inhibitory effect on the TDF's but depicted negligible influence on the gene expression of collagen I, collagen III, and decorin while there was an increase in the expression of collagen X throughout the cultivation interval. On the contrary, the nanofibers based on PLLA/collagen-I blend did not adversely affect the growth of TDF's; moreover, the blend nanofibers enhanced the gene expression of collagen I, III, X, and decorin.

Also, cells can be predisposed to growth and differentiation using electrospun nanofibers with different fiber diameters. One study investigated the effects of different diameters (320 nm, 680 nm, and 1.8 μm) of aligned polymer nanofibers on the response of TDF's (Figure 6a). It was found that the cell, proliferation, differentiation, and matrix production were regulated by varying the fiber diameters as there were higher cell number, total collagen, and proteoglycan production on the nanofiber scaffolds as compared to the control monolayer (Figure 6b–d). In contrast, the expression of the phenotypic marker of the tendon fibroblasts, such as collagen I, III, V, and tenomodulin, were enhanced for the microfibers. The mechanical properties of the scaffolds were dependent on the changes in the fiber diameters (Figure 6e).[47] Similarly, Jaiswal *et al.*,[28] demonstrated that the activations of ERK and p38 kinase in osteoblast could be regulated by using different fiber diameters. In another study, Ricotti *et al.*,[48] investigated the proliferation and skeletal muscle differentiation capacity of myoblastic cell lines on both isotropic and anisotropic electrospun nanofibers composed of poly (hydroxybutyrate). The aligned nanofiber mesh showed a decrease in the rate of proliferation; however, it provided a more positive stimulus to the differentiation of the cells and the formation of myotubes as compared to the flat films. Hence, this confirms that different topographies can be employed to control cellular fate, and this finding has been previously proven in several other studies.[40, 49] Electrical cues have been integrated with topographical cues *via* a polycaprolactone-polyaniline nanofiber blend to create synergic effects on the myoblast behaviors.[50] The polycaprolactone-polyaniline nanofiber blend was able to stimulate the differentiation of the muscle cells, making it a suitable candidate for regenerative engineering. In a similar study, Choi *et al.*,[39] designed (polycaprolactone) PCL/collagen nanofiber meshes and demonstrated that nanofibers with unidirectional orientation could induce muscle cell alignment and myotube formation in comparison to nanofibers with random orientation.

A number of investigations have shown that electrospun nanofiber scaffolds can be utilized to direct the differentiation of stem cells into musculoskeletal phenotypes specifically. Yin *et al.*,[51] confirmed this observation in a study where they investigated the effects of nanotopography on the differentiation of human tendon stem/progenitor cells using PLLA nanofiber scaffolds. They discovered that the cells were spindle-shaped and well-orientated on the aligned nanofiber and the tendon-specific gene expression was significantly higher in

the cells growing on the aligned nanofibers than those on the nanofibers with random orientations (Figure 7a–j). In addition to this observation, results from alkaline phosphatase and alizarin red staining showed that osteogenesis was induced on the randomly-orientated nanofibers, whereas the aligned fibrous scaffolds impeded the process. Besides the topographical cues provided by electrospun nanofibers, biochemical cues, such as the incorporation of ECM-like components, can be used to regulate and instruct stem cell differentiation. For example, Ravichandran *et al.,*[52] utilized nanohydroxyapatite (nHAp) coated PLLA/polybenzyl-L-glutamate/collagen nanofiber scaffold to show the effect of the presence of bioactive n-HAp molecules on the differentiation of adipose-derived stem cells (ADSC). It was found that the ECM-like components in the nanofibers were capable of directing the ADSC into osteogenic lineage. Likewise, Cobun *et al.,*[53] designed fibrous scaffolds composed of poly (vinyl alcohol) and chondroitin sulfate and evaluated their capacity to facilitate cartilage-like tissue formation. They found out that nanofiber scaffolds supported proliferation and promoted the chondrogenic differentiation of the mesenchymal stem cells used. This is indicated by the increase in the production of ECM and the expression of cartilage-specific genes. Another approach reported by James *et al.*,[54] showed that PLGA fibrous scaffolds incorporated with GDF-5 could significantly enhance the gene expression of tendon markers (*i.e.*, scleraxis and collagen type I) in primary ADSC. Interest has emerged on the need to further revolutionize the design and fabrication of electrospun nanofibers into 3D multiscale structures with high precision for tissue regeneration.[55–57] One way to achieve this high precision scaffolds is by combining electrospinning and 3D printing to obtain a matrix with both nanofiber and 3D printed components (Figure 8). The electrospun/3D printed scaffold addresses the limitations of the conventional electrospun nanofibers (such as reduced cell infiltration into the nanopores) and 3D printed scaffold (such as low print resolution). For example, Yu *et al.*[58] designed a 3D-nanostructured scaffold by infusing PCL/gelatin dispersed nanofibers into the meshes of 3D-printed PCL scaffold (Figure 8c). The PCL/gelatin/PCL nanostructured matrix showed micro-scale (100-300 μm) morphology and excellent compressive modulus (due to 3D printed mesh) as well as provided ideal platform for enhanced cell infiltration and proliferation. Similarly, using PCL, Vyas *et al.*[59] systematically evaluated the biological and morphological characteristics of a 3D-printed and electrospun scaffold with multiple mesh layers and fiber densities (Figure 8b). Results showed that cell adhesion, proliferation, and migration were improved on the dual-scale scaffold with an increasing number of mesh layers (Figure 8a). The electrospun fibers were highly aligned normal to the direction of the printed fiber and formed across the scaffold's microscale pores, promoting cell bridging and cell alignment. The bridging and alignment of the cells can be desirable in the regeneration of different tissues, and these cellular arrangements are not typically observed in the conventional 3D-printed scaffolds. The hybridization of electrospinning and 3D printing technologies is a promising development that still needs further research for its optimization.

Suitability for Sustained Release

The use of signaling molecules, such as drugs, proteins, growth factors, and DNA, is an indispensable aspect of tissue regeneration, and these chemical cues can be integrated into electrospun nanofibers for sustained and controlled delivery (Figure 9).[16, 36, 60, 61] There are two primary steps designated for nanofibers employed as a delivery system; first is the

loading capability of the nanofibers and, secondly, the controlled release of the loaded bioactive molecules.[7, 62] Depending on the type of material, bioactive molecules can be loaded into nanofibers through different ways; it could be achieved by chemically or physically binding the bioactive molecules with the fibrous materials (Figure 9a–c), by crosslinking the nanofibers to encapsulate the bioactive molecules, and by sandwiching the bioactive molecules using nanofiber layers. The chemical binding of the bioactive molecules may involve surface modifications (such as plasma treatment, wet chemical method, and surface graft polymerization) employed to improve the surface properties of nanofibers *via* the incorporation of specific functional groups. The release of bioactive molecules in a controlled and premeditated manner can be attained by modulating the nanofiber features such as porosity, diameters, and degradability. Efficacious delivery of loaded bioactive and signaling molecules is guaranteed by the high surface-area-to-volume ratio of electrospun fibrous scaffolds.[63, 64]

Cellular functions are positively imparted when signaling and bioactive molecules are involved in a sustained fashion, leading to improved tissue regeneration. Nanofibers have shown excellent prospects in their applications as vehicles for sustained and controlled release of bioactive proteins or genes. For instance, it was shown by Sahoo *et al.*,[21] that basic fibroblast growth factor (bFGF) could be randomly dispersed in PLGA-based electrospun nanofibers and released as bioactive molecules within a week interval. The sustained release of bFGF from the PLGA-bFGF matrix resulted in the activation of tyrosine phosphorylation signaling in the seeded bone marrow stem cells (BMSC). It was also discovered that the bFGF-releasing matrix promoted the proliferation of BMSC and the gene expression of tendon/ligament-specific ECM proteins. Similarly, using PLGA, Fu *et al.*,[65] designed 3D PLGA/hydroxyapatite electrospun scaffolds loaded with bone morphogenetic protein-2 (BMP-2) for bone regeneration. *In Vivo* results confirmed the sustained release properties of the fibrous scaffolds as different BMP-2 loading methods and different Hap contents were employed. The bioactivity resulting from the BMP-2 release was conserved, and this was instrumental to the enhancement of new bone formation and the subsequent healing of the defect site. The same PLGA/hydroxyapatite electrospun matrices were used to encapsulate BMP-2 plasmid, and its release from the matrices was systematically evaluated. Analyses indicated the preservation of the bioactivity of the BMP-2 plasmid released from the scaffolds, which ultimately contributed to the healing of the *In Vivo* bone segmental defects. Several other alternatives have also been reported to enhance the release potential of nanofibers *In Vivo.* For example, a hybrid hydrogel-nanofiber matrix was designed by directly embedding a heparin/fibrin hydrogel onto an electrospun PLGA nanofiber.[66] This layered system was employed to deliver Platelet-derived growth factor BB (PDGF-BB) and adipose-derived mesenchymal stem cells (ASCs) for the regeneration of dense connective tissues such as tendon. The results in a large animal model indicated favorable clinical potentials of the matrix as the heparin/fibrin allowed the simultaneous delivery of PDGF-BB and ASCs in controlled fashion and PLGA provided dimensional stability for surgical handling and implantation.[66] Another interesting approach was reported by Mo and coworkers, who developed 3D electrospun nanofibrous scaffolds for the sustained release of BMP-2-derived peptides (PEP) for bone tissue regeneration.[67] By merging techniques such as homogenization, freeze-drying, and thermal crosslinking, 3D nanofibrous structures

composed of nano-hydroxyapatite/PLLA/Gelatin (nHA/PLA/GEL) were fabricated with pre-formed electrospun nanofibers (Figure 10). The PEP was immobilized onto the 3D scaffolds using polydopamine (pDA)-assisted method. The *In Vitro* and *In Vivo (via* a rat cranial bone defect model) analyses illustrated that the nHA/PLA/GEL/PEP-based 3D nanofiber scaffolds possess favorable biocompatibility and osteoinductivity for bone regenerative engineering.[67]

Sustained release of antibiotics is necessary for repairing and regenerating musculoskeletal tissues as it can help in preventing postsurgical infections. Electrospun nanofibers have been extensively explored as carriers in several studies for the sustained release of antibiotics. One study by Shi and coworkers investigated the antibacterial activity of amoxicillin (AMX)-encapsulated nanohydroxyapatite/PLGA composite nanofibers.[68] The organic/inorganic hybrid nanofibers exhibited sustained release profile and resolute capacity to inhibit the growth of a model bacterium, Staphylococcus aureus. The same research group in a similar study demonstrated the antibacterial activity of composite nanofibers based on AMX, laponite particles (LAP), and PLGA.[62] The PLGA/LAP/AMX nanofibers enabled a sustained release of AMX, whose antimicrobial activity was not compromised. These studies demonstrated the capability and potential of these nanofibers to sustain the release of antibiotics at the site of interest with high precision and control. Nonetheless, several studies have shown that some pathogenic bacteria are becoming increasingly resistant to antibiotics. Silver nanoparticles/ions present powerful and antimicrobial tools that nullify the adverse effects of multidrug-resistant bacteria. For example, Shi *et al.*[69] prepared durable antibacterial Ag/polyacrylonitrile (Ag/PAN) hybrid nanofibers using atmospheric plasma treatment and electrospinning and then evaluated their silver-release profile over 10 days. Results showed that the silver ions were released in a slow and long-lasting manner, which was efficient in presenting excellent antibacterial activity. More recently, there have been several investigations on the use of silver nanoparticle (Ag NPs)-containing nanofibers for antimicrobial wound dressing applications. In one study, Dubey *et al.*,[70] reported the fabrication of Ag NPs-containing composite nanofibers based on poly (ethylene oxide) (PEO)/PCL as antibacterial wound dressing agents. Results showed that Ag NPs/PEO/PCL nanofiber exhibited a sustained release of Ag NPs after 24 h, which has high antibacterial potential against recombinant green fluorescent proteins expressing antibiotic-resistant Escherichia coli. Similarly, another study showed significant enhancement in the antibacterial activity of crosslinked AgNPs/chitosan/PVA nanofibers against Gram-negative bacteria Escherichia coli. The degree of crosslinking was inversely related to the rate at which the AgNPs were released as a higher crosslinking degree resulted in slower AgNPs release, and thus more potent antimicrobial activity.[71]

POLYMERIC SUBSTRATE FOR ELECTROSPINNING

Polymers and polymer-based composites have a long history of application in the design of synthetic bone grafts due to their design flexibility, biocompatibility, tunable degradability, processability, and consistent reproduction. The polymer scaffolds are enzymatically or hydrolytically degraded into smaller molecules *via* the cleavage of their functional linkages. The degradation rates can be controlled by modulating the degree of crystallinity or hydrophobicity of the polymer. Also, copolymerization and blending, along with the

compositional changes of the respective components, can be utilized to further tailor the rate of degradation of electrospun scaffolding material to specific needs. Typical polymers for electrospinning can be classified into natural polymers and synthetic polymers. Synthetic polymers possess relatively superior mechanical strength, while natural polymers are identical to the macromolecules present in the ECM, making them more biocompatible when compared to synthetic polymers. Meanwhile, electrospun material with optimal and desirable characteristics (such as mechanical properties or biocompatibility) could be obtained by blends of two synthetic polymers, natural polymers, or the combination of both in an ideal ratio. Since the pioneering work of Laurencin and colleagues on the development of polymeric electrospun nanofibers for biomedical applications, a broader range of natural and synthetic polymers have been employed in electrospinning technology for tissue regeneration and drug delivery.[72] In the following sections, we will discuss the different kinds of natural and synthetic polymers that have been investigated as substrates for electrospinning process.

Nanofibers Based On Natural Polymers

Various natural biodegradable polymers have been employed in electrospinning to produce nanofibrous substrates with anticipated properties. Owing to the similarity and striking resemblance of natural polymers to macromolecular compounds present in the body, they offer substantial benefits for use as scaffolding materials. Natural polymers, such as collagen, alginate, chitosan, silk, gelatin, glycosaminoglycan (GAG), *etc.* are the most widely investigated for nanofiber fabrication.[12, 73] They are characterized by excellent biocompatibility but suffer from relatively poor mechanical strength as compared to the synthetic counterparts. Herein, we provide a brief discussion on some of the natural polymers.

Collagen is regarded as the most abundant protein in the human body as it constitutes the main component of ECM in many musculoskeletal tissues. It possesses binding sites on its surface that helps to facilitate adhesion between substrate and cells. Structurally, Collagen has three polypeptide subunits arranged in a triple helix of elongated fibrils.[37] Reactive centers on the amino acid chemical structure can be exploited for crosslinking to augment its low young modulus. Fabrication of Collagen containing nanofiber scaffolds have been carried out using organic solvents or mixture solvents (such as 1, 1, 1,3,3,3 hexafluoroisopropanol (HFP), and formic acid/acetic acid) and the kind of solvent used can influence the morphology and cellular response of the fibrous mats. HFP is an excellent solvent for the formation of collagen fibers, but it is associated with a number of issues such as toxicity and denaturation of collagen. In an electrospinning study of pure collagen, Zeugolis *et al.,*[32] demonstrated that electrospinning of collagen, using HEP, causes some degree of denaturation, which leads to the formation of gelatin fibers. Gelatin, as well known, is a denatured form of collagen obtained through the denaturation of the triple helix. However, another study by Yang *et al.,*[35] reported that only 45% of the triple helical structure of collagen molecules could be denatured using HEP. Moreover, Lui *et al.,*[74] showed that using acetic acid, collagen nanofiber with more triple helical structures can be obtained as compared to collagen electrospun from HEP. Numerous studies have demonstrated the suitability of Collagen for the fabrication of biomimetic scaffolds that is

similar to the architecture of native human tissues. At the moment, the electrospinning of the collagen-based substrate is only possible with type I, II, III, and IV. Collagen can be electrospun into fibers with both aligned and random orientations, as these orientations serve different purposes. The fiber alignment plays a critical role in regenerative engineering as the mechanical properties and cell activity can be controlled by the arrangement of the fibers within the scaffolds. As aforementioned, collagen is inherently low in mechanical strength and, as such, crosslinking or blending with synthetic polymers is utilized to enhance the mechanical properties without a trade-off on his bioactivity. Also, collagen exhibits high water solubility and fast degradation, and thus crosslinking can be employed not only for improving strength but for reducing water solubility and improving resistance to enzymatic degradation.[37, 75] This expands its scope for regenerative engineering as rapid degradation of the scaffold may not allow enough time and support for the cells to lay out their own ECM. The incorporation of other polymers into collagen during the electrospinning process aids in modifying the morphology, strength, and biological properties of the composite nanofibers. Examples of the synthetic polymers that have been blended with collagen are as follows hydroxyapatite, PCL, poly (3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV) and PGA.[29, 76–78] It is uncommon practice to blend collagen with natural polymers. Nevertheless, previous studies by Chen *et al.,*[79] and Rnjak-Kovacina *et al.,*[20] have shown that collagen can be blended with chitosan and elastin, respectively, for the fabrication of composite nanofibers.

Gelatin is a well-investigated natural biomaterial that is a product of the denaturation of collagen. The processing methodology is deciding factor of the surface charge of gelatin as higher pH (pH 9) yields a negatively charged gelatin surface, and a lower pH (pH 5) results in a positively charged surface. Gelatin A has an isoelectric point around pH 9, whereas gelatin type B depicts an isoelectric around pH5. By using 25% glutaraldehyde as a crosslinking agent, the mechanical strength of gelatin nanofiber can be improved by crosslinking.[38] Young's modulus value of 33.8 MPa in axial direction was obtained for the crosslinked gelatin-based scaffolds as compared to the modulus value of 5-10 MPa for natural collagen. In one study, Salifu *et al.,*[42] reported the design of crosslinked electrospun orientated fibers composed of gelatin-hydroxyapatite (HA) and the investigation of the fate of human fetal osteoblast cells on them at different HA compositions. The study demonstrated that the composite with 25% HA witnessed the highest cell viability and ECM production while there was a significant improvement in the mechanical properties due to the fiber orientation. The possibility of blending gelatin with synthetic polymers, such as PCL, to produce nanofibers has been explored for uses as scaffolds for tissue regeneration. One study indicated that gelatin/PCL nanofiber blends exhibited enhanced mechanical properties and robust cell growth. Hence, the blending of natural polymers and synthetic polymers is a good strategy for augmenting the mechanical strength of natural biomaterials for regenerative engineering.

Silk fibroin is another promising natural biomaterial used for nanofibrous scaffolds. It is a natural protein fiber composed mainly of fibroin (which encompasses the core protein), and sericin (which make up the adhesive proteins). It is produced naturally from the cocoons of the larvae of silkworms. As a result of its excellent biocompatibility, extraordinary mechanical properties, and customizable degradation rates, silk has been employed as

medical material for suture in surgeries. Silk fibroin has been used in several tissue regenerative applications, including the healing and regeneration of critical-sized femur defects. Besides, the effect of processing conditions, such as degumming, on the properties of silk was investigated by Wray *et al.* It was found that cell viability, pore-forming ability, and the reproducibility of the silk were significantly imparted by degumming.[80] Drug molecules and growth factors could be loaded or immobilized into electrospun silk nanofibers for sustained release. A recent study by Mehraz *et al.,*[41] showed a controlled and slow release of the drug model (β-cyclodextrin) from β-cyclodextrin-grafted silk nanofibers. The ability to control and sustain the release of small bioactive molecules is essential for inducing differentiation of stem cells and regeneration of tissues. Nanoparticles such as hydroxyapatite can be incorporated into silk fibrous mesh to improve bone regeneration. In a rabbit study, Wang *et al.*,[81] examined the effectiveness of a silk fibroin/hydroxyapatite (SF/HA) composite for the repair of a segmental bone defect. Results indicated that the SF/HA supported the healing of the bone defect and presented a promising choice as a bone-graft substitute. Recently, Gao *et al.,*[82] discovered that the presence of Tussah silk fibroin (TSF) in a PLA/TSF composite nanofiber accelerated the nucleation and growth of hydroxyapatite on the surface of the scaffold. The PLA/TSF fibrous structure exhibited enhanced cytocompatibility, osteoblast differentiation, and mechanical properties.

Hyaluronic acid is a glycosaminoglycan, which is a component of the ECM of many soft tissues of animals, especially mammals. Over the past decades, it has witnessed an increasing interest in the biomedical field. However, electrospinning of pure hyaluronic has a posed challenging issue due to its polyelectrolytic nature as high solution viscosity is often observed at low polymer concentration. Hence, it becomes difficult to reach the critical chain entanglement concentration required for electrospinning to occur. To overcome this drawback, Kim *et al.,*[83] reported the use of sodium hydroxide: dimethylformamide (NaOH: DMF) system. The use of NaOH: DMF as an electrospinning solvent ensured the successful production of electrospun nanofibers; however, the diameter of the fibers was well above 100 nm. A recent study by Liu *et al.*,[84] showed the successful fabrication of hyaluronic acid nanofibers with a mean diameter below 100 nm using a combined solution of deionized water, formic acid, and DMF. The addition of formic acid was believed to have positively impacted the electrospinnability of the hyaluronic acid solution. In addition, a follow-up study by Brenner *et al.,*[85] showed a drastic improvement in the fiber diameter of the electrospun hyaluronic acid nanofibers using a less basic (pH 11) aqueous ammonium hydroxide (NH_4OH) and DMF solvent system. The average diameter of the fibers produced was reported to be around 39 ± 12 nm, and there was no evidence of degradation while using the NH_4OH: DMF electrospinning solvent.

Alginate is a natural multifunctional polymer that has found numerous applications in the biomedical field due to its exceptional biocompatibility. It is increasingly being utilized as hydrogels for wound healing, drug delivery, *In Vitro* cell culture, and tissue regeneration as a result of its mild gelation conditions. However, the electrospinning of alginate has been problematic as aqueous sodium alginate can only be electrospun into fibers by blending with another polymer such as polyethylene oxide (PEO). Saquing *et al.*,[86] suggested that the incorporation of PEO as a carrier polymer reduces the electrical conductivity and surface tension of the alginate solution, thereby facilitating the formation of the fibers. PEO

provides the necessary molecular entanglement for electrospinning. Alginate-based nanofibers have demonstrated great utility and potential as scaffolding materials for tissue regeneration and drug delivery systems. For example, Xu *et al.,*[24] demonstrated that alginate/PLA composite nanofiber membranes have improved mechanical properties and exhibited excellent cell adhesion, growth, and migration. In another study, Kyziol *et al.,*[87] showed that alginate/PEO composite nanofibers loaded with ciprofloxacin hydrochloride (CpHCl) can control the release of the CpHCl drug molecules for effective therapy.

Chitosan is an amino-based polysaccharide that is commercially produced from chitin by alkali deacetylation. Chitin, as a precursor of chitosan, is the second most abundant natural polymer in the world and it is obtained from crustaceans and insects. The diverse biological activity of chitosan biopolymer has made it a material of choice for most tissue repair studies. Its antibacterial and antifungal properties have been exploited for use as a potential antimicrobial agent for drug delivery and wound dressing applications. For example, Bergamo *et al.,*[33] designed electrospun double-layer chitosan scaffolds composed of polycaprolactone or polycaprolactone/cellulose acetate blend (PCL/CA) as the first layer and chitosan/poly (ethylene oxide) blend (CHI/PEO) as the second layer. The first layer provides mechanical support, while the CHI/PEO layer will be in direct contact with the wound surface. The uniform distribution of the randomly orientated fibers of the scaffolds presented interconnected porosity that allowed cell chemotaxis, adhesion, and tissue repair. Besides, the double layer porous scaffolds maintained a moist environment at the wound/dressing interface, which essential for application as dressings for skin lesions. This study suggests that chitosan nanofibers could be an excellent candidate material for regenerative engineering.

Nanofibers Based On Synthetic Polymers

Synthetic polymers constitute the largest class of biomaterials, and a good number of them have been utilized to produce electrospun nanofibers for various tissue regeneration applications. The widely used synthetic polymers and copolymers for regenerative engineering are PLA, PLGA, PCL, PEG, and poly (ethylene terephthalate) (PET).[12] Synthetic polymer nanofibers are characterized by their easy preparation and reproducibility. They are sturdy and possess excellent mechanical properties; however, they exhibit relatively low biocompatibility. This section discusses the nanofibers that are fabricated with at least a synthetic polymer component, and this comprises the synthetic polymer nanofibers, synthetic/synthetic polymer nanofibers, and synthetic/natural nanofiber fibers.

In 2002, Laurencin and colleagues developed synthetic polymer-based nanofibers that were used to modulate cellular activities.[72] The electrospun nanofibrous structures were composed of PLGA with fiber diameter ranging from 500 to 800 nm. The results of this pioneering investigation were encouraging as the structures supported cell growth and maintained phenotypic expressions. Also, there is an emerging interest in conducting polymers such as polyaniline (PANI), polypyrrole and polythiophene phenylene (PThP) since advances in cell mechanics and materials science have provided an avenue for designing scaffolds that not only serve as physical support to the cells but induce cellular responses and growths through electrical signals and cues.[88–93] A recent study by Garrudo *et*

al.,[94] reported that electrospun PANI/PCL nanofiber presented a viable and electrically conductive surface that supported the attachment and proliferation of cultured neural stem cells (NSCs) for neural tissue engineering applications. Similarly, Soleimani *et al.,*[95] showed that the incorporation of PANI and graphene into gelatin could yield electroactive PANI/graphene/gelatin nanofibrous scaffolds with optimal conductivity and cell viability, making them suitable scaffolding materials for tissue regeneration. Similar results have been reported for PLA/PANI, PANI/PEO, and polyvinyl alcohol (PVA)/PANI nanofibers, where enhanced cell-material interactions were demonstrated.[34, 96, 97] Additionally, Polypyrrole-based nanofibers have demonstrated high biocompatibility, sustained conductivity, and suitability for tissue regeneration.[98] Another study by Chan *et al.,*[99] reported the possibility of incorporating a peptide-containing arginylglycylaspartic acid (RGD) fragment into PThP conducting polymers blended with PLGA for electrospinning. The RGD-grafted PThP/PLGA nanofiber mat showed excellent cytocompatibility with human dermal fibroblasts-adult (HDFa) and human epidermal melanocytes-adult (HEMa) cells and maintained electrochemical activity. Interestingly, our group recently developed a stimuli-responsive and conductive nanocomposite matrices made up of PEDOT: poly (styrenesulfonate) (PSS), and dopamine-functionalized PCL.[93] The designed matrices enhanced the activities of myoblast muscle cells and depicted tunable conductivity and biocompatibility. In another study, we fabricated tripolymeric triaxial electrospun nanofibers with dual drug delivery capability. The tripolymeric fibrous scaffolds, which are composed of PCL (core layer), PLGA (sheath), gelatin (intermediate layer), supported mesenchymal stem cell growth and showed adequate mechanical competence while controlling the release of the model molecules, desired for regenerative engineering.[60] Another interesting class of synthetic polymers that have undeservedly attracted little attention is the polyphosphazene polymers. Polyphosphazenes have great synthetic versatility and have been extensively investigated for a variety of biomaterial applications.[5, 23] Unlike the other synthetic polymers (especially polyesters), they degrade into milder and near-neutral degradation products.[100] They have been reported by several studies to show excellent biocompatibility and high degradation tunability.[101–104] Extensive efforts have been made toward the development and use of polyphosphazene nanofibers for regenerative engineering. In one of the studies, Deng *et al.,*[101] designed dipeptide-containing polyphosphazene and PLGA blend nanofibers to imitate the dimensions of collagen fibrils present in the natural bone ECM. The polyphosphazene-based nanofiber scaffolds showed appropriate mechanical properties and presented suitable porosity that allowed the infiltration of osteoblast cells and ECM secretion. Meanwhile, the adhesion, proliferation, and phenotypic expression of the cells were well promoted on the fibrous scaffolds, suggesting high potentials for bone regenerative engineering.

CONCLUSION AND FUTURE OUTLOOK

In the past two decades, the field of biomedicine has witnessed tremendous progress and breakthroughs in several areas. The electrospinning technology is one such area that has enabled the facile fabrication of electrospun nanofibers for use as scaffolding materials for tissue regeneration. Nanofibrous materials are emerging as a promising biomimetic substrate that can mimic the properties and structural features of the native ECM and contribute to the development of optimal tissue microenvironment. The architecture of nanofiber mats plays

an essential role in modulating and regulating cellular response and subsequent tissue development as it presents desirable characteristics such as a large surface area to volume ratio, highly porous and patterned structure, and broader window for surface modifications and enhanced mechanical functions. Various biocompatible polymers, including natural and synthetic, have been utilized in the electrospinning process for regenerative engineering and other medically related applications. A variety of bioactive molecules have been incorporated into nanofibers before electrospinning, or by surface functionalization of the electrospun mats for sustained release. The improvement in the porosity and surface area of the nanostructured and nanofiber-based scaffolds ensures higher bioavailability and appropriate cues for enhanced cell-material interactions.

Despite the innovations, biomimicry, and effective drug delivery offered by electrospun nanofibers, there is still much left to be done as full regeneration has not been attained. Future directions for addressing this particular issue will be to integrate a programmable delivery system into nanofiber-based regenerative scaffolds, and this will present exciting avenues for tissue regenerations. Recently, stem cell therapy has shown excellent prospects in regenerative engineering. The optimization of the cell microenvironment using nanofiber scaffolds will be of benefits to stem cell therapy as the fiber parameters can be tailored accordingly to meet specific needs for different cell types. Recent advances in induced pluripotent stem cells (iPSCs) have provided a means of producing a variety of cell types for various regenerative purposes, and this helps in facilitating the clinical translation. It will be exciting to consider the incorporation of nanofiber fragments into 3D printing for the development of gradient scaffolds for interfacial tissue regeneration. The exploitation of the ability of nanofibers to exhibit different stiffness and drug-loading capacity will allow the design of smart 3D-nanostructured scaffolds with precise spatial and temporal control. The convergence and optimization of 3D printing and conventional electrospinning technologies could represent a game-changer that would revolutionize biomaterial-based scaffold design and strategies for effective tissue regenerations.

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VOCABULARY

Extracellular matrix (ECM)
is a non-cellular three-dimensional macromolecular network that provides structural and biochemical support to the surrounding cells.

Durotaxis
is the coordinated migration of the cells in response to the rigidity gradients, which result from differential structural characteristics of the extracellular matrix.

Osteoconductivity
is the tendency of a biomaterial to promote the attachment and subsequent growth of bone cells seeded on it

Osteoinductivity
is the ability of a biomaterial to induce osteogenesis with biomimetic substances, such as bone morphogenetic proteins.

Integrin
is a transmembrane receptor used by cells to bind unto an extracellular matrix.

Cytocompatibility
is the property of not being harmful to the cells

Dual-scale scaffold
is a scaffold with both electrospinning and 3D printing components

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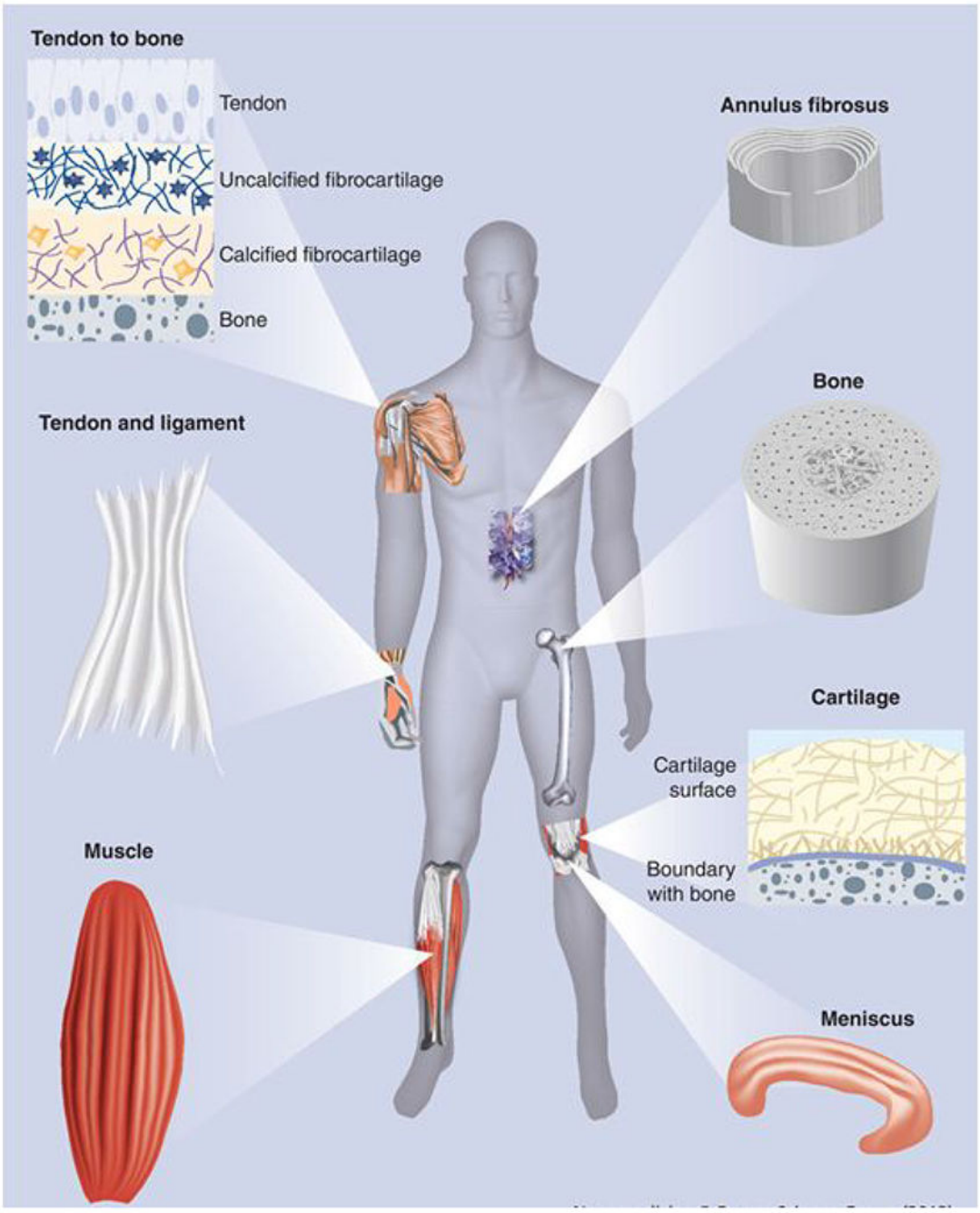

Figure 1.

The organization of collagen nanofibers found in different musculoskeletal tissues. The tendon and ligament tissues are composed of collagen nanofibers that are uniaxially aligned and wavy. Collagen fibers showed aligned-to-random orientations at tendon-to-bone insertion sites and circumferential alignment in the meniscus and annulus fibrosus. Collagen fibers in the cartilage tissues are arranged in layers. Reprinted with permission from ref 13. Copyright 2013 Future Medicine Ltd.

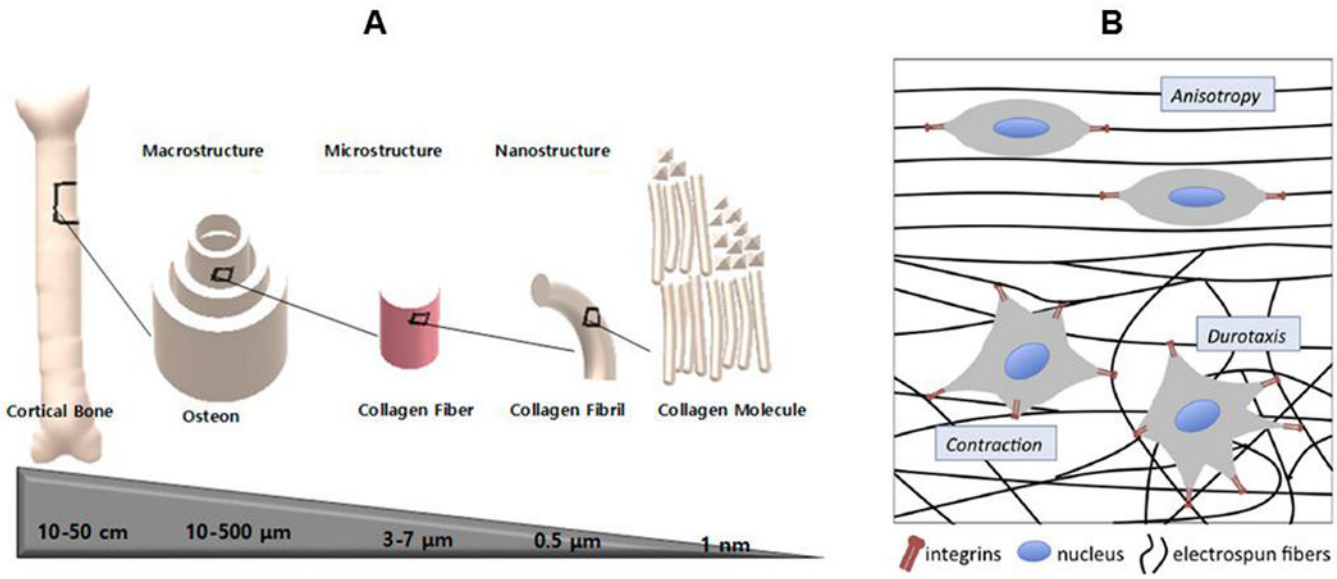

Figure 2.

(A) Diagram showing the hierarchical macrostructure constructs of bone along with the cylindrical shape of osteon construct, and microstructure/nanostructure of collagen. Reprinted with permission from ref 14. Copyright 2018 MDPI. (B) Schematics of cell-nanofiber interactions showing the attachment of cell cytoskeleton to the fibers with the aid of integrins. The anisotropic arrangement and alignment of the fibers influence the morphology of the cells and matrix stiffness regulate the cellular migration (durotaxis) as the cells exhibit contraction when in contact with soft region of the matrix. Reprinted with permission from ref 44. Copyright 2017 Elsevier.

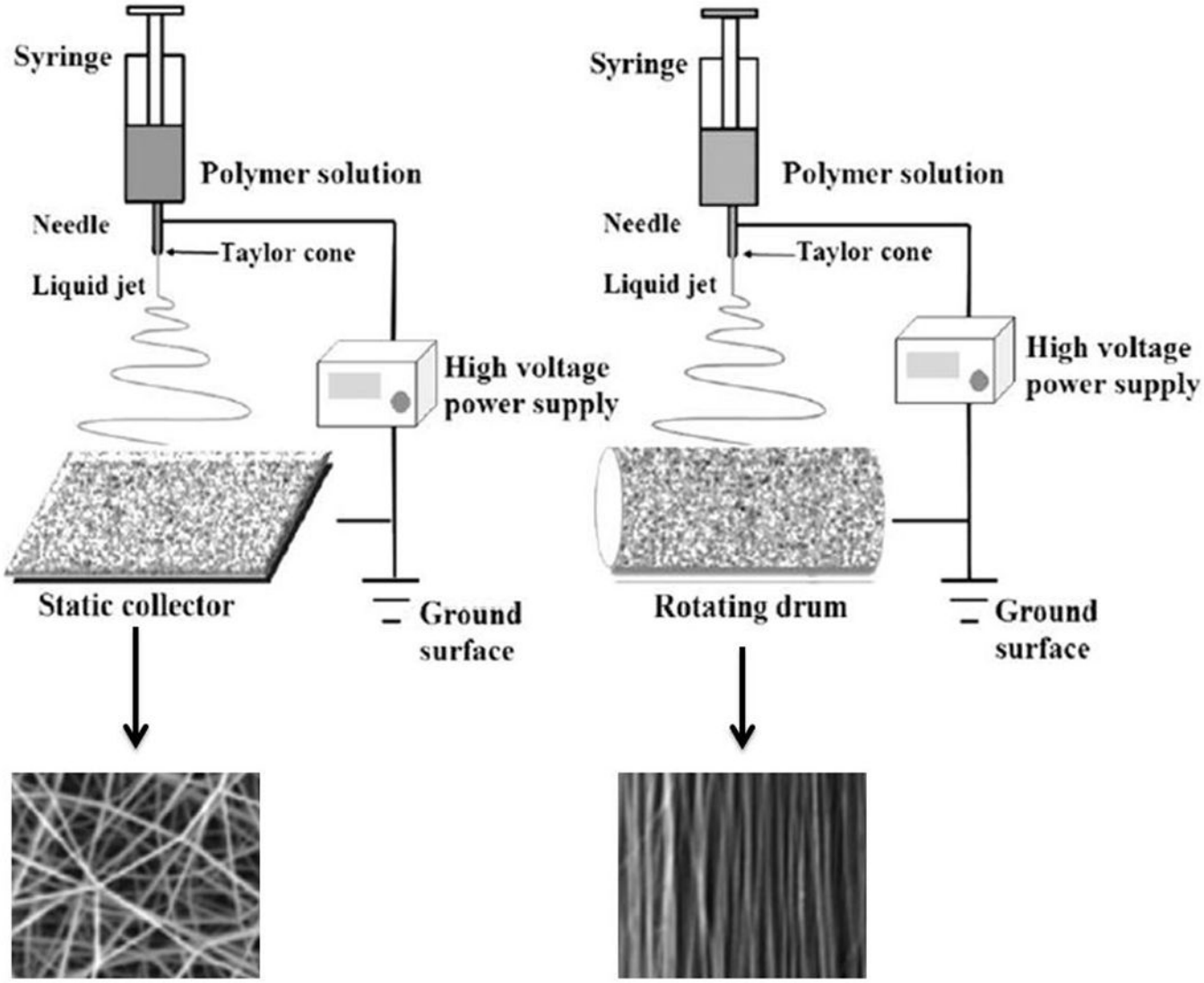

Figure 3.

Schematic diagram of electrospinning process with static and rotating collectors. Randomly-oriented nanofibers are collected on the static collector, while the rotating drum produces uniaxially aligned nanofibers. Reprinted with permission from ref 30. Copyright 2018 Georg Thieme Verlag KG.

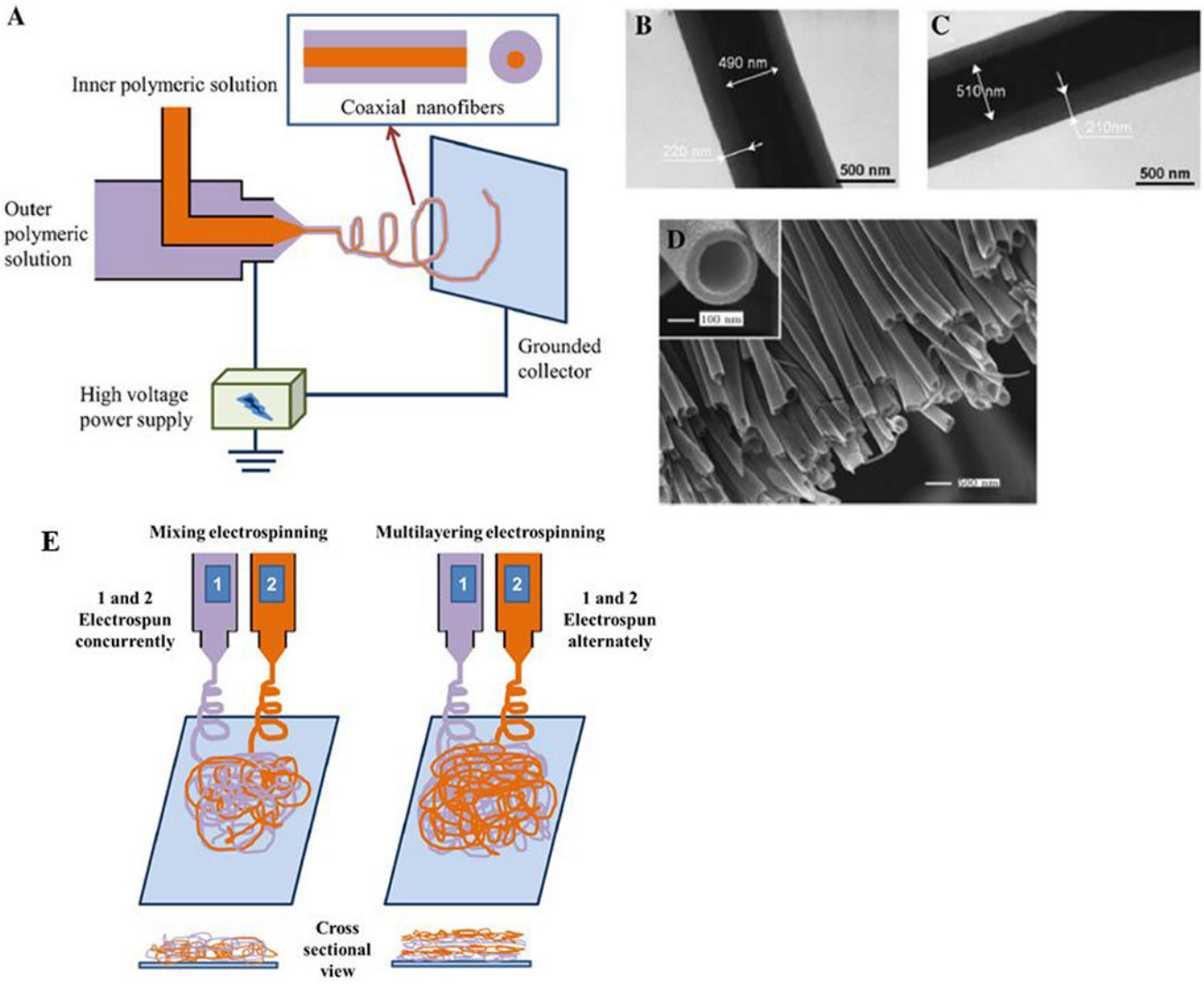

Figure 4.

Diagrammatic illustrations of coaxial, mixing and multilayering electrospinning systems **(A)** coaxial electrospinning involving the extrusion of two different polymeric solutions through the inner and outer needle coaxial spinneret **(B and C)** Images of transmission electron microscopy for coaxially electrospun nanofibers **(D)** The microscopic view of hollow nanofibers. **(E)** Mixed and multilayered nanofibers using mixing and multilayering electrospinning process respectively. Reprinted with permission from ref 37. Copyright 2017 Springer Nature.

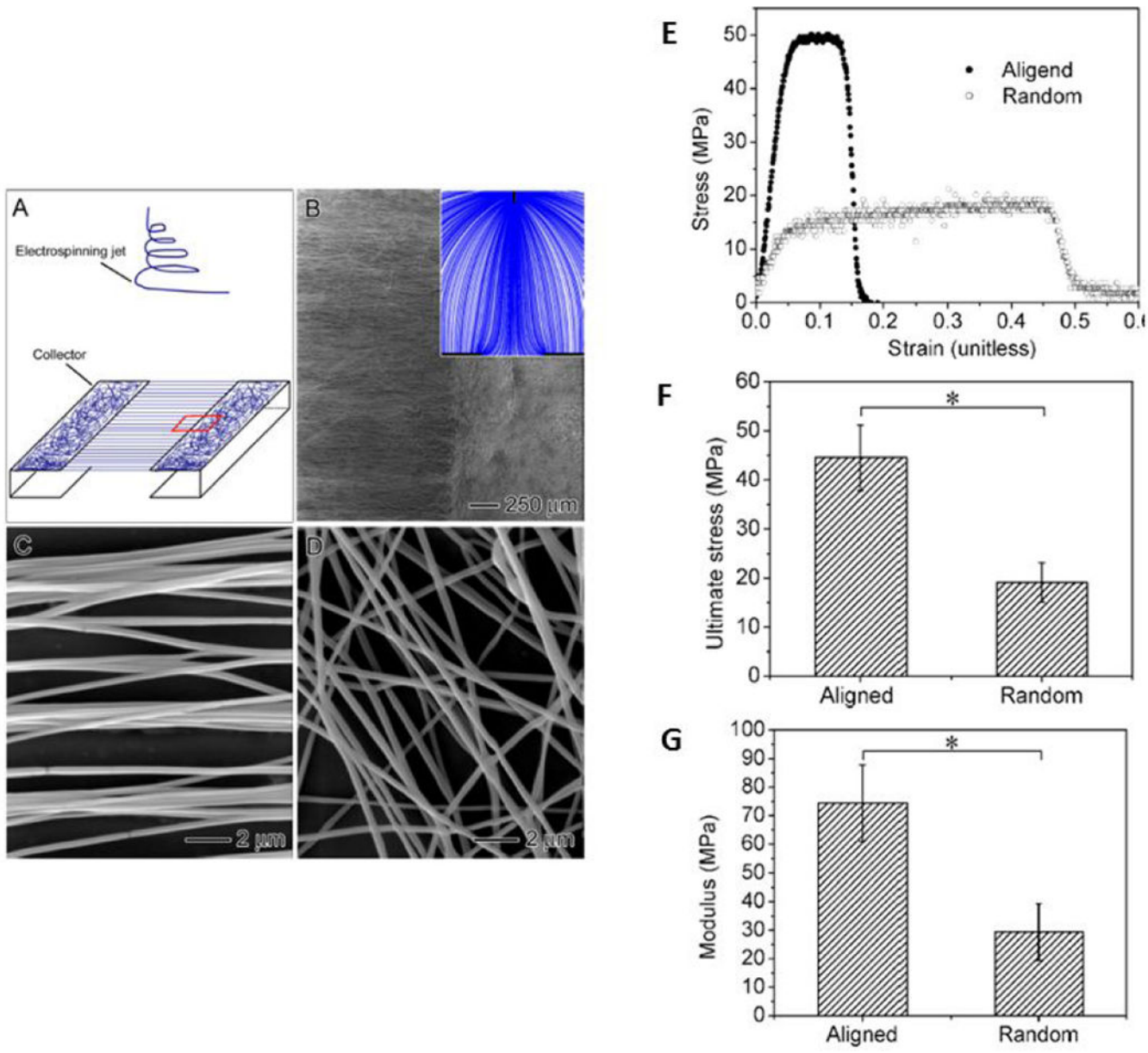

Figure 5.

Electrospun nanofibers with aligned-to-random orientations **(A)** Experimental setup employed in fabricating aligned-to-random nanofiber scaffolds **(B)** SEM images showing the random and uniaxial aligned PLGA with the random orientation on the left and aligned orientation on the right **(C-D)** Random and aligned fibers with high magnifications views **(E-G)** Mechanical properties of the aligned and random nanofiber scaffolds. Aligned nanofibers exhibited higher modulus and ultimate stress. Reprinted with permission from ref 46. Copyright 2009 Royal Society of Chemistry.

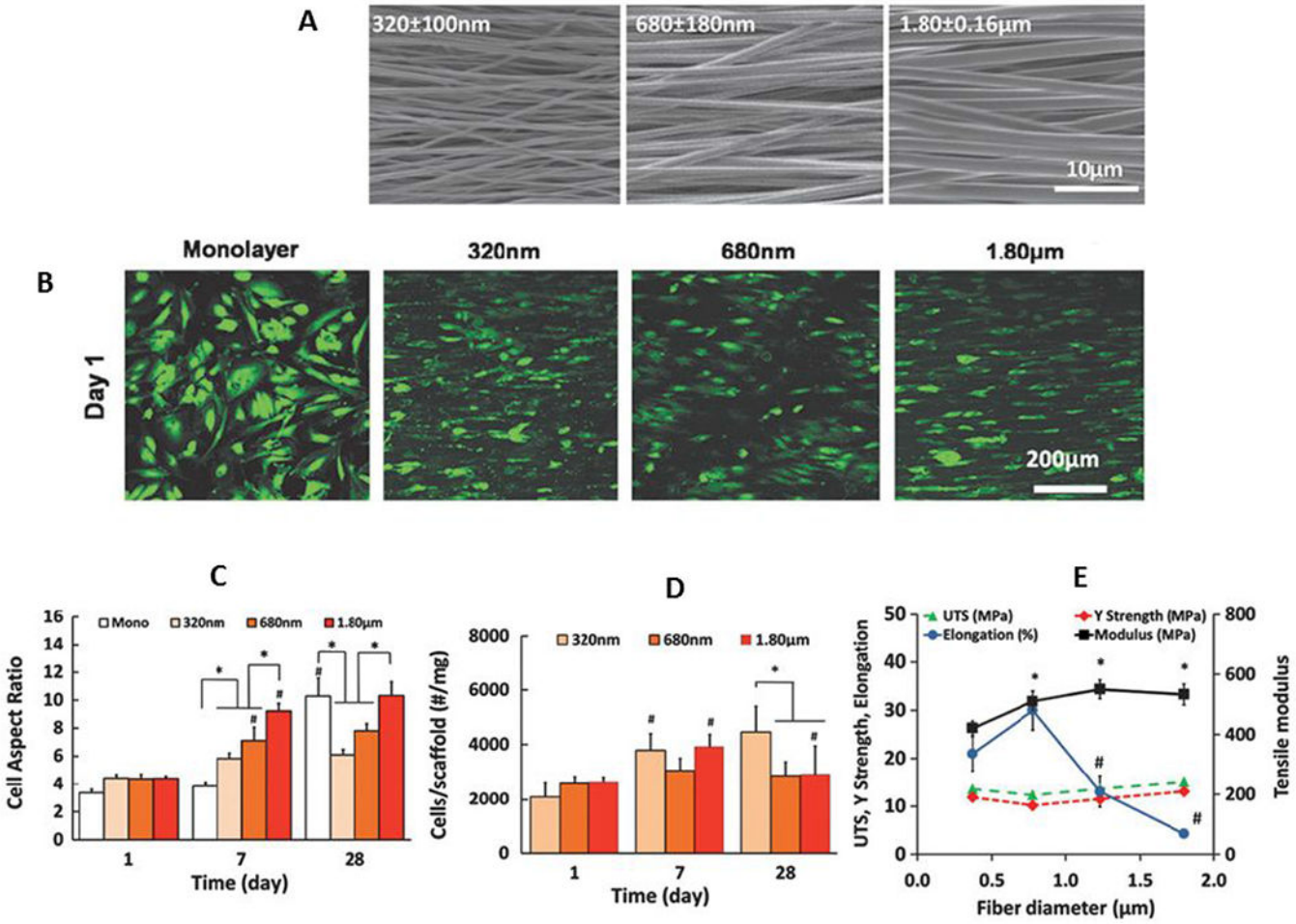

Figure 6.

Effects of fiber diameters on the cellular activities and mechanical properties of scaffolds **(A)** SEM images of nano and microfibers with different diameters **(B)** Live/dead cell viability assay on the scaffolds **(C)** Time-dependent changes of cell aspect ratios. **(D)** Proliferation of cells with culture time **(E)** the changes in tensile properties with varying diameters. Reprinted with permission from ref 47. Copyright 2013 Mary Ann Liebert, Inc.

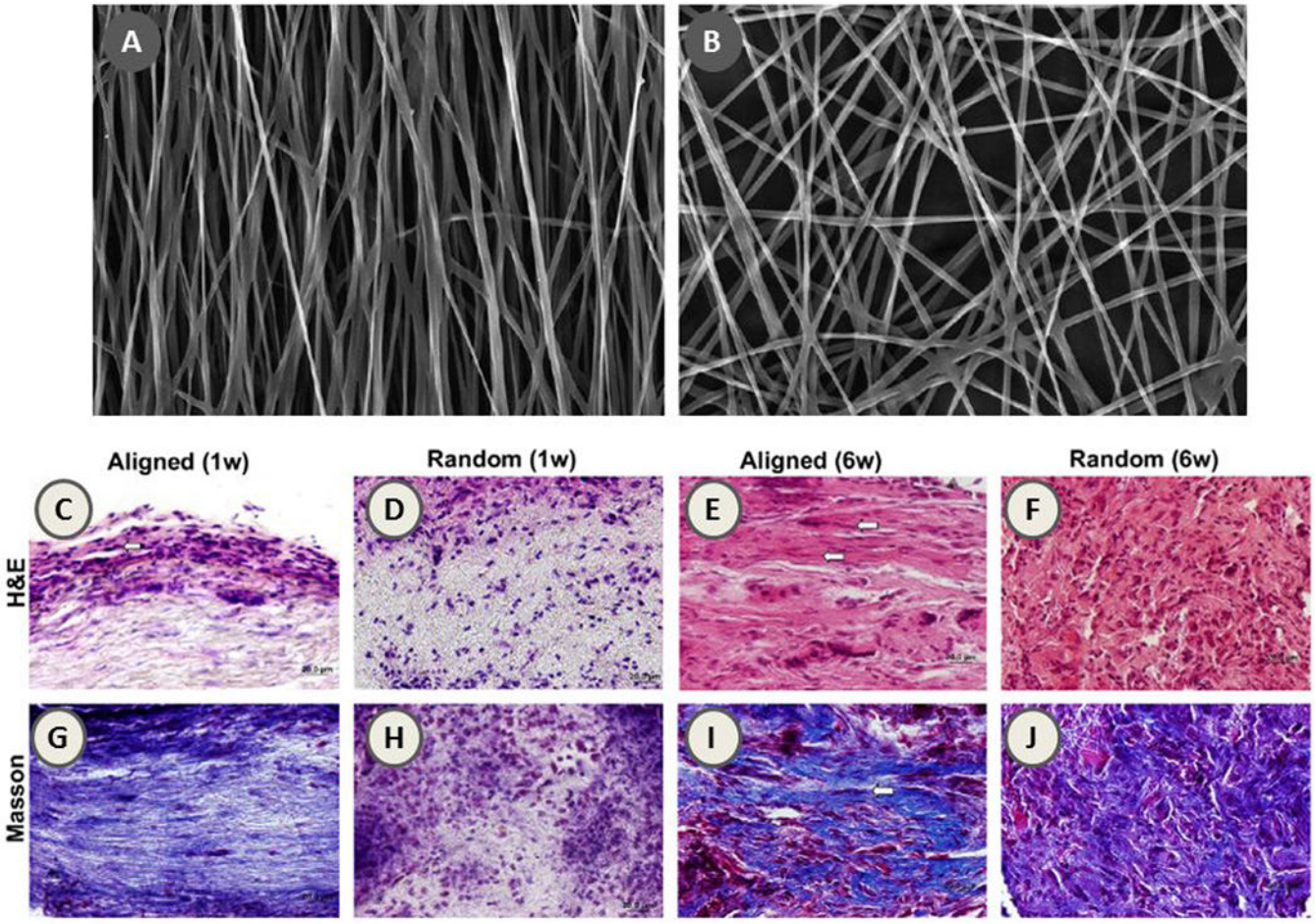

Figure 7.

Electrospun nanofibers of PLLA **(A)** SEM image of aligned nanofiber **(B)** randomly-oriented nanofiber scaffold **(C-F)** hematoxylin and eosin staining histology of nanofiber-induced tissue formation **(G-J)** Masson trichrome staining showing the formation of collagen fibers on the aligned and random fibrous scaffolds. Reprinted with permission from ref 51. Copyright 2010 Elsevier.

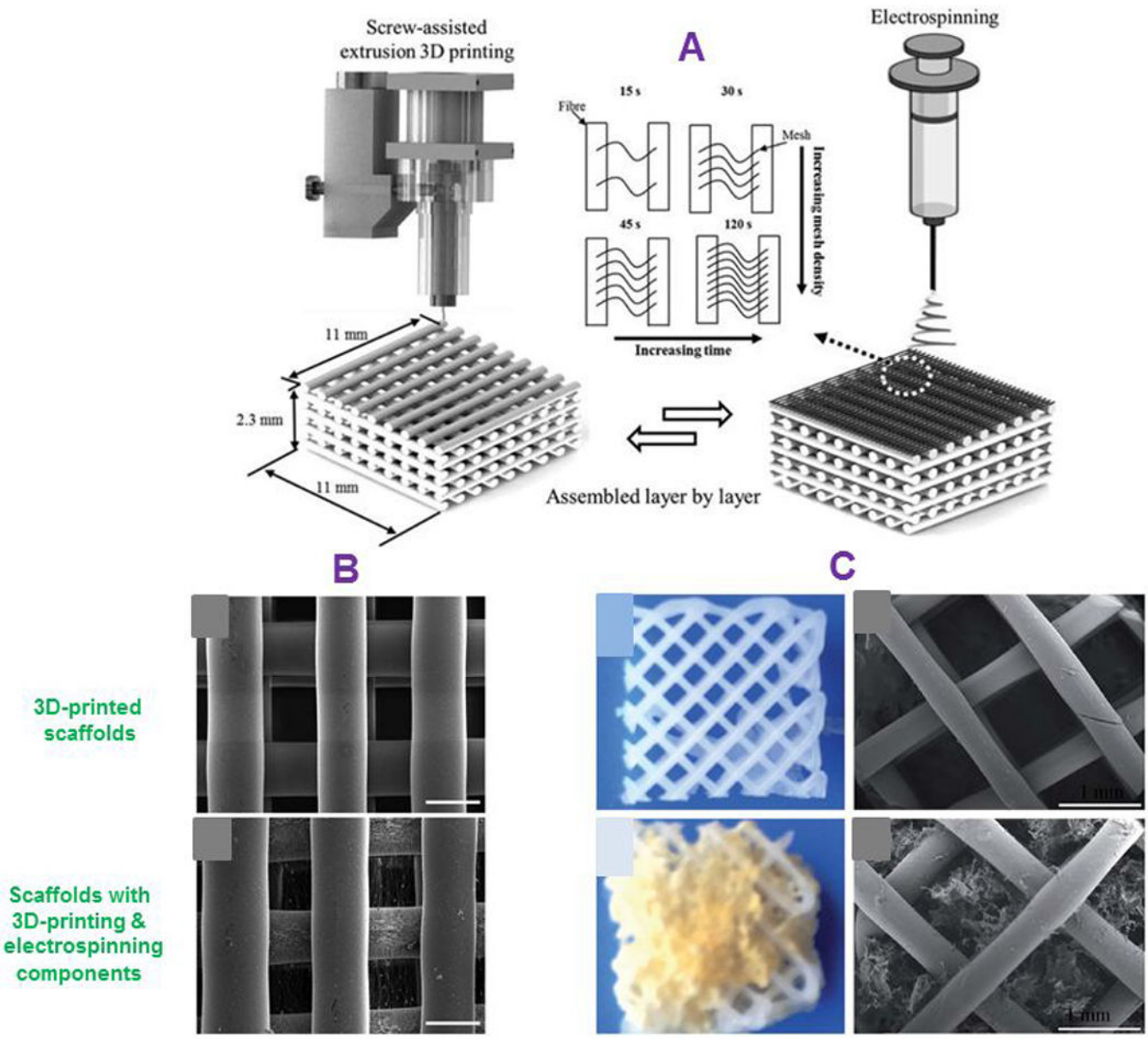

Figure 8.

Convergence of 3D printing and electrospinning Technologies (A) Fabrication of dual-scale scaffold with meshes (mesh densities of 15s, 30s, 45s, & 120s) evenly distributed throughout the scaffold by electrospinning onto the scaffold at appropriate layer during printing (B) SEM images of a 3D-printed scaffold and dual-scale scaffold with electrospun nanofibers. The dual-scale scaffold is obtained when nanofibers are electrospun directly onto the scaffold during printing [scale bar = 300μm]. Reprinted with permission from ref 59. Copyright 2020 Mary Ann Liebert, Inc. (C) Photo and SEM images of 3D-printed scaffold and 3D composite scaffold. The composite scaffold is obtained by infusing the dispersed nanofibers into the meshes of 3D-printed scaffold [scale bar = 1mm]. Reprinted with permission from ref 58. Copyright 2016 Royal Society of Chemistry.

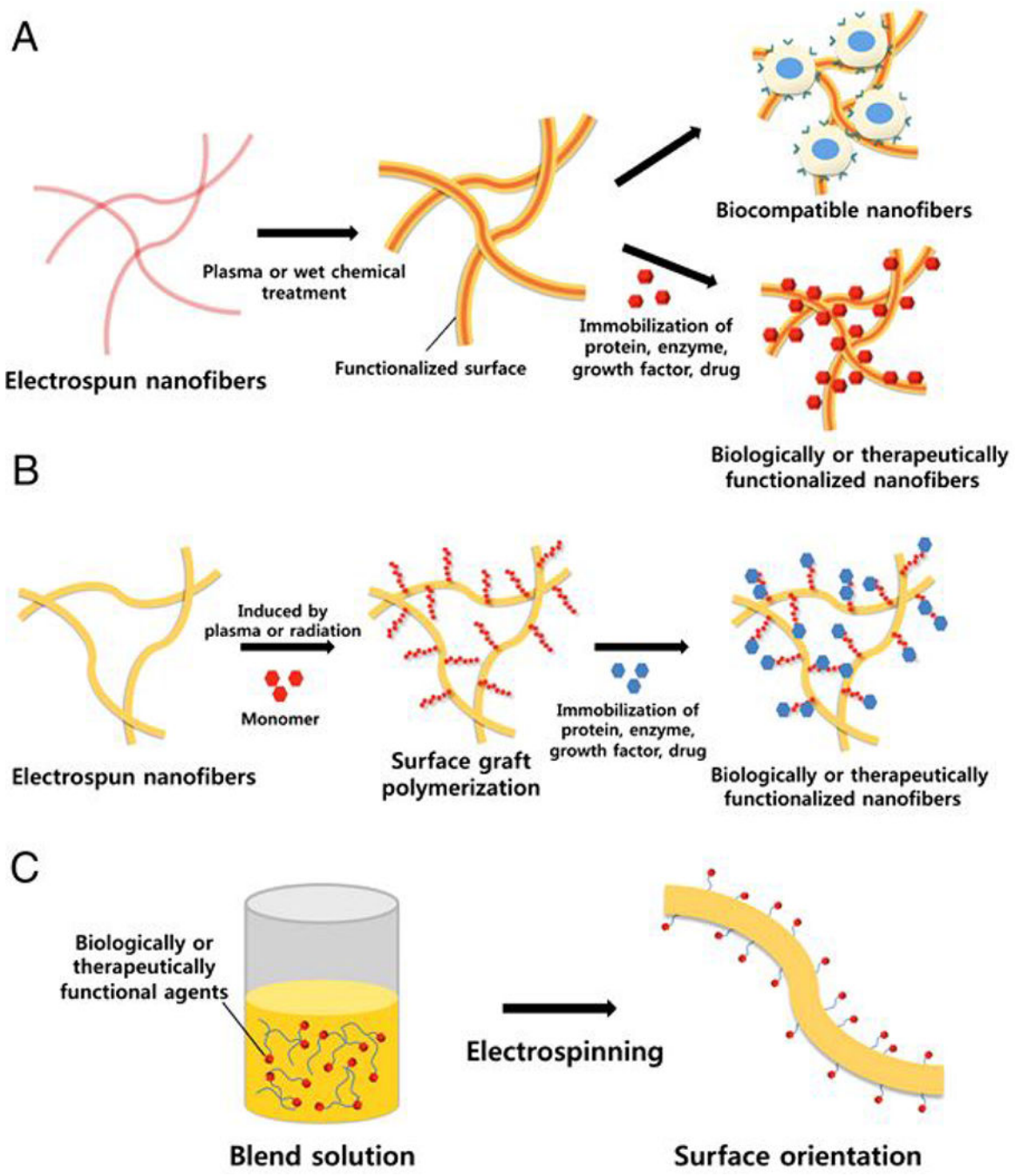

Figure 9.

Schematic diagram showing different modification methods used to integrate bioactive molecules to nanofibers **(A)** treatment by plasma **(B)** Immobilization of bioactive molecules by surface graft polymerization **(C)** co-electrospinning of substrate and bioactive agents. Reprinted with permission from ref 61. Copyright 2009 Elsevier.

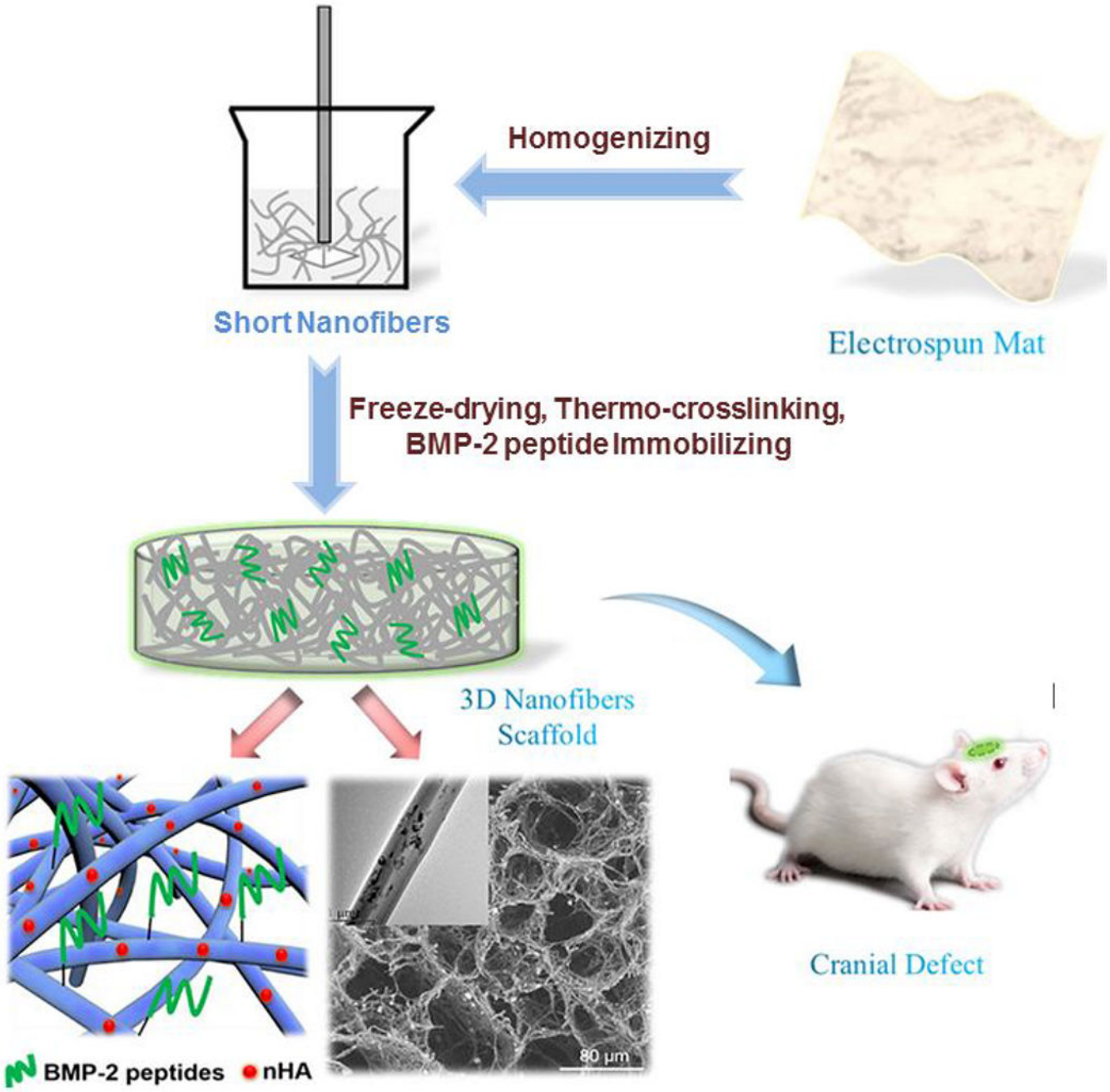

Figure 10.

Preparation of three-dimensional (3D) electrospun nanofibrous scaffold for the regeneration bone tissues using a rat cranial bone defect model. Reprinted with permission from ref 67. Copyright 2019 Elsevier.