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Electrosprayed nanoparticles and electrospun nanofibers based on natural materials: applications in tissue regeneration, drug delivery and pharmaceuticals

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Abstract

Nanotechnology refers to the fabrication, characterization, and application of substances in nanometer scale dimensions for various ends. The influence of nanotechnology on the healthcare industry is substantial, particularly in the areas of disease diagnosis and treatment. Recent investigations in nanotechnology for drug delivery and tissue engineering have delivered high-impact contributions in translational research, with associated pharmaceutical products and applications. Over the past decade, the synthesis of nanofibers or nanoparticles via electrostatic spinning or spraying, respectively, has emerged as an important nanostructuring methodology. This is due both to the versatility of the electrospinning/electrospraying process and the ensuing control of nanofiber/nanoparticle surface parameters. Electrosprayed nanoparticles and electrospun nanofibers are both employed as natural or synthetic carriers for the delivery of entrapped drugs, growth factors, health supplements, vitamins, and so on. The role of nanofiber/nanoparticle carriers is substantiated by the programmed, tailored, or targeted release of their contents in the guise of tissue engineering scaffolds or medical devices for drug delivery. This review focuses on the nanoformulation of natural materials via the electrospraying or electrospinning of nanoparticles or nanofibers for tissue engineering or drug delivery/pharmaceutical purposes. Here, we classify the natural materials with respect to their animal/plant origin and macrocyclic, small molecule or herbal active constituents, and further categorize the materials according to their proteinacious or saccharide nature.

Key words: Drug delivery, electrospinning, electrospraying, nanofiber, nanoparticle, natural material, tissue engineering.

1. Introduction

Nanotechnology is a rapidly emerging cross-discipline program that manipulates assorted synthetic and naturally occurring materials in nanoscale dimensions (1–1000 nm). The advent of nanotechnology platforms has modernized several branches of science and engineering, and contributes to society by impacting renewable energy, energy storage, electronics, the oil and gas industries, water and air filtration, military applications, design and production of cosmetics, agriculture, the food industry, healthcare, the pharmaceutical industry, and the fast-moving consumer goods (FMCG) industry (Fig. 1).[1-8]

Because of their large surface area-to-volume ratio, nanomaterials enable revolutionary advances in almost every industry, greatly improving effectiveness in production and/or delivery. For example, division of a 1-mm^3 rigid sphere into 10^{18} nanospheres of 1 nm each increases the effective surface area of a drug or a cosmeceutical by 10^6-fold. Furthermore, many physical/physicochemical, optical, and electronic properties are tunable, depending on the size, shape, and composition of the amalgamated nanomaterials. Eventually, the utilization of nanomaterials culminates in a lowering of material costs together with an increase in performance and, hence, nanomaterials are now indispensable in healthcare and medicine.

The immense promise that nanotechnology delivers has inspired researchers from numerous scientific, engineering, and healthcare disciplines to take on new challenges. Both the structure and the composition of nanomaterials can be varied and custom-designed to the end-use application by applying the appropriate synthetic method.[9,10] For instance, solid liquid nanoparticles (SLNs) are manufactured to possess a solid lipid core with an average diameter of ~10–1000 nm stabilized by surfactants, and the lipophilicity of SNLs overcomes major disadvantages of traditional drug delivery.[11] Along the same lines, liposomes

correspond to phospholipid bilayer membrane vesicles that are utilized as microscopic nanocarriers, with great promise for gene delivery and cancer therapy.[12] Quantum dots, for their part, have a wide range of application as transistors, solar cells, and light-emitting diodes, as well as in medical imaging.[13] Polymer nanoparticles also have many applications in electronics, photonics, medicine, biotechnology, pollution control, and environmental technology, and are used as conducting materials, sensors, and so forth.[14] Dendrimers mimic the properties of micelles, liposomes, and some biomolecules, and are used as drug carriers and immunogens.[15] Nanofibers find application as carriers for cell/tissue regeneration, as scaffolds in wound dressing, and as drug delivery systems.[16] Nanorods are employed as gas sensors, computer components, nanoelectric/nano-optoelectronic components, and in video displays, microelectromechanical systems, and solar energy conversion.[17]

Several methods are available for the fabrication of nanofibers and include electrospinning (to produce random, aligned, core-shell, and vertical nanofibers),[18-28] electrospraying,[29,30] rotary jet spinning,[31] self-assembly,[32] sol-gel methods,[33] phase separation,[34] melt-blown protocols,[35] and template synthesis.[36] Electrospinning surpasses the other methods in relation to efficiency and has emerged as the most efficacious technique for nanofiber production. The electrospinning process is continuous (and, therefore, scalable), applicable to many different polymers, and yields excellent control over nanofiber dimensions and orientation. A reasonable number of electrospun nanofiber formulations have now found utility as commercial products (Fig. 2). Similarly, synthesis of nanoparticles by electrospraying surmounts limitations associated with other fabrication processes, exemplified by emulsion/evaporation, coacervation, spray-drying, nanoprecipitation, and microfluidic methods. Thus, syntheses of nanoproducts by electrospinning or electrospraying is currently an essential component of nanomaterial research (Fig. 3).

Metal oxide nanofibrous composite materials are employed for their high solar energy-to-electrical energy conversion ratio, whereas a high chemical-to-electrical energy conversion ratio is achieved by the use of nanofibrous electro-catalytic materials. Additional metal oxide nanoparticle-incorporated nanofibers are utilized in the manufacture of protective clothing materials with military applications. The active antimicrobial component in the metal oxide nanofibers shows a selective interaction against bacterial pathogens, also rendering these nanofibers applicable to use as air filters and antimicrobial textiles.

Electrospun nanofibrous scaffolds and nanoparticles resemble the nanodimensional features of the native extracellular matrix (ECM), which in turn directs numerous aspects of cellular organization and survival. Due to the diversity of both the elastic modulus of solid tissues and the cellular microenvironment, the electrospinning process can be modified to generate selective scaffolds for each individual application. In addition, the diameter of electrospun nanofibers and the fiber orientation can be fine-tuned to influence cell attachment, proliferation, migration, and differentiation.[37-39] When native proteins, growth factors, or deoxyribonucleic acid (DNA) are transformed into nanofibrous or nanoparticulate scaffolds, their interactions with the body are also fine-tuned in ways that allow these natural materials to play exciting roles toward resolving previously unmet healthcare issues.

Both nanofibers and nanoparticles with suitable biocompatibility and biodegradability show utility for applications in tissue engineering[40-44] and drug delivery/pharmaceuticals.[45-47] Nanofibers have a huge advantage over conventional materials for topical pharmaceutical/cosmeceutical applications in the form of easy-to-use nanofibrous membranes (Fig. 4). Drug-loaded nanofiber stents are also under investigation by various research groups, because of the membranous nature of the stent and its capacity to form a layer on the

surface of conventional stents. Electrosprayed nanoparticles can be directly sprayed onto conventional stents, and also delivered via inhalation, oral, and ocular routes.

Injectable nanoparticles[48] have been widely studied for cardiomyoplasty applications, both by means of acellular and cellular approaches for the regeneration of the infarcted myocardium (Fig. 5).[49] In this regard, electrosprayed poly(lactic-co-glycolic acid) (PLGA) porous beads were cellularized with human amniotic fluid stem cells and tested as injectables.[50] The microporous, cellularized microparticles improved regional contractile capacity together with neovascularization and myocardial regeneration. Li et al.[51] went on to report the fabrication of oxygen-releasing, core-shell microparticles by electrospraying, and used these microparticles together with cardiosphere-derived cells and thermosensitive hydrogels made from poly(hydroxyethyl methacrylate/oligo-hydroxybutyrate) for cardiac regeneration. These findings and others support the use of nanofibers and nanoparticles as part of the next generation of more complex, combinatorial medical device products with improved drug delivery (Fig. 6)[52] and tissue engineering capabilities (Fig. 7).[53,54]

An intriguing strategy that can potentially expand the biomedical utility of electrospun nanofibers is the development of cell electrospinning (i.e., electrospinning/electrospraying of viable cells into a biopolymer matrix).[55,56] Jayasinghe et al. [57] recently electrosprayed human blood[57] and ascertained the feasibility of electrospinning or electrospraying for biomedical applications with viable cells. Subsequent improvisation in the spinning process, as well as the use of thermosensitive polymers, yielded cell-bearing, three-dimensional (3D) scaffolds. Such cell-bearing scaffolds have immense potential in the fields of diagnostics, toxicology, and drug screening and, if appropriately designed, may have the capability for producing synthetic or artificial tissues.

The sustained release of incorporated growth factors, energy supplements, or active pharmaceutical ingredients is determined by the degradability of polymer materials. In this regard, natural materials are more widely used than synthetic materials for the fabrication of nanofibrous scaffolds, where the natural components function either as polymers or as active drug ingredients (Fig. 8). Natural polymers themselves are composed of animal or plant-derived proteins or saccharides, or extracts of medicinal plants. This review presents the collective role of natural polymeric materials and drug ingredients that are nanoformulated by electrospinning or electrospraying for pharmaceutical and tissue engineering applications.

2. Electrospun and electrosprayed nanofibers/nanoparticles that contain natural products

Many plant extracts or their purified major fractions have known drug effects. For example, natural extracts derived from *Aloe vera* are antioxidant in nature and of good medicinal importance for tissue engineering.[58,59] Neem (*Azadiracta indica*) extracts containing omega fatty acids have numerous medical and cosmetic applications.[60] Ginsenosides found in the plant genus Panax (Ginseng) are often used in traditional Chinese medicine, and exhibit anticancer activity.[61] Recently, various plant extracts and active components have been formulated as nanofibers or nanoparticles for many therapeutic purposes.

Charernsriwilaiwat et al.[62] manufactured nanofibers from chitosan-ethylenediaminetetraacetic acid/polyvinyl alcohol (CS-EDTA/PVA) that incorporated assorted concentrations of *Garcinia mangostana* extract. The electrospun mats released ~80% of the predominant active ingredient, α-mangostin, within 60 min and showed a burst-release profile. The extract-loaded mat was non-cytotoxic toward fibroblast cells, but potently cytotoxic against gram-positive and gram-negative pathogens. Interestingly, the minimum inhibitory concentration and minimum bactericidal concentration values decreased by ~10-fold as the concentration of

the extract in the mat increased to 3%, indicating a synergistic role of the drug and the carrier polymer. Given that α-mangostin by itself is inactive against gram-negative pathogens, these results further suggest that the antimicrobial properties of PVA nanofibrous mats can be expanded in combination with CS-EDTA. Moreover, in a rat model of skin wound healing, fiber mats containing 3% mangostin extract displayed faster wound closure relative to control mats. The faster wound healing together with anti-infective properties suggest that nanofibrous mats containing natural active ingredients might be of therapeutic value as wound dressing materials.

Ligia and colleagues[63] electrospun a bionanocomposite membrane comprising PVA, pineapple nanofibers and *Stryphnodendron adstringens* bark extract for medical applications, and characterized the membrane via differential scanning calorimetry and thermo-gravimetric analysis. The membrane showed higher thermal stability and crystallinity than a control material because of the incorporation of the natural bark extract. Another crude bark extract derived from *Tecomella undulata* and containing the herbal drug, tecomin, was electrospun from polycaprolactone/polyvinylpyrrolidone (PCL/PVP) and investigated for prospective wound dressing applications.[64] Consequently, fiber mats containing the herbal drug (7.5% w/w) displayed potent and broad antimicrobial activity. In another work, the antimicrobial activity of a fatty acid-containing emu oil-loaded polyurethane nanofiber composite was studied for intended wound dressing and skin disease applications *in vitro*.[65] Polyurethane mats containing 10% emu oil displayed inhibitory activity against two bacterial strains.

Ultra-fine cellulose acetate (CA) electrospun fiber mats containing asiaticoside extracted from *Centella asiatica* were explored for topical/transdermal and wound dressing purposes.[66] Both herbal CA crude extract (CACE) and pure asiaticoside (PAC) were incorporated into the nanofibers, followed by analysis of transdermal diffusion of the drug through porcine

skin. The diffusion rate was 24–26% for PAC and 10–12% for CACE depending on the presence of phosphate or acetate buffer. Furthermore, the fiber membranes exhibited no toxicity against normal human dermal fibroblasts, indicating the likely advantages of the nanofibrous materials for wound dressing applications.

Many medicinal plants, in addition to those described above, have a long history of curative properties in wound healing. The high porosity and maximized surface area-to-volume ratio render plant material-loaded nanofibers suitable as scaffolds for communicating with the wound tissue via drug transportation and cell accommodation. Four different plant extracts, namely *Indigofera aspalathoides*, *Azadirachta indica*, *Memecylon edule* (ME), and *Myristica andamanica* were recently electrospun[67] with PCL for the development of skin graft substitutes. The cell proliferation capacity of human dermal fibroblasts on the PCL/ME electrospun nanofibrous scaffold was the highest among all of the electrospun scaffolds, with a 31% higher proliferation rate compared with a control underivatized PCL scaffold. Additionally, PCL/ME nanofibers showed the least cytotoxicity among the different scaffolds, as well as enhanced epidermal differentiation capacity toward adipose-derived stem cells. These findings demonstrate that PCL/ME scaffolds are promising substrates for skin tissue engineering.

Due to previous osteogenic medicinal applications of *Cissus quadrangularis* (CQ), CQ is exploited as a prospective therapeutic agent to enhance bone healing.[68-71] Recently,[72] the synergistic osteogenic effect of hydroxyapatite (HAp) and CQ extract was utilized in the fabrication of a PCL/CQ/HAp nanofibrous scaffold. The response of human fetal osteoblast cells to the scaffold was evaluated by using assessing cell adhesion/proliferation, alkaline phosphatase activity, alizarin red staining for calcium deposition, and osteocalcin expression for bone tissue regeneration. Comparison of cell proliferation on day 5 vs. day 15 showed

three-fold more proliferation and maximal proliferation for the CQ-incorporated nanofibrous scaffold vs. the other scaffolds, whereas a two-fold increase in proliferation was observed for the other scaffolds vs. the PCL control. The PCL/CQ/HAp nanofibrous scaffold apparently displayed the appropriate rough surface for optimal osteoblast adhesion, proliferation, alkaline phosphatase activity, and mineralization relative to the other scaffolds (Fig. 9).

Prior explorations of the physicochemical and biochemical/biological properties of nanocomposites suggests that nanocomposites as scaffolds have potential applications in bone tissue engineering. Emodin, an anthaquinone derivative of the Chinese herb, *Polygonum cuspidate*, was previously incorporated into PVP nanofibers prepared by electrospinning.[73] PVP was chosen to improve the solubility of emodin and to decrease its recrystallization tendency. Electrospun nanofibrous mats released the anthaquinone drug in its entirety within 2 h due to the increased dispersion of the drug within the nanofibers, along with the carrier PVP. *In vivo* studies revealed an early and accelerated wound healing effect by using the emodin/PVP nanofibrous membrane vs. the free drug and an underivatized PVP membrane. Moreover, the medicated nanofibrous material was nontoxic, non-allergenic, and highly biocompatible.

Prospective biomedical applications of the traditional Chinese herbal drug, Shikonin, were investigated by incorporating the agent into PCL/poly(trimethylene carbonate) nanofibers and assessing its antioxidant and antibacterial properties therein.[74] Shikonin (5%)-loaded fiber mats retained excellent free radical scavenging and antibacterial activities.

Turmeric is a gold-colored spice derived from C*urcuma longa* that is extensively used in the Indian subcontinent. Curcumin is the active ingredient in turmeric and imparts its yellow color. Curcumin was originally isolated approximately two centuries ago, and structurally identified as diferuloylmethane in 1910. The compound exhibits numerous biological

activities, including antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal and anticancer activities, rendering it potentially useful against cancer, diabetes, allergies, arthritis, Alzheimer's disease, and other chronic illnesses. Curcumin exerts its actions through various transcription factors, growth factors, inflammatory cytokines, protein kinases, and other enzymes. To enhance the *in vitro* and *in vivo* properties of the agent, several drug delivery strategies have been described that alter curcumin's intrinsic qualities.

For instance, electrospun as well as electrosprayed nanoparticles incorporating curcumin have been fabricated with excellent porosity and a highly functionalized surface area, permitting curcumin diffusion out from the matrix and thereby improving the mass-transfer capacity of the drug. To this end, nanosized poly(L-lactic acid) (PLLA) fibers loaded with curcumin were prepared by electrospinning, where PLLA at 8% w/v in acetone/dimethyl acetamide (2/1 v/v) was used for loading the drug (5 wt% with respect to PLLA).[75] Release kinetics studies indicated that ~50% of the curcumin was released within 24 h from the drug-loaded fiber mats.

Next, Guo et al.[76] showed that curcumin-loaded, biodegradable poly(ethylene glycol)-poly(ε-caprolactone) (PCEC) nanofibers can serve as drug delivery vehicles directed against malignant gliomas (Fig. 10). The presence of a high concentration of curcumin in the dope solution employed for electrospinning did not affect the average diameter of the individual nanofibers, but instead broadened the distribution of the fiber diameters. Antitumor activity was correlated with the amount of curcumin released from the mats.

Curcumin can suppress the Her2/neu signaling pathway in breast cancer cells.[77] Nevertheless, the toxicity of the agent against normal tissue, in conjunction with its low absorption at the tumor site due to low drug retention, is exacerbated by conventional drug delivery to tumors. Because of its high hydrophobicity, curcumin lacks good bioavailability and tissue specificity

when used as a conventional drug, limiting its efficacy as a chemotherapeutic agent against solid tumors (e.g., pancreatic, cervical, and breast cancers). However, curcumin-loaded PCL nanofibers exhibit good antioxidant potential and the ability to maintain normal cell viability under conditions of oxidative stress, as assessed by an oxygen radical absorbance capacity assay.[78]

In a systematic study, curcumin-loaded zein (a storage protein found in corn) nanoparticles were prepared by electrospraying so as to facilitate the delivery of curcumin in food ingredients.[79] The concentration of zein, the flow rate, and to a lesser extent, the applied voltage, all influenced the average diameter of the zein nanoparticles, but the addition of curcumin failed to affect particle morphology or size. Nanoparticles (average diameter, ~175–250 nm) were synthesized with high encapsulation efficiency and a curcumin content of ≤ 10% with respect to zein. The nanoparticles demonstrated improved drug delivery capacity relative to commercially available active ingredients, and the stability of the encapsulated curcumin was retained at a rate of up to 50% upon storage. The nanoparticles were well dispersed in milk to produce a yellow color, whereas the commercial ingredients remained mostly insoluble.

Aloe vera extract, curcumin, and *Aloe vera*/curcumin composite PCL nanofibrous membranes[80] were studied for their *in vitro* anticancer activity. The *Aloe vera*/PCL nanofibers showed 70% inhibition of cell viability against the A549 lung carcinoma cell line, while the curcumin/PCL nanofibers showed 65% inhibition. The addition of neem extract to the curcumin/PCL nanofibers increased the inhibition of cancer cell viability from 65% to 77%, while the addition of *Aloe vera* extract increased the inhibition to 82%. The curcumin/PCL/ *Aloe vera* combination also showed maximum inhibition against the MCF-7 breast cancer cell line. The enhanced or the synergistic activity of curcumin in combination

with either type of plant extract against the breast and lung cancer cell lines may be attributed to the interaction of the diferuloylmethane molecule with functional groups on extract-derived molecules.

Uniform, non-agglomerated spherical nanoparticles in a narrow size range of 30–150 nm were electrohydrodynamically sprayed with an ethanolic *Sophora flavescens* extract,[81] which contains a number of flavonoids with interesting biological activities. Dispersion of the electrosprayed nanoparticles onto an air filter surface increased the efficiency of the filter for the removal of the airborne bacterium, *Staphylococcus epidermidis*.[82] Notably, the bactericidal properties of the electrosprayed nanoparticles were superior to those of particles prepared by using a conventional nebulizer.

Although they are commonly exploited in traditional rather than modern medicine, the utilization of medicinal plant extract nanoconcoctions as described above might pave the way for the development of next-generation biocompatible and biodegradable formulations. Such biocompatible/biodegradable formulations could then be used as economically viable, localized drug-eluting devices.

Vitamin-loaded nanofibers have been fabricated by electrospinning for transdermal and drug delivery applications. The nanofiber membrane acts as a vitamin-encapsulated nanoreservoir, enabling the release of vitamins in small quantities and in a sustained manner. Delivery of vitamin A (Retin-A) and vitamin E was attempted by means of electrospinning vitamin-loaded CA nanofibers with a narrow fiber diameter range of 247–265 nm.[83] When the sustained release of vitamins was compared between the vitamin-loaded, as-spun CA fiber mat and a vitamin-loaded, solution-cast CA film, the nanofiber mat showed a gradual and continuous increase in Retin-A/vitamin E release, whereas the film exhibited a burst release of vitamins.

A cyanocobalamine (vitamin B_{12})-loaded, biocompatible PCL nanofibrous membrane was prepared to investigate water-soluble vitamin delivery via a hydrophobic polymer.[84] Contact angle measurements showed a reduction in surface wettability from 139° to 108° due to the presence of water-soluble vitamins. To further enhance the surface wettability, the polymer fiber was plasma-treated at different time intervals. An increase in drug release by approximately three-fold was observed for plasma-treated vs. untreated PCL mats.

Rhodamine B- and riboflavin-containing biodegradable and biocompatible nanofibers were fabricated from soy proteins to study controlled drug release and to observe desorption processes.[85] In another approach, control over drug release was established by coaxial electrospinning, where the drugs and the polymer solutions were delivered separately by using a compound spinneret containing two syringes of varying diameters.[86] By adjusting the shell-to-core flow rate ratio, drug (magnesium l-ascorbic acid 2-phosphate and α-tocopherol acetate)-containing polyacrylonitrile nanofibers were electrospun. When compared with conventional electrospinning incorporating blending of the drug and polymer, the coaxial electrospinning approach produced uniform fibers. Release kinetics studies demonstrated a more controlled release of drugs relative to the burst release observed for drug-loaded mats prepared by conventional electrospinning.

3. Electrospun and electrosprayed natural polymer nanomaterials for pharmaceutical and tissue engineering applications

The ECM is composed of a mixture of proteins and polysaccharides that embraces collagen, hyaluronic acid, proteoglycans, glycosaminoglycans, and elastin. The ECM provides mechanical and biochemical support to surrounding cells and directs many cellular activities. Concerning biomedical applications, there is a need for biomimetic nanomaterial scaffolds that can structurally and functionally mimic native ECMs, given that the ECM is an integral

part of the natural habitat of cells and tissues. Electrospun nanofibers exhibit several features of naturally occurring ECMs (e.g., fibrosity, high porosity, and high surface-to-volume ratio), engendering a surge in research efforts to develop nanofibrous 3D scaffolds.

Electrospun nanofibers are produced from synthetic polymers or from naturally occurring materials. When compared with synthetic polymers for the construction of 3D scaffolds, natural biopolymers, such as animal- or plant-derived proteins or carbohydrates, could play a significant role in shaping cell behavior, especially in regard to chemical cues and biocompatibility (Fig. 11). However, their efficacy is limited by poor mechanical properties and rapid biodegradability. Nevertheless, these drawbacks can be overcome by a post-spinning crosslinking process with suitable crosslinkers, or by blending natural products with a biocompatible synthetic polymer, potentially resulting in ideal ECMs for drug delivery and tissue engineering applications. Natural polysaccharide and protein nanostructured materials not only serve as ideal carriers for drug delivery but also as optimal skin, bone, cartilage, vascular, neural, and cardiac tissue engineering scaffolds.

Electrospun nanofibers and electrosprayed nanoparticles of natural origin are currently being explored for use as nanodevices owing to their enhanced biocompatability.[87] Electrospun collagen,[88] chitosan,[89] gelatin,[90] fibrinogen,[91] DNA,[92] collagen/poly(ester urethane) urea,[93] collagen/glycosaminoglycan,[94] gelatin/polyaniline (PANi),[95] silk fibroin,[96] PCL/gelatin/hyaluronic acid,[97] poly(L-lactic acid)-co-poly(ε-caprolactone) (P(LLA-CL))/collagen,[98] nerve growth factor (NGF)/bovine serum albumin (BSA)/polymer(ϵ-caprolactone-co-ethylene phosphate) (PCLEEP),[99] poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)/collagen,[100] human glial cell-derived neurotrophic factor/PCLEEP,[101] collagen/PCL,[102] poly(glycolic acid) (PGA)/chitin,[103] HAp/gelatin,[104] PCL/$CaCO_3$,[105] PHBV/HAp,[106] HAp/PLLA,[107,108] PCL/HAp,[109]

collagen/elastin/poly(ethylene oxide) (PEO),[110] collagen/elastin/PLGA,[111] gelatin/elastin/PLGA,[112] collagen/P(LLA-CL),[113] and silk/PEO/HAp/bone morphogenetic protein-2 (BMP-2)[114] nanofibers have all been fabricated to mimic the environment afforded by the native ECM for tissue regeneration purposes.

3.1. Collagen

Collagen is one of the most abundant structural proteins and is commonly found in connective tissues, encompassing the skin, muscle, eye sclera, cornea, cartilage, bone, blood vessels, gut, and intervertebral disc. Collagen products are generally used in biomedical applications for wound healing due to their intrinsic ability to support tissue ingrowth. Collagen nanofibers are manufactured to provide a biomimetic environment for the regeneration of various tissue types.[115] As noted above, disadvantages of biomimetic nanofibrous scaffolds, including those made of collagen, for tissue regeneration are poor mechanical stability and rapid degradation, necessitating the usage of (1) a crosslinker that interconnects the fibrous nanostructure with chemical bonds, or (2) the blending of natural products with synthetic polymers.

Blending with synthetic biodegradable and biocompatible polymers is typically more advantageous than crosslinking for extended stability while retaining the biomimetic nature of the scaffold. Osteochondral regeneration of mesenchymal stem cells (MSCs) was recently evaluated by employing a combination of collagen and electrospun PLLA nanofibers.[116] Adhesion, proliferation and differentiation of the MSCs were examined *in vitro* on a blended, electrospun collagen and PLLA nanofibrous bilayer scaffold, and osteochondral defects were created in rabbits followed by implantation of the scaffold. As a result, the collagen/PLLA nanofibrous scaffold exhibited higher osteogenic differentiation potential than a control

scaffold containing collagen alone. Furthermore, the MSC-implanted composite scaffold induced better functional repair of osteochondral defects.

A tubular scaffold composed of collagen-coated P(LLA-CL) nanofibers was engineered as a small-diameter vascular graft and used to replace a rabbit vein (Fig. 12) to show that the scaffold could sustain suturing and the implantation process.[117] A sandwich method was adopted to combine the collagen/P(LLA-CL) scaffold and chondrocytes. At 12 weeks after implantation in rabbits, the constructs exhibited a cartilage-like morphology, with mechanical properties reaching up to 77% of the relevant values for native rabbit auricular cartilage. The investigators claimed that the constructs were easy to make from acellular cartilage sheets and yielded highly reproducible results.[118]

In another study, titanium implants coated with collagen nanofibers were shown to be biomimetic and useful as oral implants. The differentiation of MSCs into osteoblasts was faster in the context of the nanofiber-coated vs. uncoated titanium implants, and a more mineralized matrix deposition was observed. Although MSC proliferation was similar with both types of implants, the pro-differentiation ability of the coated surface was double that of the uncoated surface. Therefore, the use of a collagen nanofiber coating to improve the applicability of a titanium alloy as an implant is a promising and marketable technology.[119]

Synthetic vascular grafts composed of polytetrafluoroethylene (PTFE), Dacron®, polyurethane or polyethylene terephthalate (PET) are currently in the market for assorted purposes. Nevertheless, there remains a strong need for further biomimetic materials that incorporate one or more of these synthetic materials in combination with a natural protein. Collagen and collagen/elastin blended and aligned polyurethane nanofibers have been fabricated, and their applicability to enhance smooth muscle cell (SMC) growth for vascular tissue engineering demonstrated. While elastin provides the necessary viscoelastic properties

in line with the needs of a synthetic vascular graft, collagen improves overall cell/scaffold interactions and the growth of SMCs.[120]

Prosthetic materials or autologous grafts were employed for the reconstruction of large diaphragmatic muscle defects in congenital diaphragmatic hernia. The materials currently used for this purpose are mainly a local muscle flap or a non-absorbable, expanded PTFE (ePTFE) patch. The application of a local muscle flap involves the disadvantages of invasiveness, bleeding, and infection, whereas use of the synthetic ePTFE patch can lead to a severe foreign body reaction. Hence, electrospun aligned PCL/collagen nanofibrous scaffolds were created in an attempt to reconstruct the diaphragmatic muscle in defective rats, with no herniation or retraction at up to 6 months after implantation.[121] Muscle cell migration and tissue formation were both observed, and the retrieved scaffold resembled the normal diaphragm in terms of mechanical properties. Thus, a native protein, collagen, in combination with a nanofibrous scaffolds delivered a biomimetic, biomechanically stable, implant for diaphragmatic muscle defects.[121]

In an additional approach, Willard and colleagues[122] fabricated plant-derived human collagen nanofibers and compared their efficacy with that of freeze-dried, bovine hide-derived collagen for the development of tissue-engineered skin. Both scaffold types displayed a similar architecture and supported human cell growth, as assessed by *in vitro* data, but the novel nanofibrous scaffold was associated with a reduced risk of an inflammatory/allergic response and disease transmission.[122]

Suganya *et al.*[123] used PCL/collagen blend nanofibers for skin tissue regeneration (Fig. 13) and demonstrated augmented skin repair with the incorporation of an *Aloe vera* extract. In another recent study, collagen/glycosaminoglycan biocomposites were also shown to be useful for the regeneration of skin tissue.[124]

Nanoparticles can be incorporated into natural or synthetic polymers to create functional polymeric composites suitable for tissue regeneration, where inorganic HAp is dispersed with a preferential orientation to enhance bone tissue regeneration. HAp possesses an inorganic crystalline structure reminiscent of natural bone, and is also biocompatible, bioactive, and osteoconductive. Many studies have reported that bone-mimicking composites of HAp and bioactive components (e.g., collagen, gelatin, chondroitin sulfate, chitosan, and amphiphilic peptides) are promising for biomedical applications.[125-128] For example, a collagen/HAp composite that mimics the native ECM demonstrates excellent potential for the replacement of diseased skeletal bones.

Collagen/HAp biocomposites that are used in bone remodeling are highly crosslinked compared with naturally occurring collagen. The latter exhibits poor mechanical properties, especially with respect to bone (Young's modulus, E, = ~2–5 GPa).[129] Collagen and HAp devices are preferred over synthetic PLGA devices, because they significantly inhibit the growth of bacterial pathogens commonly associated with prosthetic devices. Collagen functions to support cell adhesion and proliferation, while HAp acts as a seed for biomineralization of osteoblasts during the regeneration of bone tissue.[130-132] Due to their bioactive, osteoconductive, and osteoinductive properties, collagen and nanoformulated HAp are obvious choices for bone grafting purposes.[133-135] To reduce the antigenicity of collagen, HAp/Col composites are produced by using atelocollagen (a water-soluble form of collagen extracted from the skin of fish) by condensing a suspension of $Ca(OH)_2/H_3PO_4$.[136]

3.2. Fibrinogen

Fibrinogen is a 340-kDa protein present in the blood stream that critically functions in blood coagulation. Fibrinogen is proteolyzed to form the web-like fibrin clot, preventing blood from oozing out of the wound or injury site. Wnek et al.[91] and Boland et al.[137] first reported the

electrospinning of fibrinogen by using 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) as the solvent. The biostability of electrospun fibrinogen was improved in a human bladder SMC culture model by the inclusion of various concentrations of aprotinin, a bovine pancreatic trypsin inhibitor. While collagen production and the rate of remodeling were both decreased with increasing concentrations of aprotinin, the results provided an alternative strategy to reduce the degradation of fibrinogen.[138]

Following this work, the authors demonstrated that tissue remodeling promoted by controlling the degradation rate of electrospun fibrinogen with aprotinin was superior to that stimulated by fibrinogen mats crosslinked by glutaraldehyde.[139] The investigators further showed that electrospun fibrinogen fixed with vapor-phase glutaraldehyde displayed poorer wet elastic moduli than fiber mats hydrated in the presence of aprotinin-containing media.[140] Sell et al.[141] showed that covalent crosslinking of electrospun fibrinogen with genipin or EDC, a zero-length crosslinker, increased the wet mechanical properties and biostability of electrospun fibrinogen relative to vapor-phase glutaraldehyde crosslinking. However, cell migration and collagen production were greatly decreased after the crosslinking treatment.[141] These findings highlight the importance of balancing optimum mechanical properties and controlled degradation of electrospun fibrinogen for tissue engineering purposes.

A combined atomic force microscopy/fluorescence microscopy technique[142] was used to compare the mechanical properties of individual fibrinogen nanofibers with those of natural fibrin and electrospun collagen fibers. In the wet state, fibrinogen nanofibers displayed a low average modulus and high extensibility, similar to natural fibrin fibers. However, the modulus increased by 1000-fold in the dry state, and the mechanical properties were comparable with those of electrospun collagen type I fibers. Given the significance of matrix elasticity in regenerative medicine, these results support the hypothesis that the elasticity of

electrospun fibrinogen can be regulated to meet the requirements of specific biomedical engineering applications.[143]

In further support of this idea, recent studies showed that co-electrospinning of fibrinogen with synthetic polymers altered the wettability, mechanical properties, and thermal characteristics of the ensuing nanofibrous mat, and demonstrated a reasonable association between the achieved mechanical properties of the scaffold and cell adhesion/migration. For example, He et al.[144] showed that electrospun fibrinogen and P(LLA-CL) at a blend ratio of 40/60 or 60/40 displayed optimum mechanical properties, biostability, and an appropriate microenvironment for cell attachment and proliferation. Furthermore, Fang et al.[145] reported similar observations regarding the maximum proliferation of human umbilical vein epithelial cells when the PLCL/fibrinogen ratio was in the range of 2/1 to 1/1. Feingold-Leitman et al.[146] went on to utilize a composite scaffold material made of a polyethylene glycol (PEG)-ylated fibrinogen hydrogel and PCL nanofibers. Hydrogel-encapsulated aortic SMCs preferentially migrated toward the PCL nanofibers, and the authors attributed this phenomenon to the mechanical strength of the fibrous portion of the hybrid scaffold.

3.3. Chitosan

Chitosan is a polysaccharide, de-acetylated derivative of chitin, and is abundantly found in nature in the shells of crustaceans. Chitosan and collagen function similarly in crustaceans and higher vertebrates, respectively. The polysaccharide also plays a vital role in nanoformulated drug delivery and tissue engineering applications, especially because chitosan nanoformulations exhibit synergistic enhancement of antimicrobial and anticancer drug actions.[147-150] The significance of this commercially low-cost biopolymer include structural similarity to the glycosaminoglycans found in vertebrate bone and induction of osteoconductivity with excellent biocompatibility, tailorable biodegradability, low

immunogenicity, good mechanical properties, and inherent antimicrobial characteristics. Owing to limited chitosan solubility, which often requires harsh solvents, and its polyelectrolyte nature, preparation of defect-free, electrospun chitosan nanofibers is difficult. Nevertheless, chitosan nanofibers of various diameters (70 ± 45 to 490 nm) were successfully electrospun by using a wide variety of solvents or solvent mixtures.[150]

To improve the stability and mechanical properties of electrospun chitosan nanofibrous scaffolds for intended biomedical applications, thermal, covalent, and noncovalent crosslinking was previously attempted.[151-154] In a comparative study, chitosan was electrospun with assorted crosslinking agents, including genipin, epichlorohydrin (ECH) and hexamethylene-1,6-diaminocarboxysulphonate (HDACS), and then subjected to post-electrospinning thermal and chemical base treatments. Thermal treatment at 120°C decreased the stiffness of the mats, but increased their tensile strength and toughness. In general, covalent crosslinking or crosslinking followed by thermal/alkaline treatment diminished the favorable mechanical properties of the mats. Compared with ECH and HDACS, genipin had the least detrimental effect on mechanical properties. However, maximum stability in water and acetic acid was achieved when the nanofibers were crosslinked with HDACS and glutaraldehyde. Recently, Kiechel and Schauer[154] reported that the water and acid stability of electrospun chitosan could be improved by crosslinking with tannic acid in ethanolic NaOH.

To enhance human fetal osteoblast proliferation and bone formation, Zhang et al.[155] fabricated composite chitosan nanofibers containing 10% ultra-high-molecular-weight polyethylene by a modified two-step approach. Briefly, an *in situ* co-precipitation synthesis route was designed to overcome the issue of nanoparticle agglomeration, and electrospinning was conducted for the preparation of HAp/chitosan composite nanofibers with a higher (30 wt%) loading of HAp nanoparticles. Electron diffraction and X-ray diffraction analyses were

employed to confirm that the acetic acid used for chitosan dissolution had a minor or nonexistent influence on the crystallinity of the HAp nanoparticle incorporated within the nanocomposite structure. The bone regeneration capacity of the HAp/chitosan nanocomposite scaffold was then assessed, with the results verifying a significantly enhanced rate of osteoblast proliferation and bone formation on the Hap/chitosan nanocomposite compared with a pure chitosan scaffold.

Chitosan was also electrosprayed onto nano/micro particles for prospective drug delivery applications.[156] The effects of the solution properties of chitosan (i.e., viscosity, conductivity, and surface tension) were investigated on the size of the resulting nanoparticles, as were the effects of the processing parameters (i.e., nozzle diameter, applied field strength, and flow rate). The analysis was somewhat complicated by the fact that the electrosprayed chitosan nanoparticles failed to show a smooth and spherical morphology. Consequently, for a given polymer concentration with low conductivity, a linear correlation was obtained between the particle size and the flow rate.

In a more systematic approach, Songsurang et al.[157] likewise investigated the effects of polymer properties and processing parameters on the particle size of electrosprayed, high-molecular-weight chitosan. These authors reported the formation of smooth, spherical chitosan particles by collecting the sprayed particles in 5% tripolyphosphate. By using this approach, chitosan nanoparticles of various sizes (ranging from 200 nm to 10 μm) were obtained by optimizing the flow rate and the field strength. The synthesized nanoparticles encapsulated the hydrophobic anticancer drug, doxorubicin, with reasonable encapsulation efficiency (~68%) and controlled release rate (~70% over 72 h).

In an attempt to synthesize chitosan nanoparticles by electrospraying followed by freeze-drying, Kim and Lee[158] reported the fabrication of porous 3D non-woven structures. The

porous non-woven structures showed excellent biocompatibility and biodegradability, and may therefore expand the biomedical potential of chitosan polymers.[159]

3.4. Silk fibroin

Silk fibroin is considered to be the most promising natural fibrous protein replacement for collagen in bone tissue engineering due to its biocompatibility, slow biodegradability, and excellent mechanical properties. In the past few years, two natural silk types (i.e., silkworm *Bombyx mori* silk and spider *Nephila claVipes* dragline silk) have been processed for making nanofibers via electrospinning.

To improve the electrospinnability of silk protein solutions and to avoid potential influences of hazardous organic solvents (e.g., HFP,[160] hexafluoroacetone,[161] and formic acid[162]) on the biocompatibility of the scaffolds, an all-aqueous electrospinning was attempted by Jin et al.[163] by blending silk fibroin with PEO at ratios ranging from 1/4 to 2/3. Methanol treatment of the electrospun material rendered the scaffolds water-insoluble due to a structural conformational change into a β-sheet structure.

Recently, an elegant demonstration of the role of initial nanostructures in the dope solution on the formation of electrospun fibers was documented.[164] Here, a dope solution containing silk nanofibers displayed a concentration-dependent, shear-thickening rheology, and formed smooth fibers even at a low concentration. On the other hand, a dope solution containing nanospheres showed Newtonian fluid-like behavior and required a two-fold higher concentration to form smooth electrospun nanofibers of a similar size.

Silk-based biocomposite nanofibers containing HAp and BMP-2 were fabricated by Li et al.,[165] and enhanced bone formation was observed by culturing the nanofibers with human bone marrow-derived MSCs. The inclusion of HAp and BMP-2 in the electrospun silk fibroin

nanofibers led to the highest calcium deposition and upregulation of BMP-2 transcript levels in the MSCs relative to the other electrospun silk-based scaffolds.

Sericin is a protein obtained from *Bombyx mori* during the production of silk, and is used in the food and cosmetics industries as an antibacterial agent. Sericin is also used as a drug delivery vehicle. Najmeh et al.[166] successfully electrosprayed a servicing nanopowder by dissolving a sericin sponge in dimethyl sulfoxide. A particle size of 25 nm that consisted of small crystals and showed high absorbency was achieved by using this method.

3.5. Gelatin

Gelatin is a natural polymer derived from collagen. Indeed, gelatin corresponds to the hydrolyzed form of collagen, with a similar composition and properties. This fibrous protein is obtained from collagen-abundant bones and skin. The commercial sources of gelatin are bovine and porcine materials, while the food ingredient is commonly derived from porcine products. The low-cost collagen derivative exhibits good biocompatibility and biodegradability, and is also non-immunogenic. Hence, gelatin finds numerous applications in the food and pharmaceutical industries. A nanoformulation of gelatin as a nanofiber is applicable to tissue engineering purposes, wound dressings, surgical treatments, and controlled drug release of incorporated drug ingredients.[167-169]

Gelatin shows good water solubility, but it can also be electrospun by using organic solvents, such as 2,2,2-trifluoroethanol (TFE), trifluoroacetic acid, or formic acid.[170-172] Furthermore, gelatin can be used as a blend with PVA in an aqueous solution.[168,171] Cell proliferation is enhanced on fiber mats containing gelatin and PVA after crosslinking with glutaraldehyde to improve stability (Table 1). Additionally, gelatin can be electrospun with PLLA,[173,174] PCL,[175] PLGA,[176] and PANi.[177]

Liu et al.[183] fabricated a 3D gelatin/apatite nanofiber composite scaffold to mimic the native nanoscale architecture and to match the chemical composition of natural bone ECM. Inorganic HAp was deposited all along the 3D porous structure in a fashion ideal for controlling surface topography and chemistry within a complex nanostructure. The resulting scaffolds demonstrated excellent biocompatibility and mechanical properties, and exhibited enhanced osteoblast adhesion, proliferation and differentiation in a manner suitable for bone tissue engineering applications.

Table 1. Applications of gelatin and synthetic polymers with various crosslinking agents.

Electrospinning of gelatin	**Crosslinking agents/process**	**Cultured cells**
10/20 wt/vol% cinnamic acid-modified gelatin in HFP	Ultraviolet (UV) light Genipin	Mouse fibroblast cells (L929 cell line)[177]
20 wt% gelatin (from porcine skin) in an aqueous solution containing 2 wt% formic acid (88%) and 20 wt% ethanol	Soaking in 2 or 5 wt% glutaraldehyde solution for 72 h	Small mouse mesangial cells (SV40 American Type Culture Collection (ATTC)® CRL-1927 cell line)[179]
8.3 wt/vol% gelatin (type B, from bovine skin) in HFP	10 vol% HMDI crosslinker in isopropyl alcohol for 1 h	HEPM cells (ATCC® CRL-1486 cell line)[180]
30 wt/vol% gelatin (type A, from porcine skin) in 60/40 vol% acetic acid/double distilled water	Genipin	Vascular wall MSCs isolated from human femoral arteries[181]
Gelatin/chitosan ratios: 0/100, 50/50 and 100/0 in HFP and trifluoroacetic acid (8/2)	Glutaraldehyde (25%) crosslinking for 3 days	Porcine iliac endothelial cells[182]
PLLA/gelatin 90/10 vol/vol in dichloromethane and aqueous acetic acid	—	WI-38 human embryonic fibroblasts and the lung-derived cell line, CCL-75[183]
10 wt/vol% PCL (M_w 80,000) in TFE	—	Bone marrow stromal cells isolated by short-term adherence to plastic from

		the iliac crest of 4-month-old female New Zealand White rabbits[175]
PVA (88% hydrolyzed, M_w 88,000) and gelatin (type A, 300 bloom from porcine skin) in aqueous solution	Glutaraldehyde (50%) vapor for 8 h at 40°C	NIH 3T3 fibroblasts[171]
Gelatin (bovine skin, type B powder, bloom number 225) and PANi in HFP	EDC crosslinker in 90% ethanol for up to 2 h	H9c2 cardiac rat myoblast cells[177]
PLGA (LA/GA 85/15, M_w 200,000) and gelatin in TFE	—	Murine calvarial pre-osteoblasts (MC 3T3-E1 cell line)[176]
Gelatin and PLLA in HFP	Heating at 140°C for 48 h	MSCs isolated from rat bone marrow[174]

Cyclophosphamide is a cyclic phosphamide ester of mechlorethamine that is used to treat retinoblastoma, neuroblastoma, lung cancer, and breast cancer by crosslinking with DNA and preventing cell division. Gulfam et al.[184] demonstrated the electrospraying of gliadin nanoparticles for the controlled release of cyclophosphamide. Gliadin/gelatin composite nanoparticles loaded with cyclophosphamide were first prepared. A comparison of particle size, drug loading capacity, and drug releasability for the two nanoparticles revealed that the gliadin-based nanoparticle was superior to the gliadin/gelatin nanocomposite in terms of smaller size and slower drug release.

In another work, gelatin-loaded nanofibers were crosslinked by employing a nontoxic, natural vitamin (riboflavin)-based crosslinking protocol.[185] Riboflavin generates reactive oxygen species (ROS) during UV light treatment, assisting in gelatin crosslinking and resulting in improved tensile properties (Fig. 14). Riboflavin-loaded PCL nanofibers generated ROS *in situ* upon UV light treatment due to the crosslinking effect, which was employed to form hydrogels of gelatin and fibrinogen. Accordingly, riboflavin-encapsulated nanofibrous

membranes enabled the sustained release of ROS for stabilizing gelatin in the context of nanofibers, films, and solutions, and could hypothetically be used to crosslink any type of protein fiber or fibrous ocular, skin, or cardiac tissue.

Gelatin nanofibers with antifungal agent encapsulation were recently fabricated to facilitate topical application of five different United States Food and Drug Administration-approved antifungals (amphotericin B, natamycin, terbinafine, fluconazole, and itraconazole).[186] Relative to other antifungal-loaded fiber mats, gelatin/polyene nanofibrous scaffolds (Fig. 15) showed a two-fold increase in nanofiber diameter and effectively inhibited both yeast and filamentous fungus growth with good primary human corneal and sclera fibroblast cytocompatibility. The terbinafine-loaded mat showed activity against only three filamentous fungal species, while the itraconazole-loaded mat was potent against only a single strain of *Aspergillus*. However, the triple-helical conformation of gelatin was stabilized by the polyene component in the gelatin/polyene mat, and the hemolytic activity of the polyene was reduced in the presence of gelatin. Thus, these polyene antifungal-loaded gelatin fiber mats display enhanced antifungal activity, are devoid of significant cell toxicity, and show new promise in the management of superficial skin infections.

Gelatin boasts greater availability and is more economical than collagen, and is thus a promising natural compound that can be used to mimic the ECM and in drug delivery applications. Although scaffolds made of gelatin are readily biodegradable and have poor mechanical properties without crosslinking, they are ideal for tissue replacement purposes and as drug delivery nanoscaffolds with the appropriate choice of crosslinker.

3.6. Hemoglobin

Hemoglobin is an essential protein for oxygen transport and consists of four globular subunits in its quaternary structure. Each of the two α- and β-chain subunits contains a heme group

with a central iron (Fe^{2+}) ion. Pure hemoglobin was electrospun by Barnes et al.[187] by using several concentrations of the protein in TFE. The electrospun fibers showed a ribbon-like structure and fiber diameters of 0.5–3.5 μm. The material had a low mechanical strength, but the mechanical strength was increased by crosslinking with glutaraldehyde. Given the physiological role of hemoglobin described above, the electrospun scaffolds become a promising material for oxygen transport in wound healing. Recently, a composite electrospun nanofibrous scaffold containing gelatin, fibrinogen, and hemoglobin in a weight ratio 1/1.5/1.5 was developed for use in cardiac tissue engineering.[188]

3.7. BSA

BSA is a native globular protein that is more or less similar to human serum albumin (HSA). BSA and HSA show a sequence homology of 76%. Serum albumin is the most abundant protein in blood plasma (0.42 g/l), and some of its important functions are to regulate osmotic blood pressure and to maintain blood pH. Furthermore, serum albumin is capable of binding with high capacity to various substrates, so that it is able to regulate the adsorption, distribution, metabolism, and excretion of numerous drugs.[189, 190]

3.7.1. Spinnability of BSA

Because of the complex structure of BSA and other natural proteins, the processing of these molecules in nanofibrous form is challenging. Many inter- and intramolecular noncovalent interactions in proteins lead to stable tertiary structures. Therefore, the prior unfolding of proteins is necessary to permit the electrospinning of protein solutions.[191] Regev et al.[192] determined the solution structure of a concentrated solution of BSA dissolved in distilled water or TFE containing β-mercaptoethanol (β-ME) by small-angle X-ray scattering. A clear transition from a rigid colloid-like behavior to a random-coil conformation was observed when the water was replaced by β-ME-containing TME. The authors concluded that the

complete unfolding of BSA by β-ME is a prerequisite for the production of continuous, uniform nanofibers.

Dror et al.[193] showed that BSA nanofibers with significantly improved mechanical properties could be attained by electrospinning 10% BSA in TFE/β-ME under conditions of alkaline pH. The enhanced tensile strength and ductility observed for the electrospun fibers under these conditions was attributed to the rearrangement of intermolecular disulfide bonds, as well as to increased fiber orientation and crystallinity. On the other hand, electrospinning of BSA in aqueous solvents was achieved by blending BSA with synthetic water-soluble polymers, such as PVA and PEO.[193]

Moradzadegan et al.[194] successfully electrospun BSA blended with PVA by adding 10 mg/ml BSA to a 6% aqueous solution of PVA. The fiber diameters were comparable to those of pure PVA, although their morphology was more irregular, due to PVA/protein interactions.

The use of PEO as a supporting polymer allows an aqueous solution to be used more than once. For example, Kowalczyk et al.[195] employed a 8.7% polymer solution in water, where 85% of the polymer consisted of PEO and 15% consisted of BSA. The electrospinning process yielded insoluble fibers with a ribbon-like morphology. Gel electrophoresis and tryptophan fluorescence assays confirmed that the electrospun fibers retained the native structure of serum albumin. Whereas the PEO constituent was easily dissolved in water directly after spinning, the BSA constituent was insoluble after 2 weeks of aging.

Ravichandran et al.[196] used gold nanoparticle-mediated crosslinking of BSA/PVA nanofibers for the stabilization of BSA. The investigators then employed the scaffold prepared by "green" electrospinning (electrospinning of aqueous dispersions of water-insoluble polymers in the absence of toxic organic solvents) for cardiac regeneration (Fig. 16).[196]

3.7.2. Utilization of BSA in drug release studies

BSA was initially used in electrospinning technology primarily as a model protein (Table 2). BSA was added in small amounts to assorted electrospun fibers that consisted of various polymer materials. From there, different methods of electrospinning, such as coaxial and emulsion electrospinning or blended electrospinning, were used for the fabrication of BSA-containing nanofibers for controlled drug release and tissue engineering applications.

As a new approach, protein powders were synthesized by electrohydrodynamic atomization in a nanoparticle size range centered at ~700 nm. Tavares Cardoso et al.[197] electrosprayed BSA (as a model protein) with lactose (as a biological carrier) to produce new dry-powder nanoparticles for drug delivery. Yiquan et al.[198] similarly fabricated elastin-like polypeptide (ELP)-based, pH-responsive doxyrubicin-loaded nanoparticles with a size range of 300–400 nm by a flexible and effective electrospraying technique. The results suggested that molecular weight, ELP concentration, flow rate, and applied field strength are all key parameters for the synthesis of ELP-based nanoparticles with a homogeneous distribution of particle sizes. In addition, the particles could load up to 20% (w/w) doxyrubicin without altering particle size or morphology.

Table 2. Fabrication of BSA nanofibers for controlled drug release and tissue engineering applications.

Materials	Polymer	Solution	Reference
Core-shell fiber PCL (shell); PEG (core)	BSA	PCL in dimethylformamide/chloroform (3/7); PEG and BSA in water	[199]

Core-shell fibers P(LLA-CL) (shell); BSA (core)	BSA	P(LLA-CL) in TFE; BSA in water	[200]
Core-shell fibers P(LLA-CL) (shell); BSA/NGF (core)	BSA/ NGF	BSA and NGF (recombinant human β-NGF) in water; P(LLA-CL) in TFE	[201]
Emulsion electrospinning P(LLA-CL)	BSA	P(LLA-CL) in methylene dichloride; addition of rhodamine B, Span® 80 nonionic surfactant and BSA to water to form an emulsion	[202]
Emulsion electrospinning; gelatin	BSA	BSA in TFE; addition of Tween-20 to gelatin	[203]
Blended PVA	BSA	Aqueous PVA; fluorescein isothiocyanate/BSA solution	[204]
Blended PCL	BSA	PCL in dimethylformamide/methylene chloride (60/40); Alexa Fluor-conjugated BSA	[205]

4. Additional polysaccharide and glycoprotein nanomaterials

The utility of the polysaccharide, chitosan, for biotechnology applications is discussed above. Other polysaccharides and glycoproteins of relevance to tissue engineering and drug delivery/pharmaceutical applications include alginate, chondroitin sulfate, heparin, the xylans, and hyaluronic acid.

Alginate is an anionic polysaccharide derived from algae that is known for its capability to absorb copious amounts of water. This polysaccharide displays outstanding biocompatibility, degradability, and hydrogel formation (Fig. 17), lending it to countless biological applications.[206] Due to strong inter- and intramolecular hydrogen bonding forces and the resulting chain conformation and entanglement,[207] electrospinning of pure alginate, like BSA, is challenging. In pure water, jet instability of alginate results in the deposition of irregular

droplets. The addition of glycerol (volume ratio = 2 with respect to water) to a 2% alginate solution reduces internal hydrogen bonding, surface tension and conductivity of the solution, allowing preparation of homogeneous 200-nm fibers. The flow properties of alginate change from Newtonian fluid-like behavior in water to thixotropic behavior in the presence of glycerol.

Qi et al.[208] fabricated alginate microsphere-encapsulated PLLA microfibers by emulsion electrospinning. The composite alginate bead-embedded fibers were transformed from a beaded-fiber morphology to a spindle-like structure upon increasing the voltage. Incorporation of BSA into the microfibers resulted in controlled release of the latter from the alginate microspheres, which was not observed for PLLA microfibers containing no BSA. In another approach aimed at drug release,[209] a temperature-sensitive copolymer PLGA/PEG/PLGA hydrogel/electrosprayed alginate microsphere composite was designed for programmed release of two small-molecular-weight, water-soluble anti-inflammatory drugs.

Lastly, modification of alginate nanofibers with the cell adhesion RGD peptide led to a significant enhancement of human dermal fibroblast cell attachment, spreading, and subsequent proliferation.[210]

Chondroitin sulfate is a sulfated glycosaminoglycan with its main applications in cartilage repair and osteoarthritis treatment and control. PVA nanofibrous scaffolds modified with chondroitin sulfate as a biological cue were designed for use in articular cartilage repair (Fig. 18).[211] The porosity and low density of the scaffold allowed for immediate cell infiltration *in vitro* for optimal tissue repair, as well as enhanced chondrogenic differentiation of MSCs, as inferred from increased ECM production, cartilage-specific gene expression, and cell proliferation.

Heparin is another sulfated glycosaminoglycan with a high negative charge density. This glycosaminoglycan is used as an anticoagulant in medical devices. Heparin-incorporated gelatin,[212] PCL/chitosan,[213] and PLLA[214] electrospun nanofibers are all fabricated for vascular tissue engineering applications and the prevention of thrombosis. In addition, hemocompatible gelatin/heparin/polyurethane core-shell nanofibers[215] are fabricated as potent artificial blood vessels, and heparin-grafted PLLA/chitosan core-shell nanofibers[216] are manufactured as vascular gaskets. Apart from soft tissue engineering, heparin/chitosan-coated PLGA nanofibrous scaffolds[217] are currently under exploration for their HAp mineralization efficiency for prospective bone tissue regeneration.

Xylans comprise a group of hemicelluloses present in plant cell walls. Like alginate, xylan alone is not spinnable and, therefore, xylan/PVA nanofibers have been developed as potential scaffolds for skin[218] and cardiac tissue[219] regeneration. Hyaluronic acid, a naturally occurring straight anionic polysaccharide, widely exists in the ECMs of connective tissues. Electrospinning of hyaluronic acid[220-228] has attracted much attention, because of the remarkable wound healing ability of the compound and its utility in tissue engineering applications.

Silk fibroin/hyaluronic acid/PCL composite nanofibers were fabricated by one-step emulsion electrospinning.[228] The incorporation of hyaluronic acid into the PCL/silk fibroin nanofibers increased the hydrophilicity of the scaffold, thereby reducing nonspecific protein adsorption, fibrosis tissue thickness, and macrophage adhesion *in vivo*. Thus, hyaluronic acid-containing nanofibrous scaffolds can apparently regulate protein adsorption and cellular infiltration.

5. Conclusions and future perspectives

During the past decade, the scope of biomaterials has undergone a notable expansion thanks to the advent of electrospinning and the processing of natural or semi-synthetic

macromolecules. Although nanotechnological investigations have historically relied on synthetic nanomaterials, cost-effective natural materials are also available to greatly amplify the research efforts in numerous fields.

Initially, the focus of biomaterials was toward the development of implants based on interfacial reactions, biomechanics, fracture mechanics, fatigue testing, and retrieval analysis.[229] Interest then extended into drug delivery systems, with a focus on drug/polymer interactions and tissue engineering scaffolds for cell transplantation,[230] followed by the use of resorbable bioactive particulates or porous networks to activate mechanisms of tissue regeneration *in vivo*.[231] Other areas of interest included the surface modification of biomaterials with chemical cues, the prevention of nonspecific protein adsorption, the creation of biomimetic materials to imitate natural processes and structures, and the design of sophisticated 3D architectures to produce well-defined patterns for diagnostics.[232] Therefore, biomaterials consist of both simple devices and highly complex functional materials to control biological interactions.[233]

Nowadays, the focus of biomaterials is more far-reaching (Fig. 19) and comprises medical devices, pharmaceuticals, biotechnology, cosmetics/cosmeceutical, the food industry, etc.[234] Nanotechnology has accelerated biomaterials-based research, as exemplified by electrospun nanofibers and electrosprayed nanoparticles. Electrosprayed and electrospun nanoformulations based on natural materials (Fig. 20) offer promising therapeutic approaches that include cells, biomaterials, biomolecules, and microenvironmental factors for the promotion of tissue repair and/or functional restoration. Cell electrospinning and bio-electrospraying have emerged as vital medical techniques, and are under active investigation for many applications, such as repair or replacement of damaged/aging tissues, as well as the delivery of personalized medicines.[235,236] Controlled delivery of drugs from implantable

devices and nanoparticle formulations derived from natural sources are currently in advanced stages of clinical evaluation and/or already in the pharmaceutical market.

Despite the continuing interest in animal-based, natural material research, there is considerably less attention focused on plant-based, electrospun nanofibers, or nanomaterials for pharmaceutical applications. We believe that further research in the area of plant-based nanomaterials will be beneficial in relation to cost, availability, and other commercial issues. Additionally, unlike the restricted usage of materials of animal origin for geo-political reasons, plant-based nanobiomaterials can serve humanity in a more holistic manner.

Vitamins are essential for skincare and other applications, and transdermal approaches to vitamin delivery are widely accessible. Nevertheless, nanoparticulate vitamin formulations have not yet been attempted by electrospraying. Nanoparticulate formulations have almost limitless delivery applications via oral, pulmonary, transdermal, and ocular routes of administration. Vitamins and other natural resources are plentiful, and their nanoformulations would undoubtedly facilitate both tissue regeneration and pharmaceutical delivery.

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References

1. R. Zajtchuk. *Dis Mon.* 1999, **45**, 449-495.
2. L. Wolfgang. Application of nanotechnologies in the energy sector. Volume of the series Aktionslinie Hessen-Nanotech of the Hessian Ministry of Economy, Transport Urban and Regional Development. 2008.
3. N. Sozer and J.L. Kokini. *Trends Biotechnol*. 2009*,* **27**, 82-89.
4. Allah Ditta. *Adv. Nat. Sc*i: *Nanosci. Nanotechnol.*2012, **3**, 033002.
5. J. Altmann and A. Gubrud. 2004 Military, Arms Control, and Security Aspects of Nanotechnology. D. Baird, A. Nordmann & J. Schummer (eds.), Discovering the Nanoscale, Amsterdam: IOS Press.
6. T. Hillie and M. Hlophe. *Nature Nanotechnology.* 2007, **2**, 663–664.
7. V. Shanmugam, S. Selvakumar and C.S. Yeh. *Chem. Soc. Rev*. DOI. 10.1039/c4cs00011k.
8. K. Lia and B. Liu. *Chem. Soc. Rev*. 10.1039/c4cs00014e.
9. B.D. Gates, Q. Xu, M. Stewart and G.M. Whitesides. *Chem. Rev.* 2005, **105**, 1171-1196.
10. S. Zhang. *Nature Biotechnol.* 2003, **21**, 1171-1178.
11. R H Müller, K Mäder and S Gohla. *European Journal of Pharmaceutics and Biopharmaceutics* 2000, **50**, 161-77.
12. V. P. Torchilin. *Nature Reviews Drug Discovery* 2005, **4**, 145-160.
13. A.P. Alivisators. *Science.* 1996, **271**, 933-937.

14. J. P. Rao and K. E. Geckeler. *Progress in Polymer Science* 2011, **36**, 887-913.

15. D. A. Tomalia, A. M. Naylor and W. A. Goddard. *Angew Chemie Intl Edn*, 1990, **29**, 138–175.

16. N. M. Rodriguez. *J. Mat. Res*. 1993, **8**, 3233-3250.

17. J. P. Juste, I. P. Santos, L. M. Liz-Marzána and P Mulvaney. *Coordination Chem. Rev.* 2005, **249**, 1870-1901.

18. S. Ramakrishna, K. Fujihara, T. Wee-Eong and R. Ramaseshan. *Materials Today*. 2006, **9**, 40-50.

19. Z. Huang, Y.Z. Zhang, M. Kotaki and S. Ramakrishna. *Compos. Sci. Technol*. 2003, **63**, 2223-2253.

20. R. Sridhar, J. Venugopal, S. Sundarrajan, R. Ravichandran, B. Ramalingam and S. Ramakrishna. *J. Drug Del. Sci. Tech.* 2012, **21**, 451-468.

21. R. Jayakumar and S. V. Nair (Eds.). *Advances in Polymer Science (series)*, Vol. **246**, 1-287.

22. V. Beachley and X. Wen. *Prog. Poly. Sci.*, 2010, **35**, 868-892.

23. R. Dersch, A. M. Graeser, A. A. Greiner and J. H. Wendorff. *Aust. J. Chem*. 2007, **60**, 719–728.

24. D. Liang, B. S. Hsiao and B. Chu. *Adv. Drug Del. Rev.* 2007, **59**, 1392–1412.

25. S. Agarwal, A. Greiner and J. H. Wendorff. *Prog. Poly. Sci.*, 2013, **38**, 963-991.

26. J. Xie, X.z Li and Y. Xia. *Macromol. Rapid Commun*. 2008, **29**, 1775–1792.

27. H. S. Yoo, T. G. Kim and T. G. Park. *Adv. Drug Del. Rev*. 2009, **61**, 1033-1042.

28. D. B. Khadka and D. T. Haynie. *Nanomedicine: Nanotechnology, Biology, and Medicine*. 2012, **8**, 1242-1262.

29. N. Bock, T.R. Dargaville and M.A. Woodruff. *Prog. Poly. Sci.*, 2012, **37**, 1510-1551.

30. R Sridhar and S Ramakrishna. *Biomatter.* 2013, **3**(3), 24281-12.

31. M. R. Badrossamay, H. A. McIlwee, J. A. Goss and K. K. Parker. *Nano Lett.*, 2010, 10, 2257–2261.

32. K. Dewangan, N. N. Sinha, P. G. Chavan, P. K. Sharma, A. C. Pandey, M. A. More, D. S. Joag, N. Munichandraiah and N. S. Gajbhiye. *Nanoscale*, 2012, 4, 645-651.

33. B. B. Lakshmi, C. J. Patrissi and C. R. Martin. *Chem. Mater*. 1997, **9**, 2544-2550.

34. X. Liu and P. X. Ma. *Biomaterials*. 2009, **30**, 4094–4103.

35. C. J. Ellison, A. Phatak, D. W. Giles, C. W. Macosko and F. S. Bates. *Polymer*, 2007, **48**, 3306–3316.

36. C. M. Zelenski and P. K. Dorhout. *J. Am. Chem. Soc.*, 1998, **120**, 734–742.

37. A. S. Badami, M. R. Kreke, M. S. Thompson, J. S. Riffle and A. S. Goldstein. *Biomaterials* 2006, **27**, 596–606.

38. V. Karageorgiou and D. Kaplan. *Biomaterials* 2005, **26**, 5474–5491.

39. C. A. Bashur, R. D. Shaffer, L. A. Dahlgren, S. A. Guelcher and A. S. Goldstein. *Tissue Eng Part A*. 2009, **15**, 2435-2445.

40. N. Ashammakhi, A. Ndreu, L. Nikkola, I. Wimpenny and Y. Yang. *Regen Med*. 2008, **3**, 547-574.

41. J. Venugopal, S. Low, A. T. Choon and S. Ramakrishna. *J. Biomed. Mat. Res. Part B: Appl. Biomat.* 2008, **84**, 34-48.

42. B. Oh and C. H. Lee. *Expert Opin Drug Deliv.* 2013, **10**, 1565-1582.

43. B. Ma, J. Xie, J. Jiang, F. D. Shuler and D. E. Bartlett. *Nanomedicine (Lond).* 2013, **8**, 1459-1481.

44. A. Martins, R. L. Reis and N. M. Neves. Intl. Mat. Rev., 2008, **53**, 257-274.

45. M. Hadjiargyrou and J. B. Chiu. *Expert Opin. Drug Deliv. 2008,* **5**, 1093-1106.

46. F. Ignatious, L. Sun, C. P. Lee and J. Baldoni. *Pharmaceutical Res.*, 2010, **27**, 576-588.

47. Y. J. Son, W. J. Kim and H. S. Yoo. *Arch. Pharm. Res*. 2014, **37**, 69-78.

48. D. Zhang, I. Y. Shadrin, J. Lam, H. Q. Xian, H. R. Snodgrass and N. Bursac. *Biomaterials*. 2013, **34,** 5813-5820.

49. S. A. Doppler, M. A. Deutsch, R. Lange and M. Krane. *J Thorac Dis*. 2013, **5**, 683-697.

50. C.C. Huang, H.J. Wei, Y.C. Yeh, J.J. Wang, W.W. Lin, T.Y. Lee, S.M. Hwang, S.W. Choi, Y. Xia, Y. Chang and H.W. Sung. *Biomaterials.* 2012, **33,** 4069-4077.

51. Z. Li, X. Guo and J. Guan. *Biomaterials.* 2012, **33**, 5914-5923.

52. A. Jain, M. Betancur, G. D. Patel, C. M. Valmikinathan, V. J. Mukhatyar, A. Vakharia, S. B. Pai, B. Brahma, T. J. MacDonald and R. V. Bellamkonda . *Nat. Mat.*, 2014, **13**, 308-316.

53. M. C. Bottino, V. Thomas and G. M. Janowski. *Acta Biomaterialia* 2011, **7**, 216.

54. A. M. Martins, G. Eng, S. G. Caridade, J. F. Mano, R. L. Reis and G. Vunjak-Novakovic. *Biomacromolecules*. 2014, **15**, 635-643.

55. S. N. Jayasinghe. *Analyst*. 2011, **136**, 878-890.

56. S. N. Jayasinghe. *Analyst*. 2013, **138**, 2215-2223.

57. N. Mongkoldhumrongkul, S. Best, E. Aarons and S. N. Jayasinghe. *J Tissue Eng. Regen Med*. 2009, **3**, 562-566.

58. Q. Hu, Y. Hu and J. Xu. *Food Chem*. 2005, **91,** 85–90.

59. R. Maenthaisong , N. Chaiyakunapruk, S. Niruntraporn and C. Kongkaew. *Burns* 2007, **3,** 713–718.

60. R. Seenappa. Dinkal Agro Inc: Organic for Healthy Living. 2009.

61. L.L. Murphy. "American Ginseng in the Prevention and Treatment of Human Breast Cancer". Southern Illinois University Carbondale, 2000.

62. N. Charernsriwilaiwat , T. Rojanarata, T. Ngawhirunpat, M. Sukma and P. Opanasopit. *Int. J. Pharm*. 2013, **452**(1-2), 333-343.

63. M.M.C Ligia, M.O. Gabriel, M.C. Bibin, L.L. Alcides, F.S. Sivoney and F. Mariselma. *Indust. Crops Prod*. 2013, **41**, 198– 202.

64. S. Suganya, T. Senthil Ram, B.S. Lakshmi and V.R. Giridev. *J Applied Polym. Sci.* 2011, **121**, 2893–2899.

65. R.U Afeesh, P. Tirupathi, G. Gopalsamy, S. Kalaiselvi, A.M.B Nasser and K. Hak Yong. *Colloids Surf. A: Physicochem. Eng. Aspects.* 2012, **415,** 454-460.

66. O. Suwantong, U. Ruktanonchai and P. Supaphol. 2010. *J. Biomed. Mater. Res A*. **94**(4), 1216-1225.

67. G. Jin, M.P. Prabhakaran, D. Kai, K. Arunachalam and S. Ramakrishna. *Biomaterials*. 2013, **34**, 724-734.

68. M. Garima, S. Saurabh and B.P. Nagori. *Int. J. Pharm. Tech. Res*. 2010, **2**, 1298–310.

69. E. Mohanambal, K. Shobana, S.M. Sowmya, G.M. Kusuma, K. Satish, B. Vijayakumar. *Intl. J. Novel. Tr. Pharm. Sci.* 2011, **1**, 6-9.

70. S.S. Chopra, M.R. Patel, L.P. Gupta and I.C. Datta. *Indian J. Med. Res.* 1975, **63,** 824-828.

71. K.N. Udupa and G.C. Prasad. *Indian J. Med. Res.* 1964, **52**, 480-487.

72. S. Suganya, J. Venugopal, S. Ramakrishna, B.S. Lakshmi and V.R. Giri Dev. *J. Appl. Polym. Sci*. 2013, DOI: 10.1002/app.39835.

73. X.Y. Dai, W. Nie, Y.C. Wang, Y. Shen, Y. Li and S.J. Gan. *J. Mater. Sci. Mater. Med.* 2012, **23**(11), 2709-2716.

74. J. Han, T.X. Chen, C.J. Branford-White and L.M. Zhu. *Int. J. Pharm.* 2009, **382**(1-2), 215-221.

75. T. T. T. Mai, T. T. T. Nguyen, Q. D. Le, T. N. Nguyen, T. C. Ba, H. B. Nguyen, T. B. H. Phan, D. L. Tran, X. P. Nguyen and J.S. Park. *Adv. Nat. Sci.: Nanosci. Nanotechnol.* 2012, **3**, 025014.

76. G. Guo, S. Fu, L. Zhou, H. Liang, M. Fan, F. Luo, Z. Qian and Y. Wei. *Nanoscale*. 2011, **3**, 3825-3832.

77. V. Gupta, A. Aseh, C. Ríos, B. Aggarwal and A. Mathur. *Internat. J. Nanomed*. 2009 , **4**, 115–122.

78. G. Jonathan, Cato T. Laurencin, Alex F. Chen and S. Lakshmi. *Clin. Exp. Pharmacol. Physiol.* 2009, **36**(12), 1149–1156.

79. J. Gomez-Estaca, M.P Balaguer, R. Gavara and P. Hernandez-Munoz. *Food Hydrocolloids*. 2012, **28**, 82-91.

80. R. Sridhar, S. Ravanan, J. R. Venugopal, S. Sundarrajan, D. Pliszka, S. Sivasubramanian, P. Gunasekaran, M. Prabhakaran, K. Madhaiyan, A. Sahayaraj, K. H. Lim and S. Ramakrishna. *J Biomater Sci Polym Ed*. 2014, 1-14. DOI: 10.1080/09205063.2014.917039.

81. J.H. Jung, J.E. Lee and G.N. Bae. *J. Aerosol Sci.*2013, **57**, 185–193.

82. J.H. Jung, S.Y. Park, J.E. Lee, C.W. Nho, B.U. Lee and G.N. Bae. *J. Aerosol Sci.* 2011, **42**, 725–736.

83. P. Taepaiboon, U. Rungsardthong and P. Supaphol. *Eur J Pharm Biopharm*. 2007, **67**(2), 387-397.

84. K. Madhaiyan, R. Sridhar, S. Sundarrajan, J. R. Venugopal and S. Ramakrishna. *Int J Pharm*. 2013, **444**(1-2), 70-76.

85. S. Khansari, S. Duzyer, S. Sinha-Ray, A. Hockenberger, A. L. Yarin and B. Pourdeyhimi. *Mol Pharm*. 2013, **10**, 4509-4526.

86. X. M. Wu, C. J. Branford-White and D. G. Yu, N. P. Chatterton and L. M. Zhu. *Colloids Surf B Biointerfaces*. 2011, 82, 247-252.

87. J. Venugopal, M.P. Prabhakaran, S. Low, A.T. Choon, Y.Z. Zhang, G. Deepika and S. Ramakrishna. *Curr. Pharm. Des.* 2008, **14**(22), 2184-2200.

88. J.A. Matthews, G.E. Wnek, D.G. Simpson and G.L. Bowlin. *Biomacromolecules.* 2002, **3**, 232-238.

89. K. Ohkawa, D. Cha, H. Kim, A. Nishida and H. Yamamoto. *Macromol Rapid Commun* 2004, **25**, 1600-1605.

90. Y.Z. Zhang, S. Ramakrishna and Z.M. Huang. *J. Biomed. Mater. Res*. 2005 , **72B**, 156-165.

91. G.E. Wnek, M.E. Carr, D.G. Simpson and G.L. Bowlin. *Nano Lett.* 2003, **3**, 213-216.

92. Y.K. Luu, K. Kim, B.S. Hsiao, B. Chu and M. Hadjiargyrou. *J. Control. Release* 2003, **89**, 341-353.

93. J.J. Stankus , J. Guan and W.R. Wagner. *J. Biomed. Mater. Res.* 2004, **70A**, 603-614.

94. S.P. Zhong, W.E. Teo, X. Zhu, R. Beuerman, S. Ramakrishna and L.Y. Yung. *Biomacromolecules.* 2005, **6,** 2998-3004.

95. M. Li, Y. Guo, Y. Wei and P.I Lelkes. *Biomaterials*. 2006, **27**, 2705-2715.

96. B.M. Min, G. Lee and W.H. Park. *Biomaterials*. 2004, **25**, 1289-1297.

97. J. Venugopal, S. Low, A.T. Choon, A. Bharath Kumar and S. Ramakrishna. *Artif. Organs* 2008, **32**(5), 388–397.

98. I.K. Kwon and T. Matsuda. *Biomacromolecules*. 2005, **6**, 2096-2105.

99. S.Y. Chew, J. Wen, E.K.F. Yim and K.W Leong. *Biomacromolecules.* 2005, **6**, 2017-2024.

100. W. Meng, S.Y. Kim, Y. Jiang, J. Chul, K. Naoki and K. Inn-Kyu. *J. Biomater. Sci. Polym. Ed.* 2007, **18**, 81-94.

101. S.Y. Chew, R. Mi, A. Hoke and K.W. Leong. *Adv. Func. Mater.* 2007 , **17**, 1288-1296.

102. J.R. Venugopal, Y.Z. Zhang and S. Ramakrishna. *Artif. Organs*. 2006, **30**, 440-446.

103. K.E Park, H.K. Kang, S.J Lee, B.M. Min and W.H. Park. *Biomacromolecules.* 2006, **7**, 635-643.

104. H.W. Kim, J.H. Song and H.E. Kim. *Adv. Func. Mat.* 2005, **15**, 1988-1994.

105. K. Fujihara, M. Kotaki and S. Ramakrishna. *Biomaterials*. 2005, **26**, 4139-4147.

106. Y. Ito, H. Hasuda, M. Kamitakahara, C. Ohtsuki, M. Tanihara, I.K. Kang and O.H. Kwon. *J. Biosci. Bioeng.* 2005,**100**, 43-49.

107. H.W. Kim, H.H. Lee and J.C. Knowles. *J. Biomed. Mater. Res.* 2006, **79A,** 643-649.

108. X.L. Deng, M.M. Xu and X.P Yang. *Key Engineering Materials.* 2007, **330-332**, 393-396.

109. J. Venugopal, S. Low, A.T. Choon, A. Bharath Kumar and S. Ramakrishna. *J. Biomed. Mater. Res*. 2008, **85A**, 408–417.

110. L. Buttafoco, N.G. Kolkman and J. Feijen. *Biomaterials*. 2006, **27**, 724-734.

111. J. Stitzel, J. Liu, S.J. Lee , M. Komura, J. Berry, J.J. Yoo and A. Atala. *Biomaterials*. 2006, **27**, 1088-1094.

112. M. Li and P.I. Lelkes. *J. Biomed. Mater. Res.* **79A**, 963-973.

113. W. He, Z. Ma, T. Yong, W.E. Teo and S. Ramakrishna. *Biomaterials*. 2005, **26**, 7606-7615.

114. C. Li, C. Vepari, H.J. Jin, H.J. Kim and D.L. Kaplan. *Biomaterials.* 2006, **27**, 3115-3124.

115. C.M. Kelleher and J.P. Vacanti. *J. R. Soc. Interface*. 2010, **7** Suppl 6, S717-S729.

116. S. Zhang, L. Chen, Y. Jiang, Y. Cai, J. Ji, P. Shi and H.W. Ouyang. *Acta. Biomater*. 2013, **9**(7), 7236-7247.

117. W. He, Z. Ma, W. E. Teo, Y. X. Dong, P. A. Robless, C. T. Lim and S. Ramakrishna. *J Biomed Mater Res A*. 2009, **90**, 205-216.

118. X. He, W. Fu, B. Feng, W. Wang and J. Zheng. *Regen. Med.* 2013, **8**(4), 425-436.

119. M. Iafiscol, N. Quirici, I. Foltran and L. Rimondini. *J. Nanosci. Nanotechnol.* 2013, **13**(7), 4720-4726.

120. C.S. Wong, X. Liu, Z. Xu, T. Lin and X. Wang. *J. Mater. Sci. Mater. Med.* 2013, **24**(8), 1865-1874.

121. W. Zhao, Y.M. Ju, G. Christ, A. Atala, J.J. Yoo and S.J. Lee. *Biomaterials.* 2013, **34**(33), 8235-8240.

122. J.J. Willard, J.W. Drexler, A. Das, S. Roy, S. Shilo, O. Shoseyov and H.M. Powell. *Tissue Eng. Part A*. 2013, **19**(13-14), 1507-1518.

123. S. Suganya, J. R. Venugopal, S. A. Mary, S. Ramakrishna, B. S. Lakshmi and V. R. Giri Dev. *Iran Poly J*. 2014, **23**, 237-248.

124. K.I. Clarke, S.E. Graves, A.T.C. Wong and J.T. Czernuszka. *J. Mater. Sci. Mater. Med.* 1993, **4,** 107-110.

125. M. Kikuchi, S. Itoh, S. Ichinose , K. Shinomiya and J. Tanaka. *Biomaterials.* 2001, **22**, 1705-1711.

126. F. Chen, Z.C. Wang and C.J. Lin. *Mater. Lett.* 2002, **57**, 658-662.

127. S.S. Liao, F.Z. Cui and Q.L. Feng. *J. Biomed. Mater. Res*. 2004, **69B,** 158-165.

128. J. Wei, Y.B. Li, W.Q. Chen and Y. Zuo. *J. Mater Sci.* 2003, **38**, 3303-3306.

129. F.J. O'Brien, B.A. Harley, I.V. Yannas and L. Gibson. *Biomaterials.* 2004, **25,** 1077-1086.

130. S.H. Teng, E.J Lee, P. Wang and H.E Kim. *Materials Letters*. 2008, **62**, 3055-3058.

131. G.A. Carlson, J.L. Dragoo, B. Samimi, D.A. Bruckner and P. Benhaim. *Biochem. Biophys. Res. Commun*. 2004, **321**, 472-478.

132. W.J. Landis, M.J. Song, A. Leith, L. McEwen and B.F. McEwen. *J. Struct. Biol.* 1993, **110,** 39-54.

133. D. Wahl and J.T. Czernuszka. *Euro. Cells Materials.* 2006, **11**, 43-56.

134. D.A. Wahl , E. Sachlos, C. Liu and J.T. Czernuszka. *J. Mater. Sci. Mater. Med.* 2007, **18**, 201-209.

135. A. Porter, N. Patel, R. Brook and W. Bonfield. J. Mater. Sci. Mater. Med. 2005, **16**, 899-907.

136. J. Venugopal, S. Low, A.T. Choon, T.S. Sampath Kumar and S. Ramakrishna. *J. Mater. Sci. Mater. Med.* 2008, **19**, 2039-2046.

137. E.D. Boland, D.G. Simpso, G.E. Wnek and G.L. Bowlin. *Polymer Preprints (American Chemical Society, Division of Polymer Chemistry).* 2003, **44**(2), 92-93.

138. M. McManus, E. Boland, S. Sell, W. Bowen, D. Simpson and G. Bowlin. *Biomedical Materials (Bristol, United Kingdom).* 2007, **2(4),** 257-262.

139. M. McManus, E. Boland, D. Eugene, D. Simpson, C.P. Barnes and G.L. Bowlin. *J. Biomed. Materi. Research, Part A.* 2007, **81A**(2), 299-309.

140. M. McManus, E. Boland, D. Eugene, D. Simpson and G.L. Bowlin. *Acta Biomaterialia.* 2006, **2**(1), 19-28.

141. S.A. Sell, M.P. Francis, K. Garg, M. McClure, D.G. Simpson and G.L. Bowlin.

Biomedical Materials. 2008, **3B** 045001/1-045001/11.

142. C.R. Carlisle, C. Coulais, M. Namboothiry, D.L. Carroll, R. Hantgan and M. Guthold. *Biomaterials.* 2009, **30**(6), 1205-1213.

143. A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher. *Cell*. 2006, **126**, 677-689.

144. C.L. He, X.H. Xu, F. Zhang, L. Cao, W. Feng, H.S. Wang and X.M. Mo. *J. Biomed. Materials Res. Part A.* 2011, **97A**(3), 339-347.

145. Z. Fang, W. Fu, Z. Dong, X. Zhang, H. He and Y. Wang. *Appl. Surf. Sci.* 2011, **257(9),** 4133-4138.

146. D. Feingold-Leitman, E. Zussman and D. Seliktar. *J. Bionanosci.* 2009, **3(1),** 45-57.

147. J.K. Smith, A.R. Moshref, J.A. Jennings, H.S. Courtney and W.O. Haggard. *Clin Orthop Relat Res.* 2013, DOI:10.1007/s11999-013-2988-5.

148. H.D. Han, C.K. Song, Y.S. Park, K.H. Noh, J.H. Kim, T. Hwang, T.W. Kim and B.C. Shin. *Int. J. Pharm.* 2008, **350**(1-2):27-34.

149. K. Y. Lee, L. Jeong, Y. O. Kang, S. J. Lee and W. H. Park. *Adv Drug Deliv Rev.* 2009, **61**, 1020-1032.

150. M. Ignatova, N. Manolova and I. Rashkov. *Macromol Biosci*. 2013, **13**, 860-872.

151. M.S. Austero, A.E. Donius and C.L. Schauer. *J. R. Soc. Interface.* 2012, **9**, 2551-2562.

152. A.E. Donius, M.A. Kiechel, C.L. Schauer and U.G. Wegst. *J. R. Soc. Interface.* 2013, **10**, 20120946.

153. J. D. Schiffman and C. L. Schauer. *Biomacromolecules*. 2007, **8**, 594-601.

154. M. A. Kiechel and C. L. Schauer. *Carbohydr Polym*. 2013, **95**, 123-133.

155. Y.Z. Zhang, J.R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, and C.T. Lim. *Biomaterials.* 2008, **29,** 4314-4322.

156. S. Zhang and K. Kawakami. *Int. J. Pharmaceutics* 2010, **397**, 211–217.

157. K. Songsurang, N. Praphairaksit, K. Siraleartmukul and N. Muangsin. *Arch. Pharm. Res.* 2011, **34**, 583-592.

158. M.Y. Kim and J. Lee. *Carbohydrate Polymers.* 2011, **84**, 1329-1336.

159. S. J. Hollister. Nat Mater. 2005;4:518-24.

160. S. Zarkoob, R.K. Eby, D.H. Reneker, S.D. Hudson, D. Ertley and W.W. Adams. *Polymer.* 2004, **45,** 3973-3977.

161. S.H. Kim, Y.S. Nam, T.S. Lee and W.H. Park. *Polymer J.* 2003, **35**, 185-190.

162. K. Ohgo, C. Zhao, M. Kobayashi and T. Asakura. *Polymer.* 2003, **44,** 841-846.

163. H.J Jin, S.V. Fridrikh, G.C. Rutledge and D.L. Kaplan. *Biomacromolecules.* 2002, **3**, 1233-1239.

164. F. Zhang, B. Zuo, Z. Fan, Z. Xie, Q. Lu, X. Zhang, D. L. Kaplan. Biomacromolecules. 2012, **13**, 798-804.

165. C. Li, C. Vepari, H.J. Jin, H. Kim and D. Kaplan. *Biomaterials.* 2006, **27,** 3115-124.

166. H. Najmeh, T. Hossein and M. Ali Reza. *Sci. Technol. Adv. Materials.* 2012, **13**, 035010/1-7.

167. Z.M. Huang, Y.Z. Zhang and S. Ramakrishna. *Polymer*. 2004, **45** (15), 5361-5368.

168. N. T. B. Linh, Y.K. Min and H.Y. Son. *J Biomed. Mater. Research Part B: Applied Biomaterials.* 2010, **95B** (1), 184-191.

169. Y.Z. Zhang, J. Venugopal, Z. M. Huang, C. T. Lim and S. Ramakrishna. *Polymer,* 2006, **47**(8), 2911-2917.

170. J. Ratanavaraporn, R. Rangkupan and H. Jeeratawatchai. *Internat. J. Biol. Macromol*. 2010, **47**(4), 431-438.

171. C. Yang, X.M. Wu and Y.H. Zhao. J. Appl. Polym.Sci. 2011, **121**(5), 3047-3055.

172. D. Yang, Y. Li and J. Nie. Carbohyd.Polym. 2007, **69**(3), 538-543.

173. S.Y. Gu, Z.M. Wang and J. Ren. Mater. Sci. Eng C. 2009, **29**(6), 1822-1828.

174. Y. Moon, H. Uyama and S. Inoue. *Chemistry Letters*, 2006, **35** (6), 564-565.

175. Y. Zhang, H. Ouyang, C.T. Lim and S. Ramakrishna. J. Biomed. Mater. Res. Part B: Applied Biomaterials. 2005, **72B** (1), 156-165.

176. Z.X. Meng, Y. Wan and C. Ma. Mater. Sci. Eng C. 2010, **30**(8), 1204-1210.

177. M. Li, Y. Guo and Y. Wei. *Biomaterials,* 2006, **27**(13), 2705-2715.

178. J. Ko, H. Yin and J. An. *Macromole. Res*. 2010, **18**(2), 137-143.

179. H.C. Chen, W.C. Jao and M.C. Yang. Polym. Adv. Technol. 2009, **20**(2), 98-103.

180. M. Li, M.J. Mondrinos and M.R. Gandhi. *Biomaterials*, 2005, **26**(30), 5999-6008.

181. S. Panzavolta, M. Gioffrè and M.L. Focarete. *Acta Biomaterialia*, 2011, **7**(4), 1702-1709.

182. Y.F.Qian, K.H. Zhang and F. Chen. *J Biomater. Sci. Polym. Ed.* 2011, **22**(8), 1099-1113.

183. X. Liu, L.A. Smith, J. Hu and P.X. Ma. *Biomaterials.* 2009, **30**, 2252-2258.

184. M. Gulfam, J. Kim, J.M. Lee, B. Ku, B.H. Chung and B.G. Chung. *Langmuir* 2012, **28**, 8216−8223.

185. R. Sridhar, K. Madhaiyan, S. Sundarrajan, A. Góra, J. R. Venugopal and S. Ramakrishna. *J. Mater. Chem. B*, **2014**, *2*, 1626-1633.

186. R. Lakshminarayanan, R. Sridhar, X. J. Loh, M. Nandhakumar, V. A. Barathi, M. Kalaipriya, J. L. Kwan, S. P. Liu, R. W. Beuerman and S. Ramakrishna. *Int J Nanomedicine*. 2014, **9**, 2439-2458.

187. C.P. Barnes, M.J. Smith and G.L. Bowlin. *J. Eng. Fibers Fabrics.* 2006, **1**(2), 16-29.

188. R. Ravichandran, V. Seitz, J. Reddy Venugopal, R. Sridhar, S. Sundarrajan, S. Mukherjee, E. Wintermantel and S. Ramakrishna. *Macromol. Biosci.* 2013, **13**(3), 366-375.

189. M. Fasano, S. Curry and E. Terreno. *IUBMB Life*, 2005, **57**(12), 787-796.

190. U. Kragh-Hansen, V.T. Chuang and M. Otagiri. *Biol. Pharmac. Bulletin*. 2002, **25**(6), 695-704.

191. J. Xie and Y.L. Hsieh. J. Mater. Sci. 2003, **38**(10), 2125-2133.

192. O. Regev, R. Khalfin and E. Zussman. International *J. Biol. Macromol.* 2010, **47**(2), 261-265.

193. Y. Dror, T. Ziv and V. Makarov. *Biomacromolecules*. 2008, **9**(10), 2749-2754.

194. A. Moradzadegan, S.O. Ranaei-Siadat and A. Ebrahim-Habibi. Eng. Life Sci. 2010, **10**(1), 57-64.

195. T. Kowalczyk, A. Nowicka and D. Elbaum. *Biomacromolecules.* 2008, **9**, 2087-2090.

196. R. Ravichandran, R. Sridhar, J. R. Venugopal, S. Sundarrajan, S. Mukherjee and S. Ramakrishna. *Macromol Biosci*. 2014, **14**, 515-525.

197. C.M.A. Tavares, M. Talebi, P. Soares, C.U. Yurteri and J.R. Van Ommen. *Intl. J. Pharm*. 2011, **414**, 1-5.

198. W.J. Yiquan. M. Andrew, R.M. Jonathan, C. Ashutosh and L.C. Robert. *Biomacromolecules.* 2009, **10**, 19-24.

199. H. Jiang, Y. Hu and Y. Li. *J. Controlled Release.* 2005, **108**(2-3), 237-243.

200. C.M. Valmikinathan, S. Defroda and X. Yu. *Biomacromolecules*. 2009, **10**(5), 1084-1089.

201. S. Yan, L. Xiaoqiang and T. Lianjiang. *Polymer*, 2009, **50**(17), 4212-4219.

202. S. Yan, L. Xiaoqiang and L. Shuiping. *Colloids Surfaces B: Biointer.* 2009, **73**(2), 376-381.

203. S.P. Liu, Y. Su and Y.M. Chen. *J. Biomat. Sci. Poly Edn.* **22**(7), 2011, 945-955.

204. J. Zeng, A. Aigner and F. Czubayko. *Biomacromolecules.* 2005, **6**(3), 1484-1488.

205. M. Gandhi, R. Srikar and A.L. Yarin. *Molecular Pharmaceutics*. 2009, **6**(2), 641-647.

206. Y. M. Kolambkar, K. M. Dupont, J. D. Boerckel, N. Huebsch, D. J. Mooney, D. W. Hutmacher and R. E. Guldberg. *Biomaterials*. 2011, **32**, 65-74.

207. H. Nie, A. He, J. Zheng, S. Xu, J. Li and C. C. Han. *Biomacromolecules*. 2008, **9**, 1362-1365.

208. H. Qi, P. Hu, J. Xu and A. Wang. *Biomacromolecules*. 2006, **7**, 2327-2330.

209. J. Zhao, B. Guo and P. X. Ma. *RSC Adv*., 2014, **4**, 17736-17742.

210. S. I. Jeong, M. D. Krebs, C. A. Bonino, S. A. Khan and E. Alsberg. *Macromol Biosci*. 2010, **10**, 934-943.

211. J. M. Coburn, M. Gibson, S. Monagle, Z. Patterson and J. H. Elisseeff. *Proc Natl Acad Sci U S A*. 2012, **109**, 10012-10017.

212. H. Wang, Y. Feng, Z. Fang, R. Xiao, W. Yuan and M. Khan. *Macromol. Res*., 2013, **21**, 860-869.

213. F. Du, H. Wang, W. Zhao, D. Li, D. Kong, J. Yang and Y. Zhang. *Biomaterials*. 2012, **33**, 762-770.

214. K. T. Kurpinski, J. T. Stephenson, R. R. Janairo, H. Lee and S. Li. *Biomaterials*. 2010, **31**, 3536-3542.

215. H. Wang, Y. Feng, H. Zhao, R. Xiao, J. Lu, L. Zhang, and J. Guo. *Macromol. Res*., 2012, **20**, 347-350.

216. T. Wang, X. Ji, L. Jin, Z. Feng, J. Wu, J. Zheng, H. Wang, Z. W. Xu, L. Guo and N. He. *ACS Appl Mater Interfaces*. 2013, **5**, 3757-3763.

217. W. Liu, Y. C. Yeh, J. Lipner, J. Xie, H. W. Sung, S. Thomopoulos and Y. Xia. *Langmuir*. 2011, **27**, 9088-9093.

218. R. Krishnan, R. Rajeswari, J. Venugopal, S. Sundarrajan, R. Sridhar, M. Shayanti and S. Ramakrishna. *J Mater Sci Mater Med.* 2012, **23**, 1511-1519.

219. J. Venugopal, R. Rajeswari, M. Shayanti, R. Sridhar, S. Sundarrajan, R. Balamurugan and S. Ramakrishna. *Mater Sci Eng C Mater Biol Appl*. 2013, **33**, 1325-1331.

220. Y. Liu, G. Ma, D, Fang, J. Xu, H. Zhang and J. Nie. *Carbohydrate Polymers* 2011, **83**, 1011–1015.

221. Y. Ji, K. Ghosh, B. Li, J. C. Sokolov, R. A. Clark and M. H. Rafailovich. *Macromol Biosci*. 2006, **6**. 811-817.

222. I. C. Um, D. Fang, B. S. Hsiao, A. Okamoto and B. Chu. *Biomacromolecules*. 2004, **5**, 1428-1436.

223. Y. Ji, K. Ghosh, X. Z. Shu, B. Li, J. C. Sokolov, G. D. Prestwich, R. A. Clark and M. H. Rafailovich. *Biomaterials*. 2006, **27**, 3782-3792.

224. J. Li, A. He, J. Zheng and C. C. Han. *Biomacromolecules* 2006, **7**, 2243-2247.

225. T. G. Kim, H. J. Chung and T. G. Park. *Acta Biomaterialia* 2008, **4**, 1611–1619.

226. G. Ma, Y. Liu, C. Peng, D. Fang, B. He and J. Nie. *Carbohydrate Polymers* 2011, **86**, 505-512.

227. R. Uppal, G. N. Ramaswamy, C. Arnold, R. Goodband and Y. Wang. *J. Biomed. Mat. Res. B: Appl. Biomat.* 2011, **97B**, 20-29.

228. L. Li, Y. Qian, C. Jiang, Y. Lv, W. Liu, L. Zhong, K. Cai, S. Li and L. Yang. *Biomaterials* 2012, **33**, 3428-3445.

229. L. L. Hench. *Biomater Med Devices Artif Organs*. 1979, **7**, 339-350.

230. R. Langer, L. G. Cima, J. A. Tamada and E. Wintermantel. *Biomaterials*. 1990, **11**, 738-745.

231. L. L. Hench. *Biomaterials*. 19987, **19**, 1419-1423.

232. B. D. Ratner and S. J. Bryant. *Annu Rev Biomed Eng*. 2004, **6**, 41-75.

233. Editorial. *Nature Mater*. 2009, **8**, 439.

234. S. Ramakrishna. *Materials Today* 2013, **16**, 102-103.

235. S. N. Jayasinghe. *Analyst*. 2011, **136**, 878-890.

236. A. Townsend-Nicholson and S. N. Jayasinghe. *Biomacromolecules*. 2006, **7**, 3364-3369.

Figure Captions:

Figure 1. Nanotechnology in our life style: Various areas, industries.

Figure 2. Marketed electrospun nanomaterial products.

Figure 3. Electrospun & Electrosprayed nanomaterials: Their applications explored in diverse domains with number of publications and patents in parenthesis. a. SEM image of Poly(Vinyl alcohol) (PVA)/Curcumin nanofiber. b. SEM image of BSA/PVA/Curcumin nanofiber without further crosslinking. c. SEM image of BSA/PVA/Curcumin nanofiber with glutaraldehyde crosslinking. d. AFM image of 5% Riboflavin incorporated PCL nanofiber. e. 3D AFM image of 5% Riboflavin incorporated PCL nanofiber. f. TEM image of PCL/BSA and Curcumin core-shell nanofiber; BSA & Curcumin in core and PCL in shell. g. TEM image of Gold nanoparticle incorporated BSA/PVA nanofiber. h. PVA/Poly acrylic acid (PAA) nanofiber coated with Polyethylenimine (PEI). i. PVA/Poly acrylic acid (PAA) nanofiber incorporated with silver nanoparticle and coated with Polyethylenimine (PEI); Ag-PEI complexation seen as nanorods deposition on nanofibers. j. TEM image of Vitamin/PLGA core-shell nanoparticle. k. SEM image of Vitamin/PLGA core-shell nanoparticle.

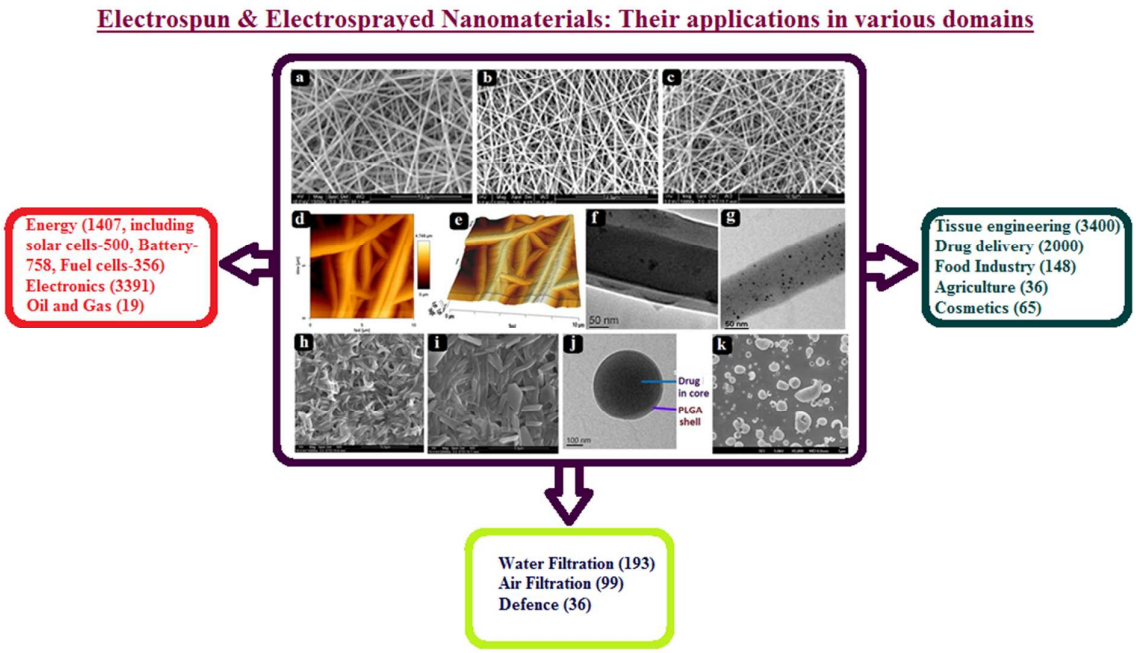


Figure 4. Electrospun biomaterial with perspective medical device application.

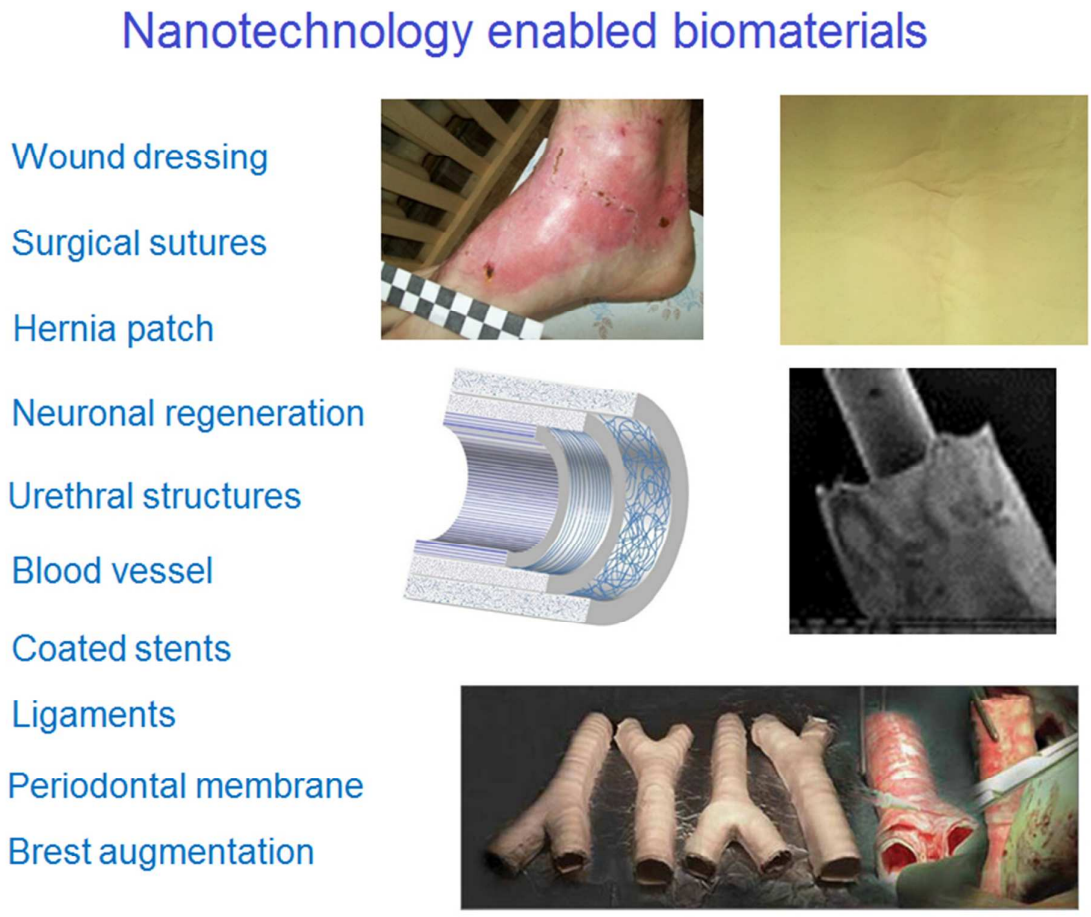


Figure 5. Future concepts for regenerative therapies. Future therapies in terms of myocardial regeneration might be a consolidation of transplantation of cells with "true" regenerative potential, tissue engineering with various scaffolds and cell types, a stimulation of resident cell sources by cytokines or growth factors or a direct reprogramming of scar tissue by delivery of various transcription factors or miRNAs. ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells; NRG1, neuregulin 1; p38 MAP KI, p38 MAP kinase inhibitor; P, periostin; miRNA, micro RNA. (Reproduced with copyright permission from ref. 49)

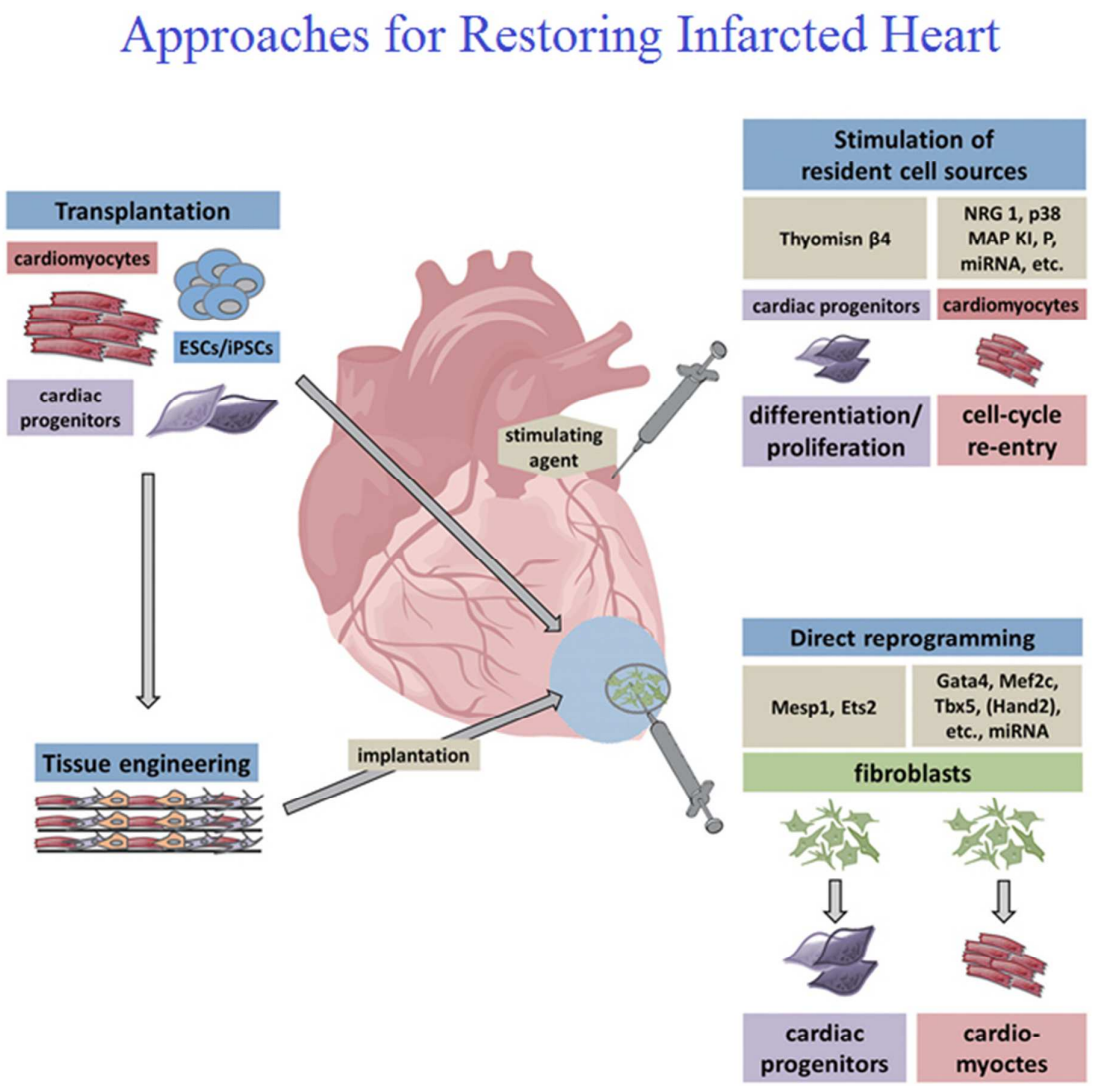


Figure 6. Schematic and image of conduit inserted into a rat brain. a, Representation of the tumour guide containing a nanofibre film inserted into a rat brain. Three-dimensional view (left) and coronal view (right) of the brain and conduit. b, Digital image of extracted brain containing a conduit. Apoptosis staining of glioblastoma cells in the cyclopamine hydrogel-filled conduits. The Dead End TUNEL System was used to stain for apoptotic cells. c, Schematic of a conduit containing 4 mm of the nanofibre film followed by the hydrogel region (2 mm in length). d, Tumour cells were present in the collagen hydrogel (left panel) and in the cyclopamine-conjugated collagen hydrogel (right panel). Scale bars, 400 μm. e, Bright-field images of cells in collagen hydrogel and cyclopamine-conjugated collagen. The left panel shows the tumour cells in the collagen-filled conduit region. It can be seen that most of the cells were viable owing to the lack of DABC staining. The right panel shows a darker brown staining, representing DABC cells in the cyclopamine-conjugated collagen hydrogel. Scale bars, 500 μm. f, Bright-field images of tumour cells in brain tissue, tumour cells in the collagen, and tumour cells in cyclopamine-conjugated collagen. The image on the right has a darker brown stain, signifying apoptotic cells. Scale bars, 100 μm. (Reproduced with copyright permission from ref. 52)

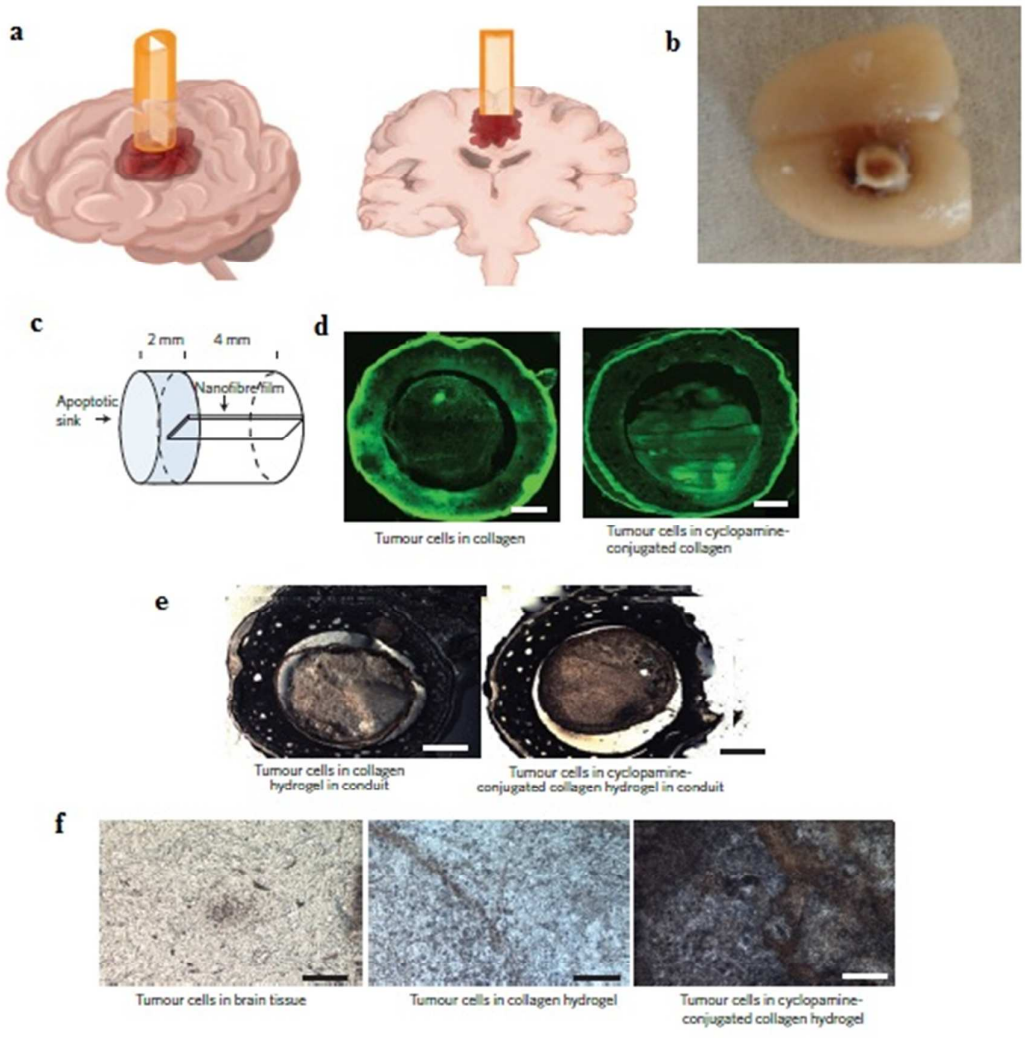

a
b
c
2 mm
4 mm
Apoptotic sink
Nanofibre film
d
Tumour cells in collagen
Tumour cells in cyclopamine-conjugated collagen
e
Tumour cells in collagen hydrogel in conduit
Tumour cells in cyclopamine-conjugated collagen hydrogel in conduit
f
Tumour cells in brain tissue
Tumour cells in collagen hydrogel
Tumour cells in cyclopamine-conjugated collagen hydrogel

Figure 7. Schematic of spatially designed and functionally graded electrospun membrane for periodontal regeneration. (Reproduced with copyright permission from ref. 54)

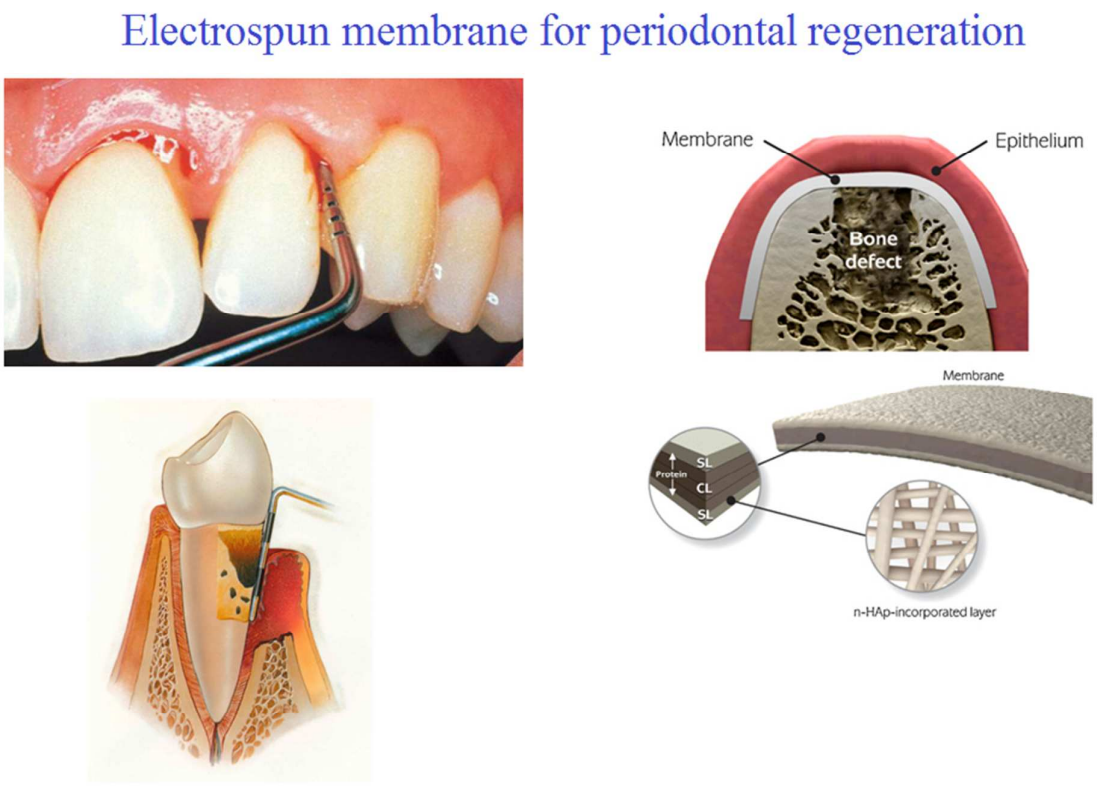


Figure 8. Schematic of electrospun and electrosprayed animal and plant based materials.

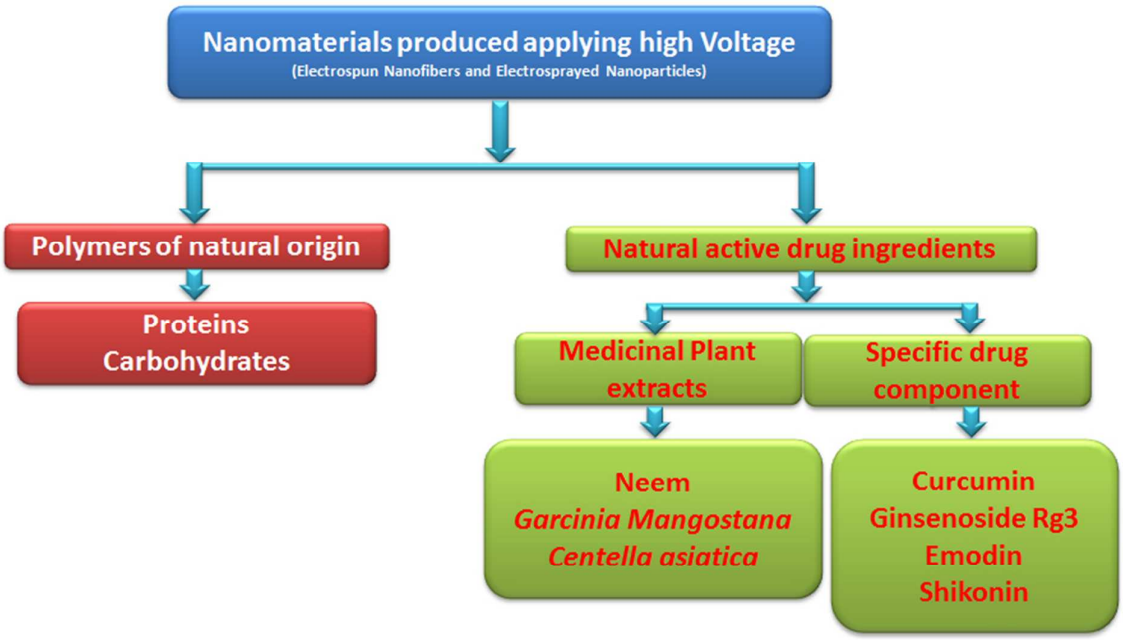


Figure 9. FESEM images showing the cell-biomaterial interactions on Day 10 on (a) TCP, (b) PCL, (c) PCL-CQ, and (d) PCL-CQ-HA nanofibrous scaffolds at 5000x magnification. Higher level of mineralization observed on the surface of PCL-CQ and PCL-CQ-HA nanofibrous scaffolds forming a thick layer along with ECM production by cells. (Reproduced with copyright permission from ref. 72)

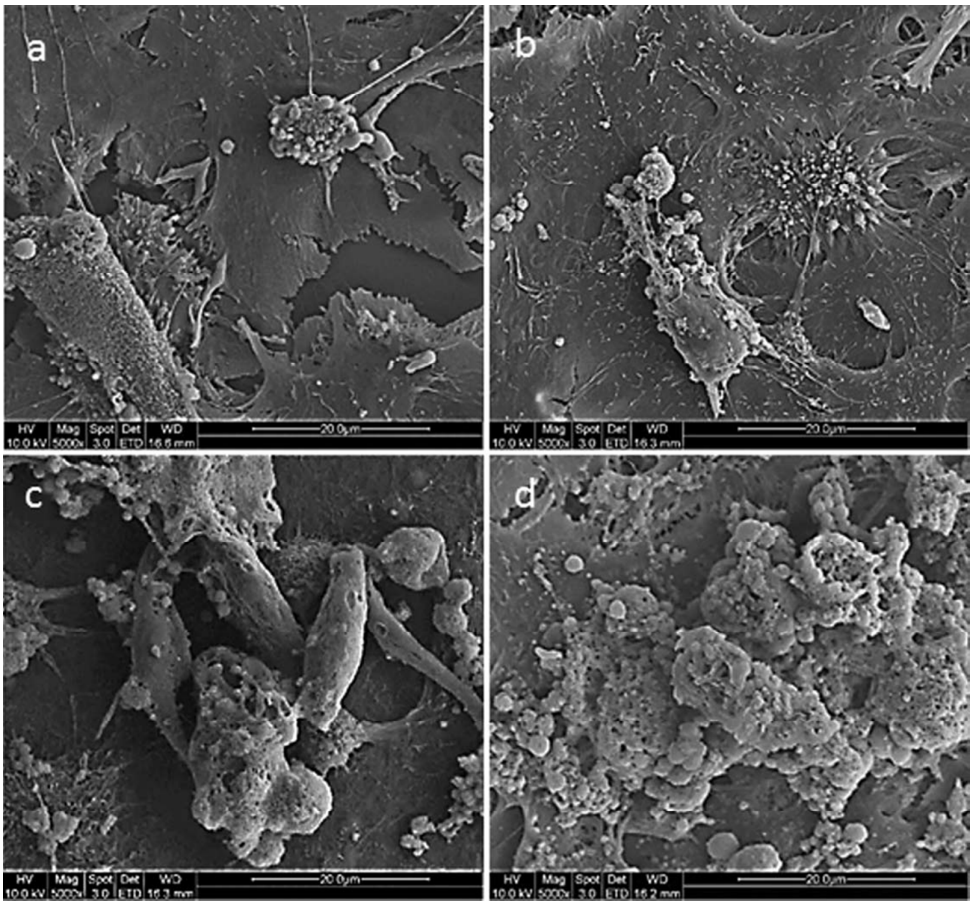
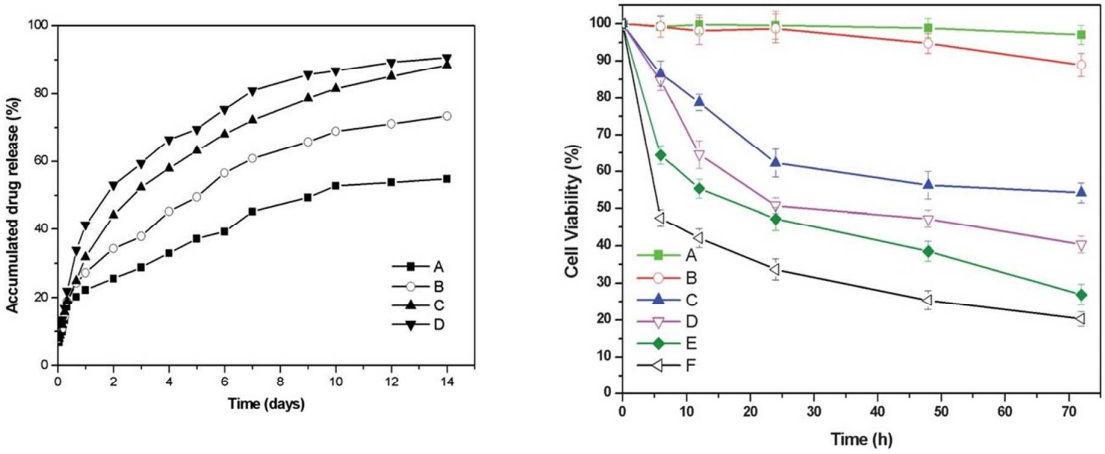


Figure 10. Release profiles of curcumin from the curcumin/PCEC electrospun fibers. curcumin content in the fibers: (A) 5 wt. %; (B) 10 wt.%; (C) 15 wt.%; (D) 20 wt.%. Cytotoxicity of the blank PCEC and curcumin/PCEC fibers to the rat Glioma 9L cells. Curcumin content in the fibers with respect to PCEC: (A) blank control; (B) 0% (blank PCEC); (C) 5%; (D) 10%; (E) 15%; (F) 20%. The curcumin equivalent concentrations to cell culture medium were 0, 0, 25, 50, 75, and 100 mg/ml, respectively. (Reproduced with



copyright permission from ref. 76)

Figure 11. Schematics of polymeric scaffolds and source of materials.

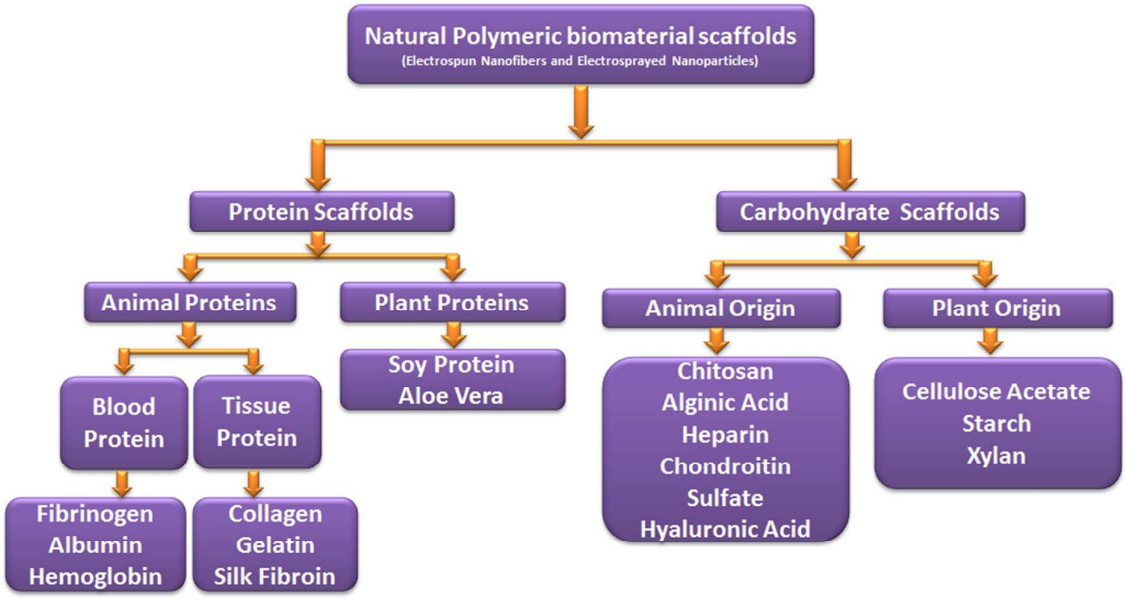


Figure 12. (a) Electrospun graft. (b) Image of the exposed femoral vein in the rabbit model. (c) Image of the replacement of the femoral vein with the tubular nanofiber scaffold (length: 1 cm, inner diameter: 1 mm). (d) H&E staining image of the oblique-section of the explanted scaffold after 7-week implantation into the rabbit model. The scale bar in (c) is 100 μm. (Reproduced with copyright permission from ref. 117)

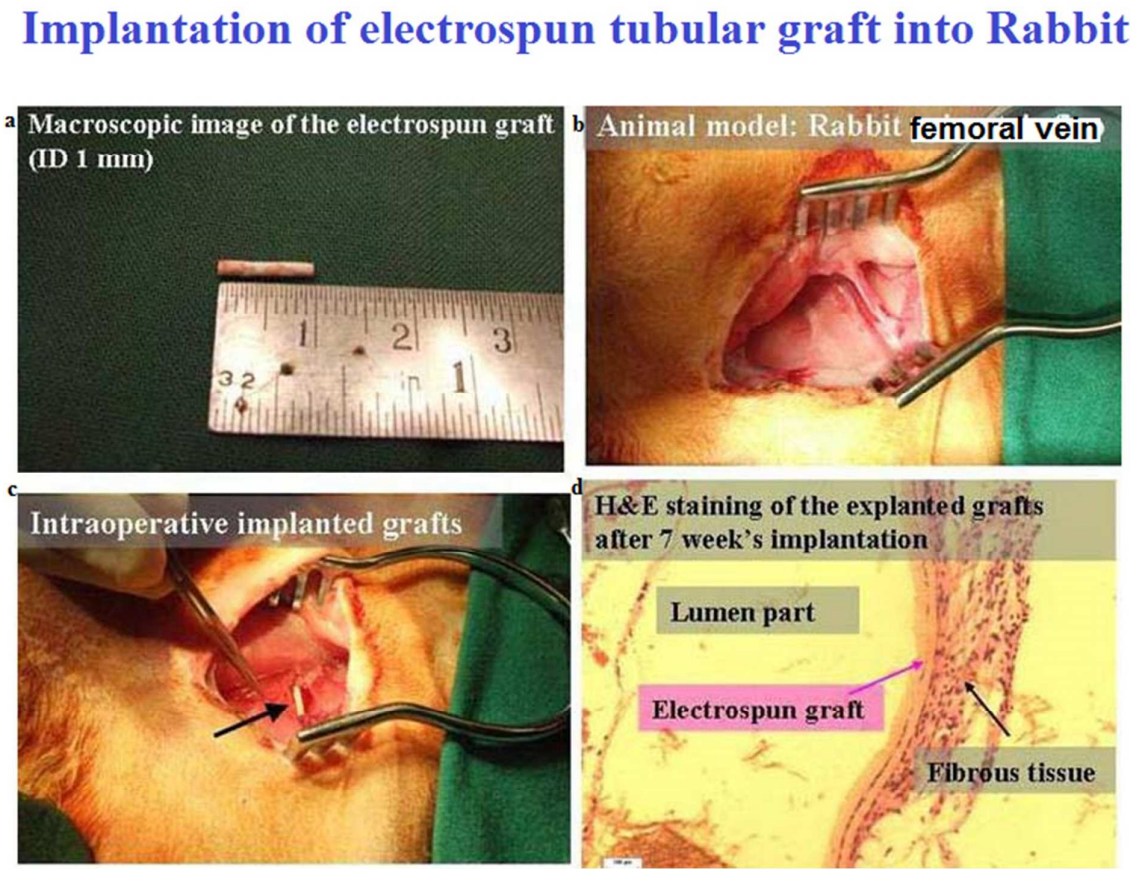


Figure. 13. F-actin expression of fibroblast cells on: a TCP, b PCL, c PCL-AV 5 %, d PCL-AV 10 % and e PCL/Collagen nanofiber scaffolds at 960 magnification. (The cells' cytoskeleton were labeled (red) with TRITC conjugated phalloidin and nucleus (blue) with DAPI). (Reproduced with copyright permission from ref. 123)

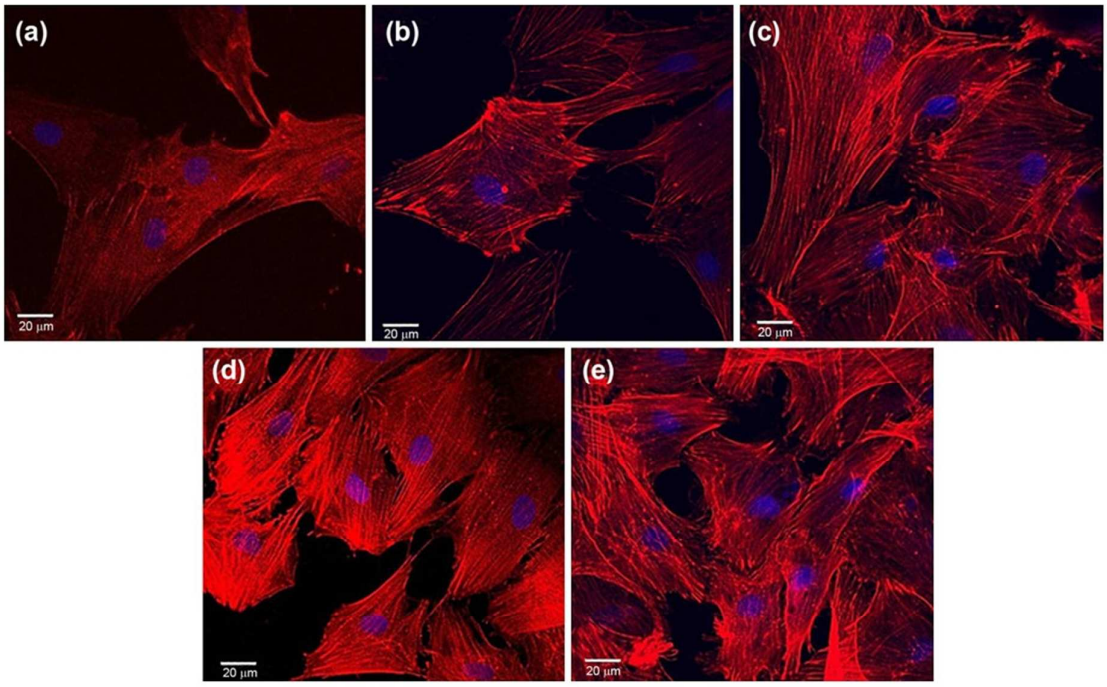


Figure 14. Riboflavin (Rib) nanofibers and protein crosslinking: AFM images of (a) Rib-Gelatin nanofibers without UV treatment (b) Rib-Gelatin nanofibers after UV crosslinking (c) Decrease in surface roughness as a measure of protein nanofiber or film crosslinking. SEM images of Rib mediated protein solution crosslinking (d) Freeze dried Fibrinogen hydrogel after ROS crosslinking (e) Freeze dried Gelatin hydrogel after ROS crosslinking. (Reproduced with copyright permission from ref. 185)

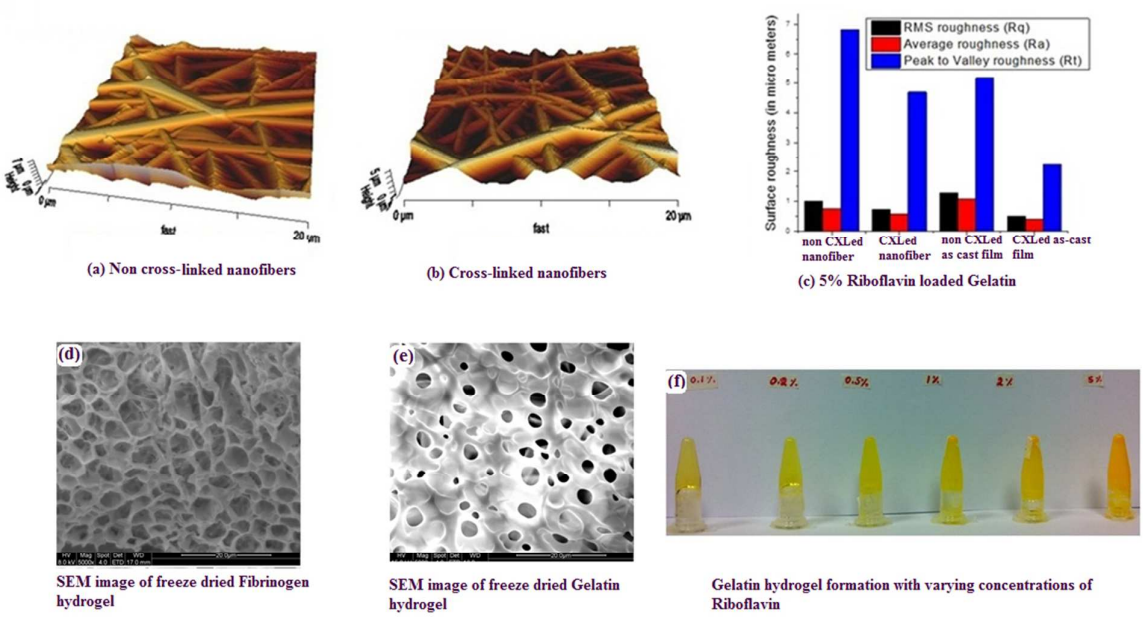


Figure 15. Antifungal activity of amphotericin B-loaded electrospun gelatin fibers: Disc diffusion assay showing (a) zone of inhibition of gelatin (upper panel) and amphotericin B-loaded gelatin (lower panels) fiber mats with Yeast strains; (b) zone of inhibition of gelatin (upper panel) and amphotericin B-loaded gelatin (lower panels) fiber mats strained with filamentous fungus; (c) SEM image showing *Candida albicans* morphology on the amphotericin B-loaded gelatin. The fungus cell wall disruption and oozing out cellular organelles can be visualized. (Reproduced with copyright permission from ref. 186)

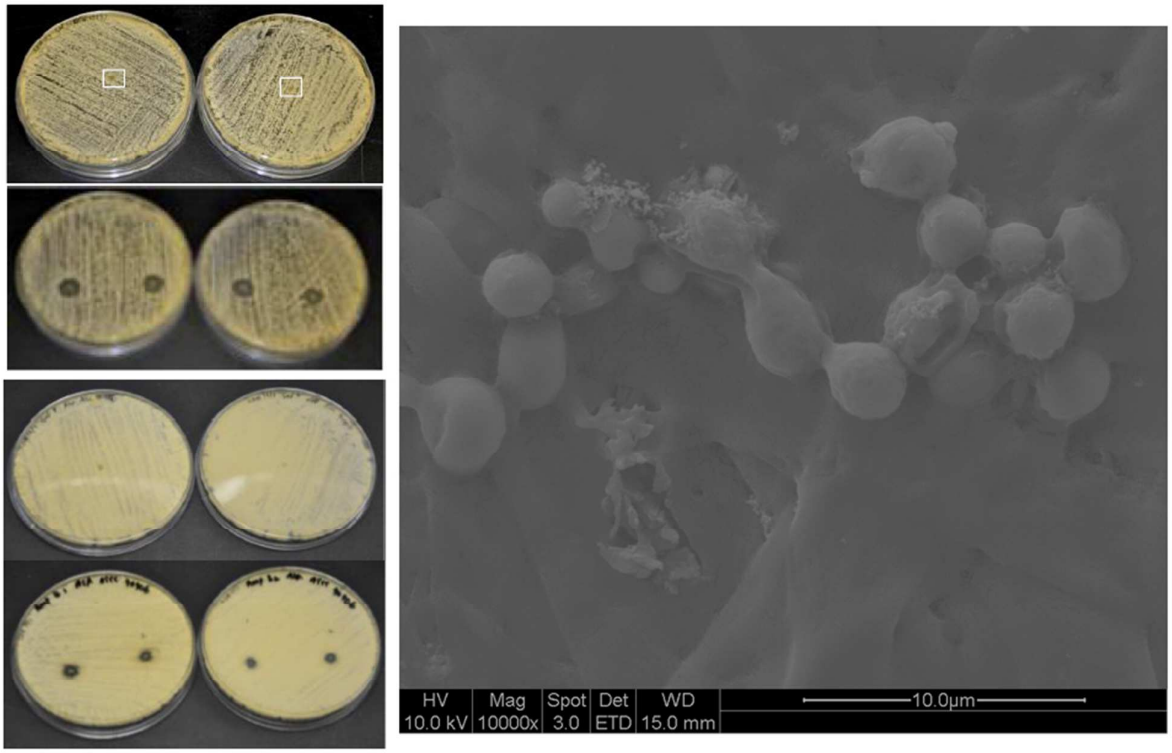


Figure 16. Immunocytochemical analysis showing the expression of gap junction protein Cx43 by differentiated contractile MSCs on (a) TCP (b) BSA/PVA (2:1), (c) BSA/PVA/Au (2:1:0.1), (d) BSA/PVA/Au (2:1:0.4) loaded nanofibers at 60x magnification, (e) TEM image of BSA/PVA/Au (2:1:0.4) nanofibers, (f) SEM image of BSA/PVA/Au (2:1:0.4) loaded nanofibers. Nucleus stained with DAPI. Scale bar represents 20 mm. (Reproduced with copyright permission from ref. 196)

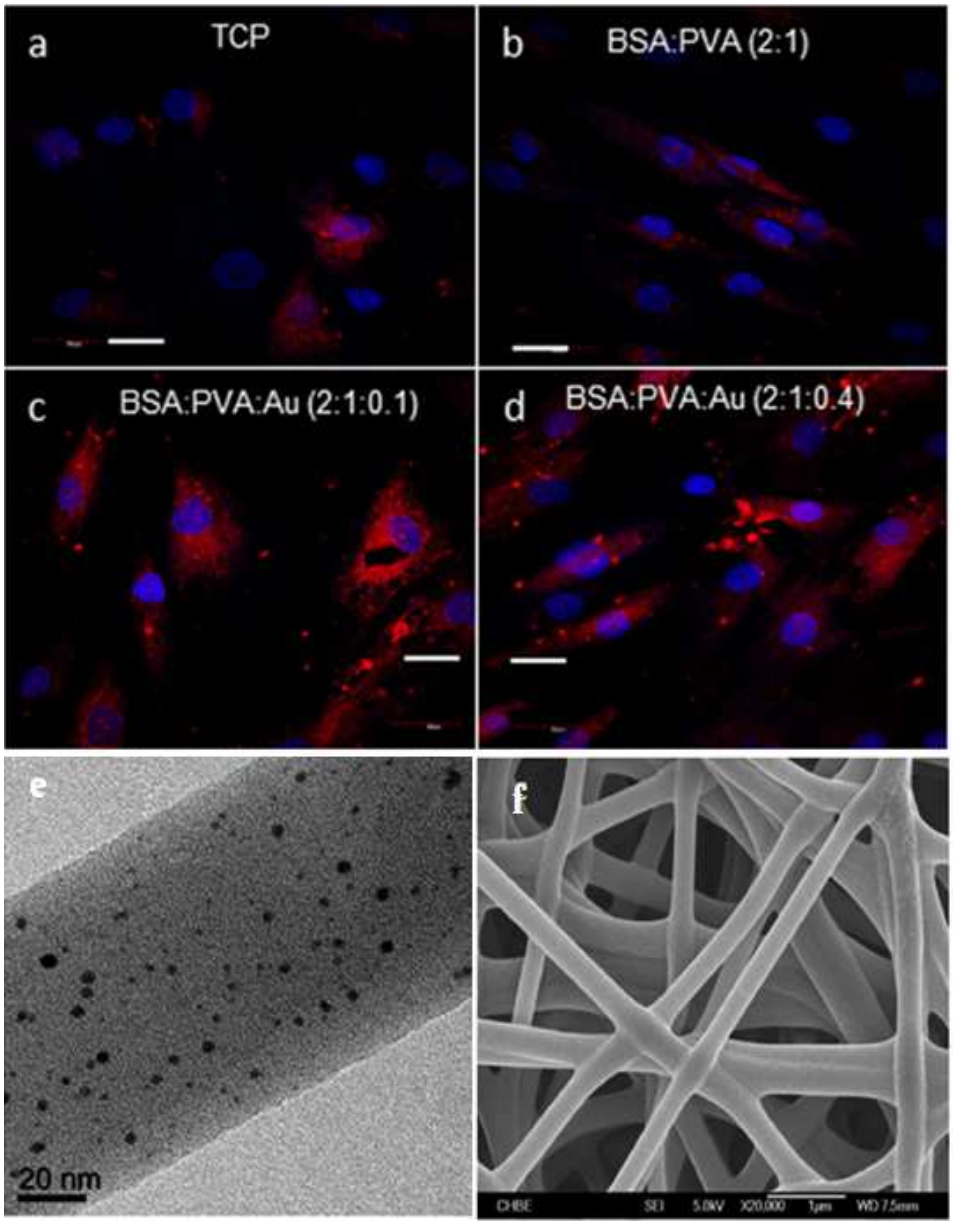


Figure 17. (A) Nanofiber mesh tubes and alginate hydrogel for surgery. SEM image of electrospun nanofiber mesh illustrating the smooth and bead-free nano-scaled fibers. (B) Hollow tubular implant without perforations made from nanofiber meshes. (C) Tubular implant with perforations. (D) Scheme of implant in segmental bone defect. Modular fixation plates are used to stabilize the femur. A nanofiber mesh tube is placed around the 8 mm defect. In some groups, alginate hydrogel, with or without rhBMP-2 is injected inside the hollow tube. (E) Picture of defect after placement of a perforated mesh tube. The alginate inside the tube can be seen through the perforations. (F) A specimen was taken down after 1 week and the mesh tube was cut open. The alginate was still present inside the defect, with hematoma present at the bone ends. (G) Alginate release kinetics over 21 days in vitro. Sustained release of the rhBMP-2 was observed during the first week. (Reproduced with copyright permission from ref. 206)

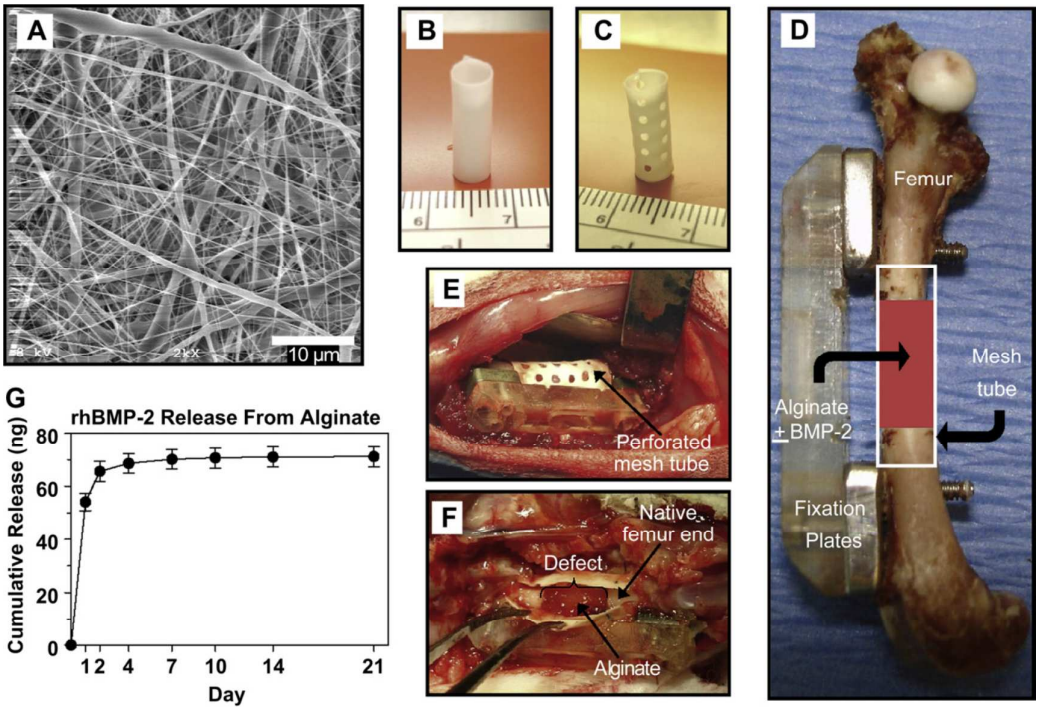


Figure 18. Schematic of experimental design. (A) Electrospinning was carried out by using a system in which the nanofibers were collected into an ethanol bath and (B) removed at predefined time intervals. (C) In vitro characterization of chondrogenesis of bone marrow derived-MSCs was performed over 42 d. (D) Nanofibers were implanted into osteochondral defects in the trochlear groove of rat hind limbs and evaluated after 6 wk. (E) SEM imaging of water-insoluble CS and PVA fibers depicted the fibrous morphology. (F) Size distribution of CS and PVA fibers. (Reproduced with copyright permission from ref. 211)

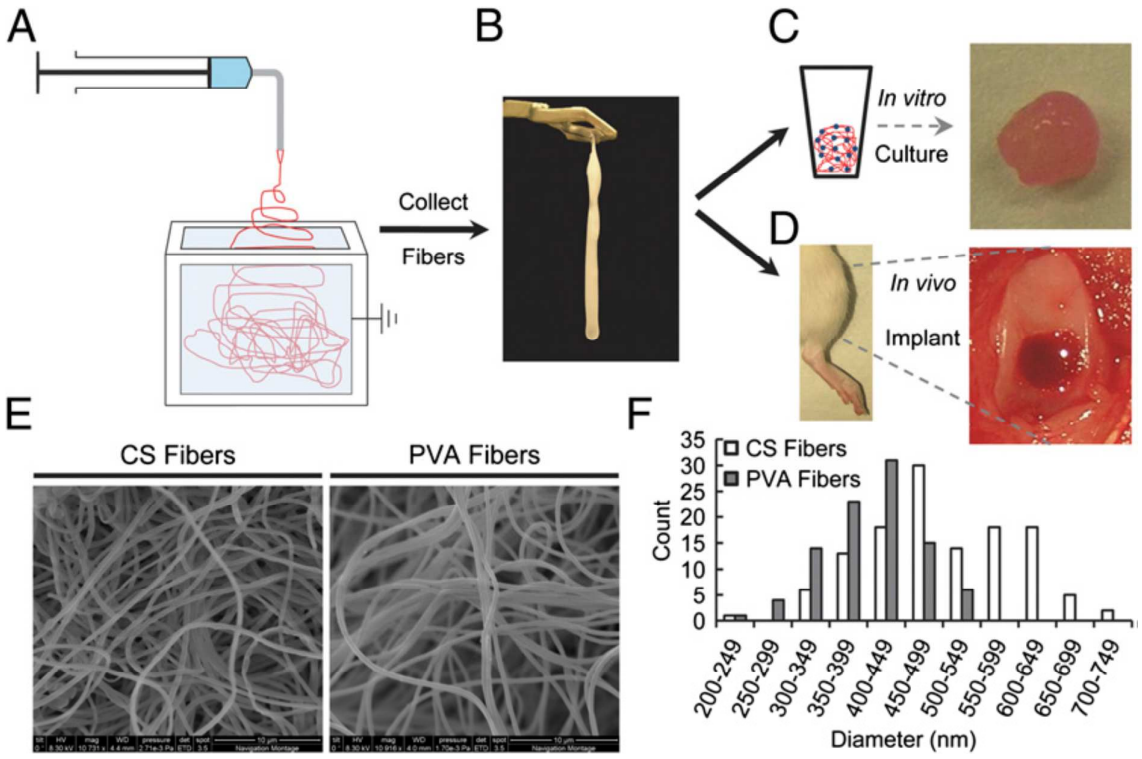


Figure 19. Expanding scope and Applications of biomaterials in healthcare, Pharma, food and personal care industry. (Reproduced with copyright permission from ref. 234)

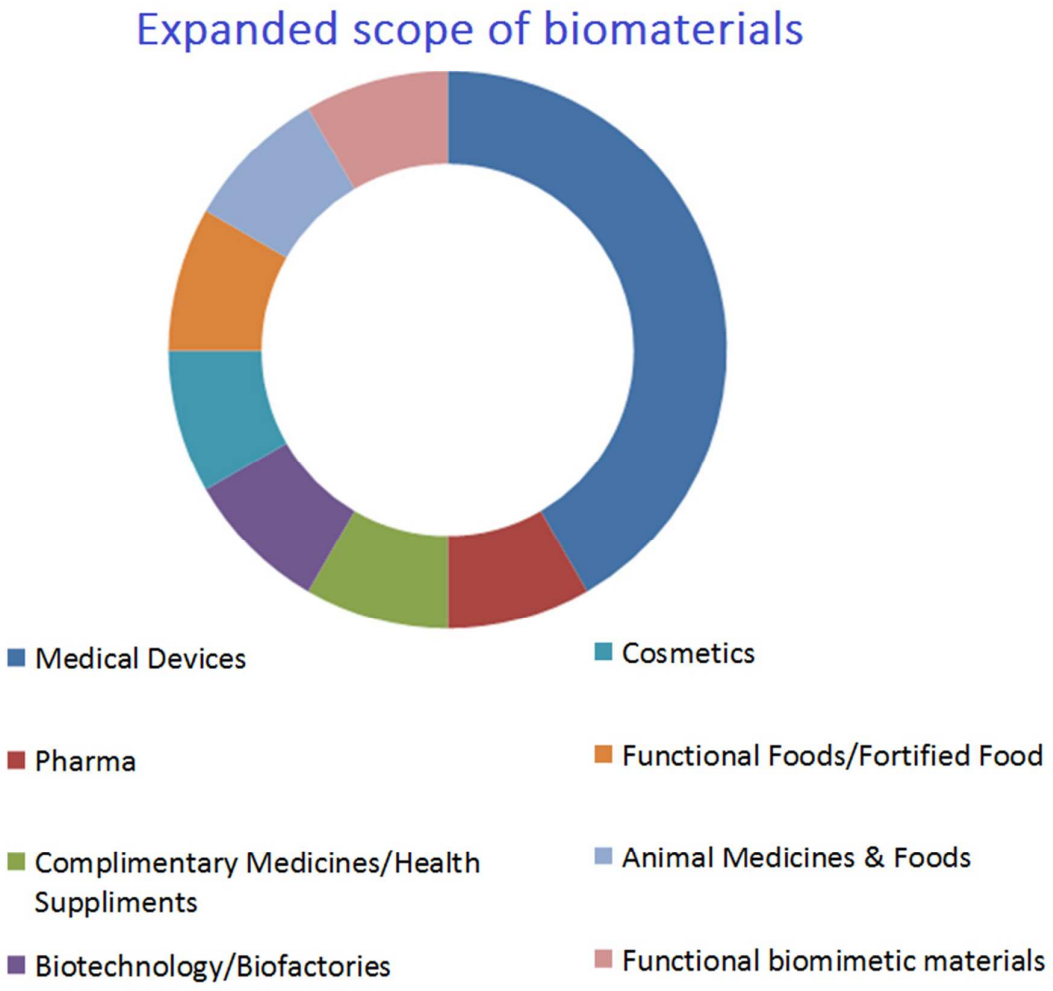
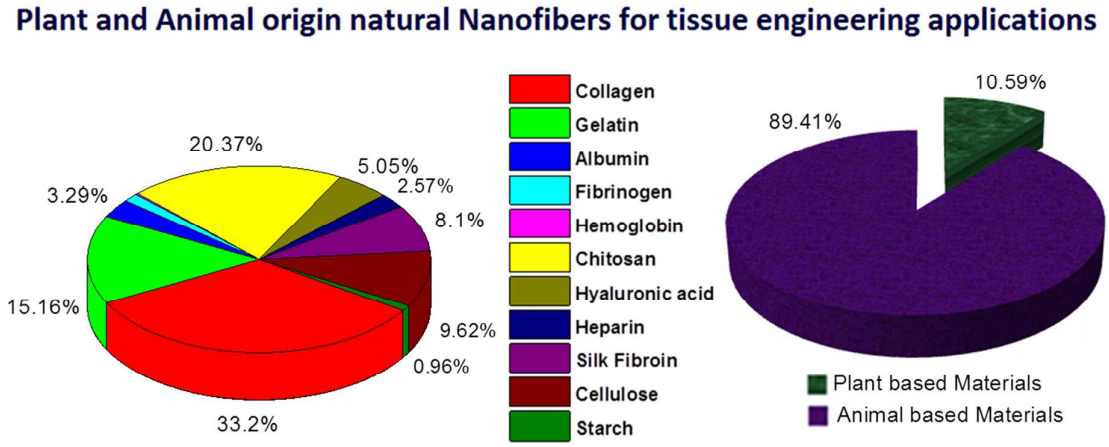


Figure 20. Global statistical data of plants and animal origin proteins, glycosaminyglycans carbohydrates, and polysaccharides for developing nanofibers for tissue engineering and drug delivery applications.



Graphical Abstract: Tissue engineering and drug delivery applications of Nanoformulated natural polymers, plant extracts.

Nanomaterials fabricated from natural source mimic natural scaffold materials. Nanoformulated herbal and plant extracts have medicinal importance and can be delivered effectively. This review presents the collective role of natural polymer materials and natural drug ingredients nano-formulated by electrospinning or electrospraying for pharmaceutical and tissue engineering applications.