Methods of Producing Three Dimensional Electrospun Scaffolds for Bone Tissue Engineering - A Review

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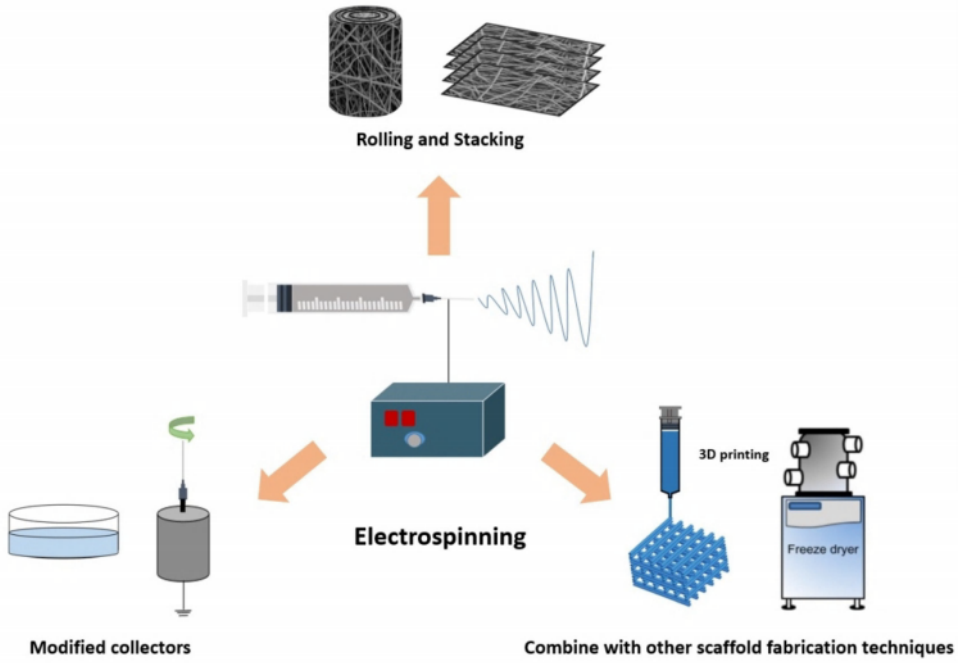

ABSTRACT
Bone is a dynamic, living tissue that exists and renews itself continuously in a 3D manner. Nevertheless, complex clinical conditions require a bone substitute to replace the defective bone and/or accelerate bone healing. Bone tissue engineering aims to treat bone defects that fail to heal on their own. Electrospinning provides an opportunity to create nano- to micro-fibrous scaffolds that mimic the architecture of the natural extracellular matrix (ECM) with high porosity and large specific surface area. Despite these advantages, traditional electrospun meshes can only provide a 2D architecture for cell attachment and proliferation rather than the 3D attachment in native tissue. Fabrication of 3D electrospun scaffolds for bone tissue regeneration is a challenging task, which has attracted significant attention over the past couple of decades. This review highlights recent strategies used to produce 3D electrospun/co-electrospun scaffolds for bone tissue applications describing the materials and procedures. It also considers combining conventional and coaxial electrospinning with other scaffold manufacturing techniques to produce 3D structures which have the potential to engineer missing bone in the human body.

Keywords:
Electrospinning, bone tissue engineering, three dimensional 3-D, core and shell, scaffold

INTRODUCTION

Bone is a complex, highly specialised connective tissue which plays a major role in critical functions in human physiology, including mechanical support and protection of critical organs, body movement, blood cells production, mineral storage and homeostasis, blood pH regulation, and provide housing for multiple progenitor cells (mesenchymal, hemopoietic). At the nanostructural level, bone is a natural nanocomposite consisting of an organic matrix made up predominantly of oriented collagen Type I fibrils (about 30-80 nm in diameter, 100 nm–1 μm in length), reinforced by an inorganic mineral phase comprising of rod or plate-shaped non-stoichiometric hydroxyapatite particles. The size of apatite crystals varies depending on the location of the crystals, whether within the ends of collagen fibrils (smaller) or between collagen fibrils (larger) (Figure 1), with the thickness ranging over 2–10 nm, the length over 20–50 nm, and the width over 15–30 nm with a rod-like (or sometimes plate-like) structure. This composite structure gives bone its balance of stiffness, strength, toughness and vibrational damping properties. In order to maintain the structure–function relationship bone tissue continuously forms and remodels throughout life to adapt to changes in biomechanical forces, and to remove the old, microdamaged bone and replace it with new, mechanically tougher and stronger bone to help maintaining bone strength.[1–6]

Despite bone's self-repair ability, there are many clinical conditions that require substantial volumes of bone regeneration, such as bone defects created by trauma, infection, tumour resection and skeletal abnormalities, as well as conditions such as avascular necrosis, atrophic non-union and osteoporosis in which the regenerative process is compromised. Worldwide, approximately 2.2 million bone graft procedures are performed annually. Although autografts are considered as the "gold standard" treatment for bone defects being histocompatible, non-immunogenic and able to induce new bone growth, they have significant limitations including donor site morbidity and limited supply. On the other hand, allografts which can be obtained from living donors or cadavers can overcome harvesting and quantity problems associated with autografts, but they are expensive, and can cause host-immune response in addition to the possibility of donor-to-recipient disease transmission.

Tissue Engineering has been defined in various ways including the internationally agreed "The use of a combination of cells, engineering materials and suitable biochemical factors, to improve or replace biological functions in an effort to improve clinical procedures for the repair of damaged tissues and organ".[8] Bone tissue engineering aims to provide a suitable alternative to conventional treatments of bone disease and to combat their limitations.[9,10] It requires understanding of bone structure, formation and mechanics, and aims to develop artificially designed biological substitutes that restore, preserve, or improve tissue function by using synergistic combination of biomaterials, cells and growth factor therapy.[11–13]

Tissue engineering technologies are mainly based on the successful interaction between the following three components (also known as the tissue engineering triad) (Figure 2):

(1) A scaffold or matrix that closely mimics the natural extracellular matrix and has the ability to hold the cells together;

(2) Implanted and cultured cells to create new tissue; and

(3) Biological signalling molecules, such as growth factors, differentiation factors and adhesion molecules that guide cells to form the desired tissue.[14–16]

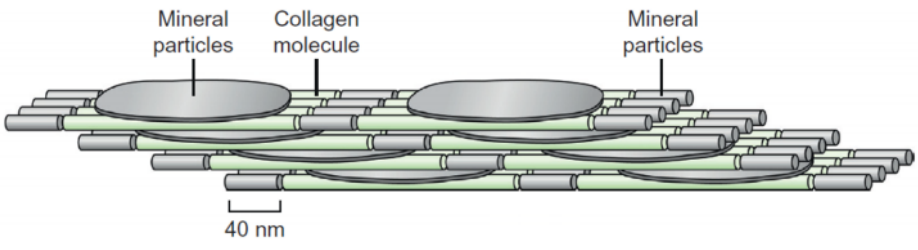

Figure 1 Schematic arrangement of mineral particles (grey) either interleaved between the collagen fibrils when plate shaped or between the ends of collagen fibrils when rod shaped. Reprinted with permission from Ref (7). Copyright 2013 Elsevier.

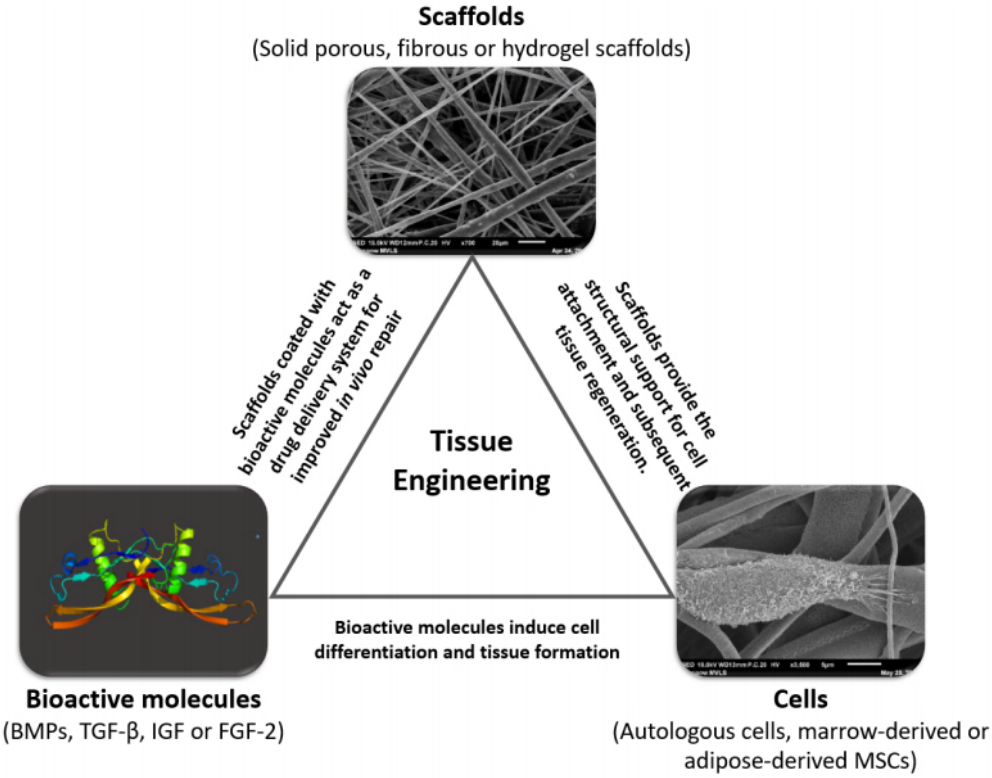

Figure 2 The tissue engineering triad showing the interactions between scaffolds, cells and bioactive molecules in tissue engineering.

Bone scaffolds must satisfy various macro and micro structural properties to ensure successful new tissue growth. These properties include excellent biocompatibility with, preferably, bioactivity, adequate mechanical properties to ensure the mechanical integrity and protection for the developing tissues, biodegradability with controllable degradation rates to match the bone regeneration and high porosity with open and fully interconnected pores with pore size ranging from 100 to 400 μm for optimal bone tissue ingrowth.[12,17–21]

Cells and tissues in the human body are organised into various three-dimensional architectures depending on the type of tissue. Thus, to engineer these functional tissues and organs, scaffolds have to provide three-dimensional space which mimics the architecture of the native extracellular matrix, to facilitate the cell distribution and guide the regeneration of new tissue. Over the years, various methods to design and fabricate 3D biomimetic scaffolds have been developed for tissue engineering and regenerative medicine and choosing the appropriate technique depends on several factors including: the required shape and properties of the scaffold, types of materials used, shape and size of pores as well as their interconnectivity and the distribution of the materials.[22,23] Scaffold manufacturing techniques include solvent casting, phase separation, gas foaming, 3D printing and electrospinning.[24–26]

Among these technologies, electrospinning has gained wide popularity over the past few decades due to the diversity of fabricating micro/nanofibers featuring large specific surface area, high porosity, adjustable structural and mechanical properties, and surface functionalisation.[27] In addition to the conventional electrospinning, recent efforts have focused on producing scaffolds with more complex and thus functional fibres such as core and shell, hollow and triaxial-channel fibres for use in various biomedical applications using coaxial electrospinning. The core-and-shell design has emerged as a promising approach for delivering therapeutic molecules and stem cells. Using core-and-shell fibres can improve biocompatibility, biodegradability, hydrophilicity and mechanical properties of the scaffolds, while allowing greater control of degradation rate.[28–30]

PRINCIPLES OF ELECTROSPINNING

Electrospinning was originally described early in the 20th Century, with the theory developed by Taylor[31–33] and has been recognised as a simple and efficient technique for the fabrication of ultrafine fibres with diameters ranging from few nanometres to several micrometres using solutions of both natural and synthetic polymers and high electric potentials.[34,35] The basic setup needed for laboratory scale electrospinning is relatively simple and requires a polymer solution, a syringe to pump the polymer solution at a controlled speed with a needle which acts as the spinneret, syringe pump, high DC voltage power supply (usually 5-50 kV) and an earthed collector. The process is usually carried out at room temperature and atmospheric pressure, with either vertical or horizontal setup (Figure 3).[34,36]

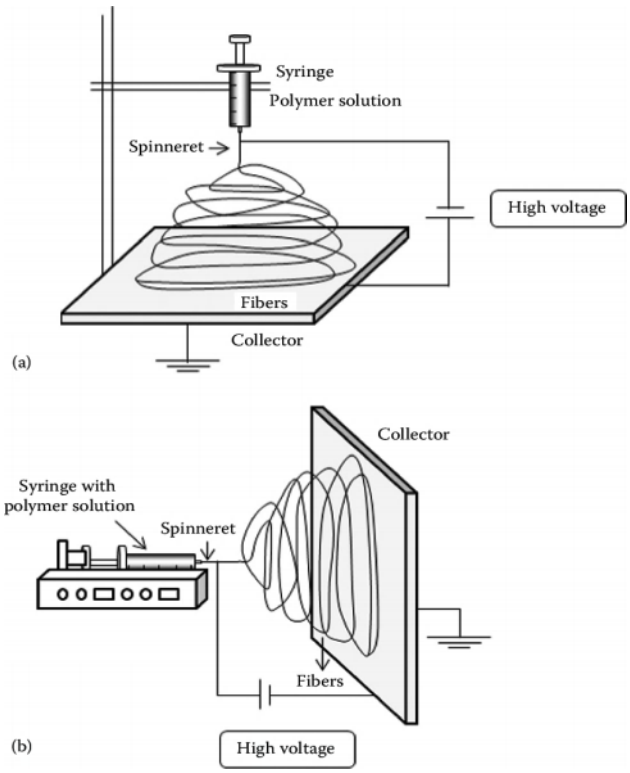

Figure 3 Schematics of electrospinning equipment: (a) vertical setup and (b) horizontal setup. Reprinted with permission from Ref (34). Copyright 2010 Elsevier

After loading the syringe with a polymer solution and connecting it to the high voltage, the pendant polymer droplet at the syringe nozzle becomes electrically charged with the induced charges evenly distributed over the droplet surface. This charge accumulation starts to distort the normal spherical shape of the droplet, created by surface tension, and the tip of the polymer droplet becomes conical forming a "Taylor cone". As the electric field strength increases beyond a critical value, the repulsive electrostatic force overcomes the surface tension and a jet of charged polymer solution is ejected from the Taylor cone and passes through stretching and whipping processes due to electrical instabilities forming a series of spiralling loops. These whipping or bending instabilities result in long narrow thread formation. Simultaneously, the solvent starts to evaporate, leaving solid polymer fibres that settle in layers on the electrically ground collector.[37,38]

Although electrospinning is relatively straight forward to set up, there are numerous parameters that can be manipulated to produce electrospun fibres with different architectures. These parameters may affect the fibre morphology individually in some way, or they may all work in harmony with one another, and can be broadly classified into three categories: polymer solution parameters (solution concentration and/or viscosity, surface tension, conductivity, dielectric constant and solvent volatility) process parameters (applied voltage, flow rate, tip-to-collector distance and collector geometry) and ambient parameters (temperature and humidity).[38–42]

PRINCIPLES OF COAXIAL ELECTROSPINNING

Coaxial electrospinning or co-electrospinning[43,44] has emerged as a subset of electrospinning and an effective alternative to emulsion electrospinning for producing core-and-shell or hollow structured fibres.[45,46] This technique has attracted attention in medical and pharmaceutical fields and has been used for producing antibacterial nanofibers, wound dressings, drug delivery materials and tissue engineering scaffolds.[47]

The basic set up of coaxial electrospinning is largely similar to conventional electrospinning, but the spinneret is modified to have two concentrically capillaries, resulting in a coaxial configuration.[48] The outer capillary is connected to the shell solution reservoir while the inner capillary is attached to the core solution reservoir (Figure 4). When high voltage is applied, a charge accumulation develops on the surface of the shell solution. Due to charge-charge repulsion, the meniscus of the shell solution on the tip of the spinneret elongates and stretches to form a Taylor cone with two concentric layers. The stresses generated in the shell solution create shearing of the core solution via viscous dragging and contact friction. The core solution then deforms into conical shape with the shell solution to create a compound Taylor cone with a core-shell jet initiating from the tip of the spinneret. Under the whipping force of electrostatic repulsion, the core-shell fibres are formed and again ultimately deposited on the grounded collector.[49]

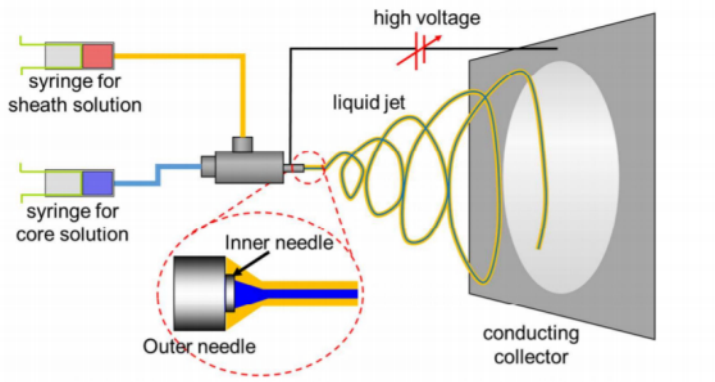

Figure 4 Schematic diagram of coaxial electrospinning showing the co-axial capillaries and sheath-core in the jet. Reprinted with permission from Ref (50). Copyright 2017 American Chemical Society.

Coaxial electrospinning has been used extensively because it is a simple one-step process. Additionally, it allows the use of core materials which alone cannot form electrospun fibres, to be electrospun through the protection and guidance of the sheath solution. Unstable compounds, such as antibiotics, growth factors and living cells, can be isolated from a harsh environment and electric charges via the shell layer. The mechanical properties and degradation rate of coaxial fibres can be tailored by choosing suitable components for the core and shell solutions, while the size of fibres can be controlled by adjusting solution concentration and processing parameters.[44]

Since the process of coaxial electrospinning is similar to that of conventional electrospinning, all conventional electrospinning parameters which control fibre morphology and the quality of the process also affect the behaviour of coaxial electrospinning.[51]

3D ELECTROSPUN SCAFFOLDS FOR BONE TISSUE ENGINEERING

Electrospun polymeric scaffolds have significant potential in the field of bone tissue engineering due to their topographical features which mimic the extracellular matrix, allowing control of key cellular activities. However, the main limitation associated with conventional electrospinning is that the scaffolds produced are usually two dimensional (2D) dense mats rather than three dimensional (3D) porous structures, which limit their applications in tissue engineering which ideally requires 3D constructs. In addition, the small pore sizes of densely packed 2D fibrous mats can restrict the access of cells to the interior of electrospun scaffolds. Thus, cells mainly spread over the surface and only distribute down to a limited depth below the surface.[52,53] Recently, several techniques have been explored to fabricate 3D electrospun scaffolds for various applications in tissue engineering. These techniques mostly include manual stacking, twisting, or rolling of scaffolds into 3D structures, redesigning the electrospinning collector, modifying the electrospinning process, or combining electrospinning with other scaffold fabrication methods. These methods have been proven to be successful in inducing bone tissue formation in vivo, especially when combined with other biomimetic stimulation methods such as growth factor delivery.[54]

ROLLING AND STACKING TO PRODUCE ELECTROSPUN SCAFFOLDS

The most common approach to create 3D bone scaffolds is to stack seeded electrospun scaffolds on top of each other in a layer-by-layer manner. Li et al.[55] were the first to introduce this approach, where they stacked electrospun uniaxially aligned nanofibers into multi-layered structures with controllable hierarchical for bone regeneration. However, they did not conduct any in vitro or in vivo studies on the scaffolds. Srouji et al.[56] and Paşcu et al.[57] also used the stacking method to produce functional 3D-stacked electrospun scaffolds, but their fibres were randomly oriented instead of aligned, thus, they could not create patterned structures or any anisotropy in the mat surface. The in vivo results of Srouji et al.[56] indicated that 3D scaffolds do support cell infiltration and neovascularisation.

Rolling scaffolds into cylinders is another popular method for creating 3D electrospun bone scaffolds. For instance, Pişkin et al.[58] manufactured 3D spiral-wound polycaprolactone (PCL) structures for reconstruction of cranial bone defects by simply rolling 2D electrospun simvastatin-loaded PCL scaffolds. The technique showed increased bone formation and mineralisation in vivo as compared to the control, a defect without a scaffold. To mimic the complex hierarchical structures of bone tissue, Deng et al.[59] constructed a 3D biomimetic scaffold by rolling electrospun nanofiber matrices with an open central cavity to imitate native bone osteons structurally and mechanically. They found that this biomimicry resulted in stress–strain curves similar

to those of native bone with a compressive modulus in the mid-range of values for human trabecular bone. 3D scaffolds have also encouraged osteoblast infiltration and ECM secretion, bridging the gaps of concentric scaffold walls during in vitro culture. Moreover, Hejazi and Mirzadeh[60] prepared 3D PCL/gelatin scaffolds with natural coral microparticles for load bearing bone defects by cutting the 2D mats into strands with desired width and length, and then rolling them into cylinders (Figure 5). In vitro cytotoxicity evaluation showed no release of cytotoxic materials from the scaffolds or coral particles. Furthermore, the fabricated 3D scaffolds exhibited comparable mechanical properties to those of natural cortical bone.

With the aim of mimicking the osteon structure, the chief structural unit of compact (cortical) bone, Vashisth and Bellare[61] designed a 3D hybrid scaffold composed mainly core-shell PCL-gelatin/hydroxyapatite (HA) nanofibrous sheets, manufactured using coaxial electrospinning, which were tightly coiled into spiral-rings and reinforced with a hydrogel (gellan/HA) matrix via crosslinking (Figure 6). In addition to mimicking the bone structure, the reinforcement of nanofibrous spiral coils in the hydrogel matrix enhanced the overall mechanical strength of the 3D scaffold, an essential requirement for bone tissue applications.

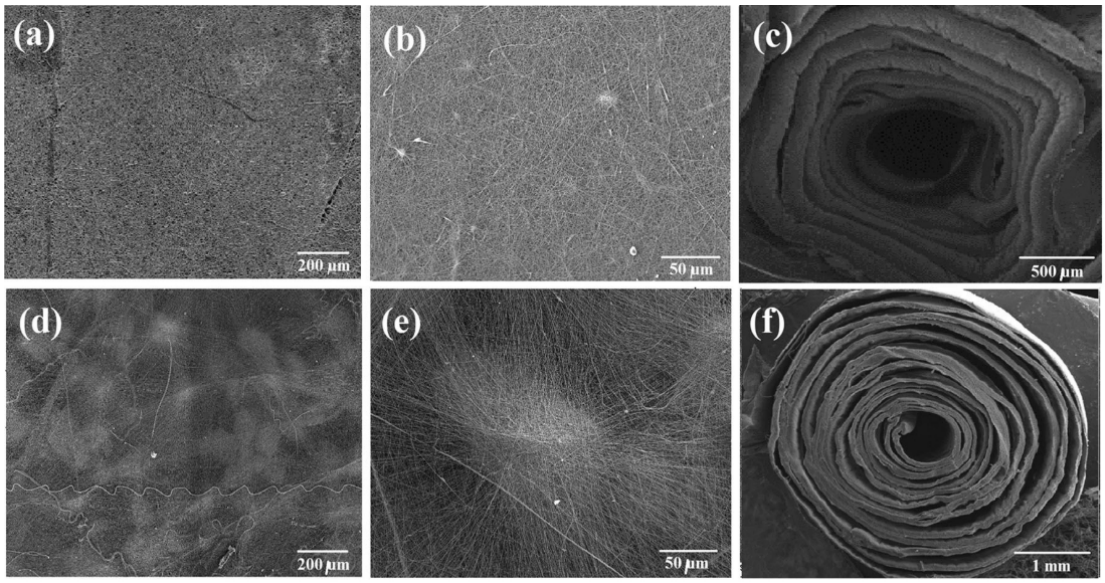

Figure 5 SEM images of the PCL/Gelatin (PG) nanofibrous mat and the related roll scaffold without (a-c) and with coral micro particle-loaded PG (d-f) nanofibrous mat and the related roll scaffold at a range of magnifications (a & d scale bar = 200µm, b & e scale bar = 50µm c & f scale bar = 500µm). Reprinted with permission from Ref (60). Copyright 2016 Springer Nature.

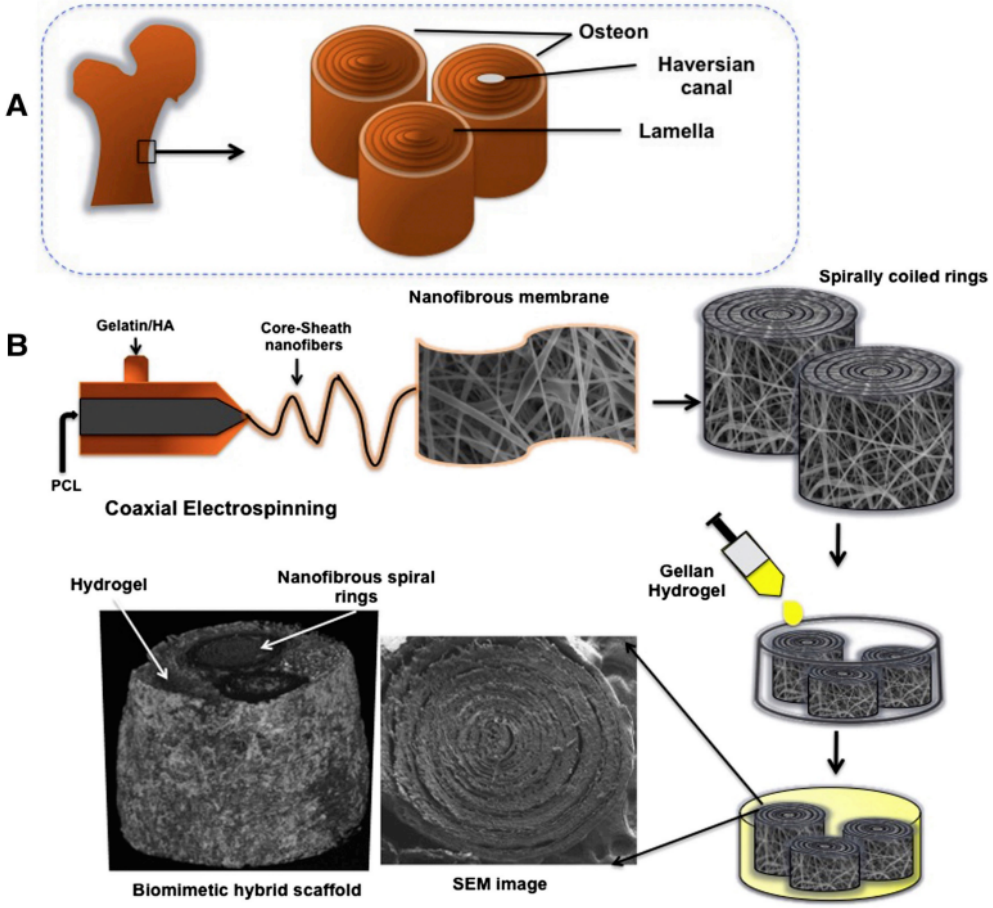

Figure 6 A) the natural structure of cortical bone with osteons and B) the production of 3D hybrid scaffold composed of coaxial electrospun nanofibers with hydrogel matrix reinforcement. Reprinted with permission from Ref (61). Copyright 2018 Elsevier

MODIFIED ELECTROSPINNING COLLECTORS

Using a 3D collection template to collect the electrospun nanofibers instead of the traditional 2D flat collector is another common option for obtaining 3D controllable electrospun scaffolds. Different collector designs have been proposed over the years such as liquid collectors (wet electrospinning), rotating drum or needle collectors and structured metallic collectors.[62,63]

Wet electrospinning is an effective technique for manufacturing electrospun 3D nanofibrous scaffolds without using sophisticated devices or chemical additives. Instead of the earthed metal collector plate used in conventional electrospinning, nanofibers are delivered into a liquid collector to produce 3D sponge-like structures.[64] Wet electrospinning was first introduced by Yokoyama et al.[65] when they fabricated spongiform polyglycolic acid (PGA) nanofibers with controlled fibre density using a novel wet electrospinning system. They used three different solutions, individually, to fill a stainless steel bath and compared the form and apparent density of the nanofibers produced in each solution. The collection solutions were pure water and tertiary-butyl alcohol (t-BuOH) at 50% alcohol concentration and 99% alcohol concentration. All solvents produced 3D spongiform nanofibers scaffolds with lower bulk density than the 2D nanofiber mats produced using a conventional electrospinning collector system. Ki et al.[66] also used the same method to collect 3D nanofibrous scaffolds in a grounded methanol bath also to produce bone tissue scaffolds. Physically, the resultant 3D scaffolds were 10 times thicker than the 2D scaffolds with higher pore size and increased porosity, while cell culture studies showed significantly higher proliferation of MC3T3-E1cells on the 3D than on 2D scaffolds after 5 and 7 days of culture.

Zhang et al.[67] created a 3D coating of porous zein/poly-L-lactic (PLLA), where zein is a prolamine protein obtained from maize, produced as coaxial nanofiber sheets on cylinders of calcium phosphate cement (CPC) to produce 3D tubular composites for bone augmentation (Figure 7). Their results indicated that the coaxial electrospun coating had preserved the physical and chemical performance of the CPC and led to the formation of a hydrophilic surface, increased mechanical properties and biocompatibility and an increased number of viable cells.

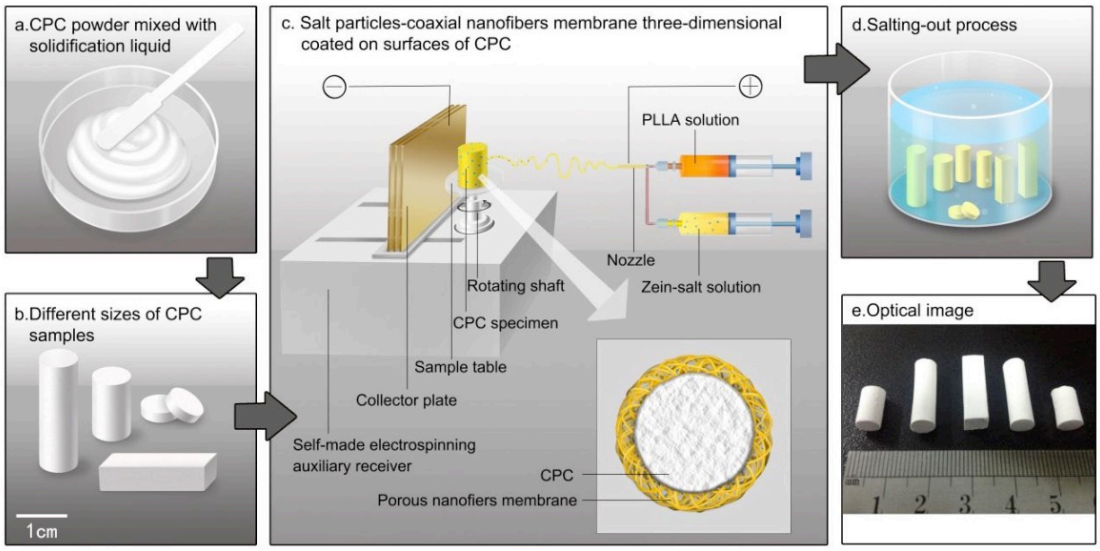

Figure 7 Schematic diagram showing the process of manufacturing a 3D zein/PLLA coaxial nanofiber membrane coating on calcium phosphate cement (CPC). Starting with a) and b) the preparation of the CPC core of different sizes then c) coating with coaxial nanofibres, followed by d) leaching out of the salt and finally e) the prepared specimens. Reprinted with permission from Ref (67). Copyright 2017 Elsevier

A different approach was used by Kareem et al.,[68,69] who fabricated 3D tubular core-shell electrospun scaffolds using coaxial electrospinning and a rotating needle collector. A composite of micron-sized sintered HA in polylactic acid (PLA) was electrospun as the shell component for coaxial scaffolds to increase the bioactivity and osteoconductivity while PCL was selected as the core material of the fibres to provide the mechanical stability to the scaffolds. The scaffolds were electrospun on a custom-built rotating needle collector with G16 (OD=1.35mm) or G21 (OD=0.67mm) stainless steel collector needles. The resulted coaxial tubular scaffolds (Figure 8) exhibited high bioactivity (apatite formation) upon immersion in simulated body fluid (SBF) for 12 weeks and exhibited gradual reductions in their mechanical properties over 12 weeks in PBS or SBF, but still retained their structural integrity. Results also showed that using a rotating needle collector has increased fibre alignment compared to a stationary collector, without affecting fibre diameter significantly, but adding HA increased the variability in the fibre diameters.

Zaiss et al.[70] electrospun PCL onto an array of concave structured metallic collectors (Figure 9) to produce 3D batch-to-batch similar scaffolds with an average fibre diameter of 15 μm and an average pore size of 250–300 μm on the concave side, 20–80 μm on the convex scaffold side. Their results indicated that the 3D structured PCL scaffolds were favourable for osteoblast cultures.

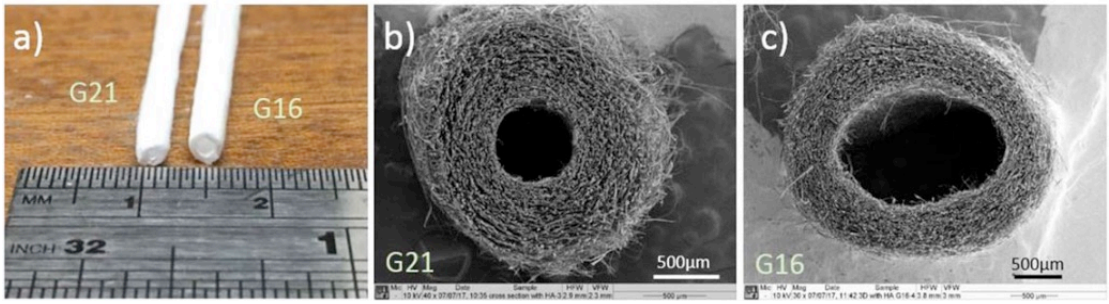

Figure 8 (a) Macroscopic structures of the coaxial tubular scaffolds, (b) and (c) SEM images of G16 and G21 tube cross sections, respectively (marker bars (b)) and (c)=500 μm). Reprinted with permission from Ref (68). Copyright 2019 IOP Publishing

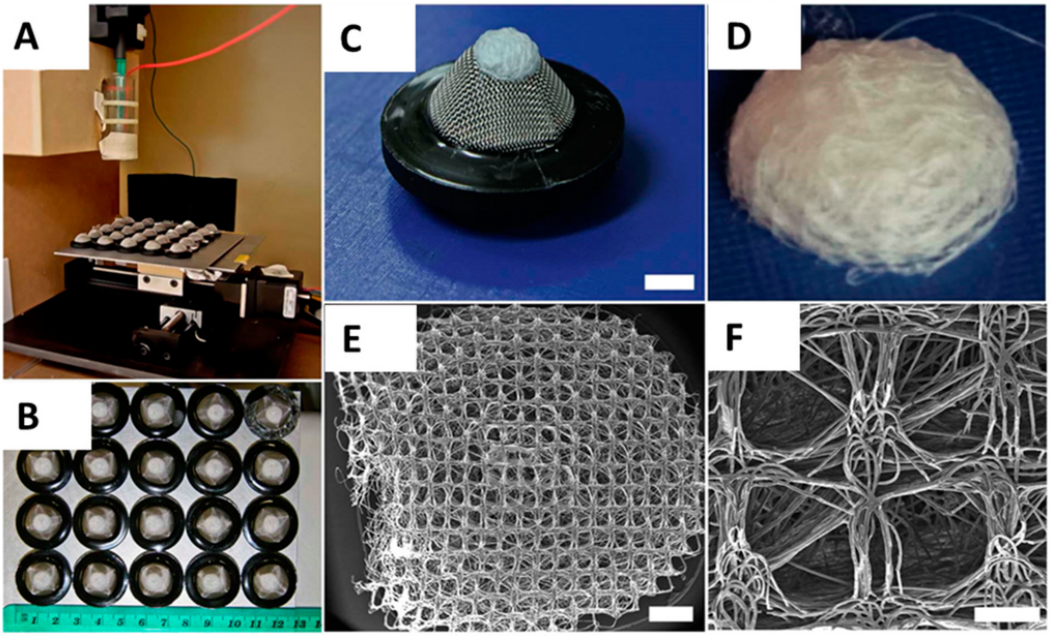

Figure 9 (A, B) Electrospinning of PCL onto 20 structured metallic collectors which produced homogenous and replicable batches of porous scaffolds; (C, D) Dome-shaped scaffolds with a concave side towards the collector and a convex shape on the opposite side; (E,F) SEM images of concave scaffold side retaining the square-shaped porous pattern of the collector (marker bar= 1mm for E and 200μm for F). Reprinted with permission from Ref (70). Copyright 2016 MDPI

COMBINING ELECTROSPINNING WITH OTHER PROCESSES

One of the main drawbacks of electrospinning process, beside the 2D architecture of electrospun mats, is the poor mechanical properties of the resultant scaffolds which limit their use in load bearing bone tissue engineering applications. Therefore, researchers started to combine conventional/coaxial electrospinning with other techniques to create 3D bone-like templates for bone regeneration and to enhance the mechanical and biological properties of the fabricated scaffolds.

Li et al.,[71] for example, combined electrospinning with solvent casting to produce bi-layered membranes used to treat oral bone defects. A dense PLGA film was first manufactured using solvent casting to act as a barrier for guided bone regeneration and then a layer of a loose electrospun micro-nano bioactive glass (MNBG) fibres was layered on the top of it to create a bi-layered membrane. In addition to the barrier function, the resultant MNBG/PLGA membranes showed stable mechanical properties and enhanced bioactivity and osteogenesis in vitro.

Martins et al.[72] combined electrospinning and 3D printing in order to create a 3D bone-like scaffold. The technique is very similar to the stacking method discussed earlier, but it incorporates layers of electrospun nanofiber scaffolds within the microfiber meshes produced by 3D printing. The resultant scaffolds exhibited significantly higher proliferation and ALP activity after 7 days in culture compared to scaffolds produced by 3D printing alone.[72,73] Similarly, Yu et al.[74] also combined electrospinning with 3D printing to produce 3D bone tissue scaffolds. However, they infused PCL/gelatin dispersed nanofibers into the meshes of PCL printing scaffold to fabricate 3D composite scaffolds (Figure 10). The scaffold were lyophilised for 24 hours, immersed into 2.5 % glutaraldehyde solution for 20 min, and then washed three times with deionized water before freezing-dried again. Their 3D composite scaffold had a micro-scale porous structure and exhibited significantly higher compressive modulus compared to lyophilised electrospun scaffold as well as enhanced proliferation infiltration on the composite scaffold compared to the PCL control scaffold, which they explained by the microporous structure of electrospun scaffold.

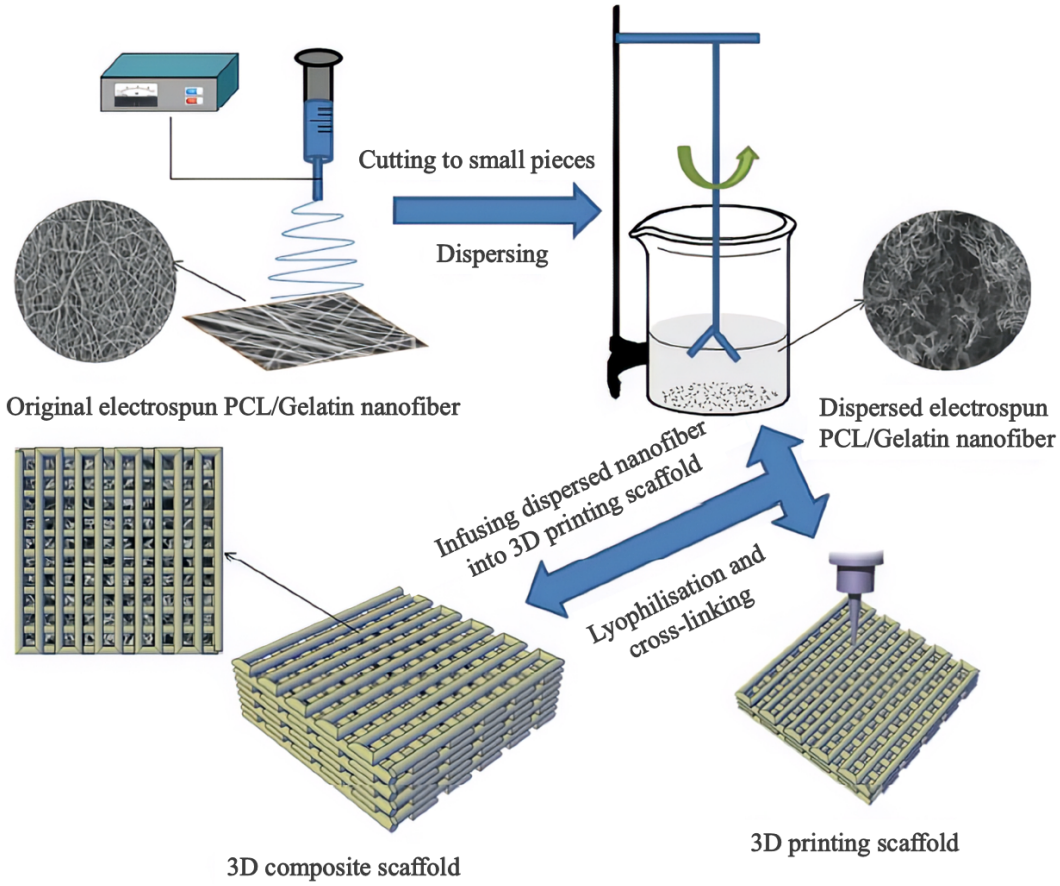

Figure 10 3D composite bone tissue scaffolds composite scaffold using electrospinning to produce PCL/Gelatin nanofibres and their production into 3D printed scaffolds. Reprinted with permission from Ref (74). Copyright 2016 Royal Society of Chemistry

In contrast, Kun et al.[75] fabricated PCL/Fe_3O_4/icariin 2D magnetic fibrous membranes via electrospinning and then expanded them to 3D scaffolds through depressurisation of supercritical CO_2 fluid. Electrospinning was performed using rotary and plate collectors, but the expanding behaviour of the electrospun membranes was more noticeable on the membrane collected from the rotary collector than the ones collected from the plate collector. The resulted 3D scaffolds exhibited enhanced in vivo cell infiltration, internal collagen deposition and angiogenesis due to the increased porosity and the action of icariin.

As mentioned earlier, coaxial electrospinning gives the option of incorporating bioactive proteins in the core component protected by the shell coating and released in a sustained and controlled manner, thus enabling a certain concentration of bioactive proteins to be maintained in target area. Hu et al.[76] prepared 3D coaxial electrospun scaffolds for bone tissue engineering in dental applications using a coaxial electrospinning and thermally induced self-agglomeration method. The coaxial scaffold was composed of bone morphogenetic protein-2 (BMP-2), as the core component, and a combination of poly (lactide-co-glycolide) (PLGA) and PCL as the shell. In order to electrospin BMP-2, the protein was dissolved in distilled deionized water with bovine serum albumin (BSA) added as a protein stabilizer. To produce the 3D structure, the coaxial electrospun scaffolds were cut into small pieces, and suspended uniformly in 2 mL of gelatin aqueous solution using a homogeniser. The fibre suspension was transferred into a glass bottle and immersed in water at 55°C for 2 min. This induced the small pieces to spontaneously agglomerate into a 3D scaffold with 12–14 mm diameter and 2 mm thickness. The 3D scaffold was put in ice water for 2 min to prevent further shrinkage and then freeze-dried. The resultant scaffold showed significantly enhanced osteogenic differentiation of rat adipose-derived stem cells (rADSCs) with BMP-2 incorporation in the fibre core.

Zhao et al.[77] designed a new form of core and shell scaffold to mimic natural bone structure by using electrospun PCL/collagen/HA as the shell of the scaffold while the core was made by freeze dried collagen with icariin (ICA)-loaded chitosan microspheres (Figure 11). The resulted drug-loaded 3D scaffold showed excellent cell attachment in vitro while in vivo studies performed in rabbit tibial plateaux demonstrated that abundant new bone was formed on the scaffold.

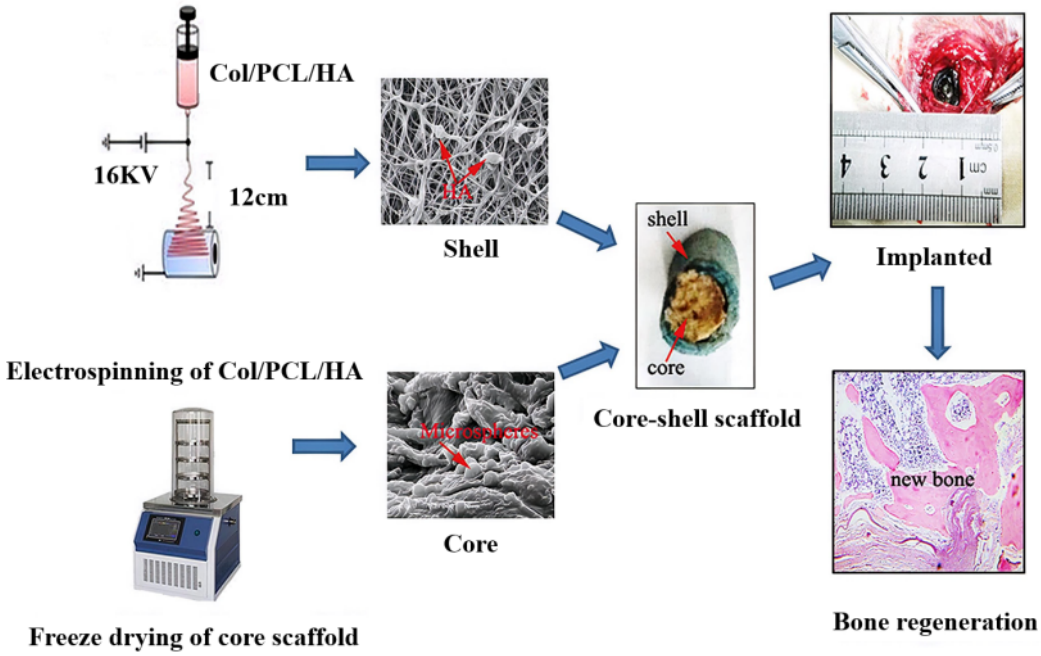

Figure 11 3D core and shell scaffold using electrospinning and freeze drying techniques and the response when implanted into a rabbit tibial plateau. Reprinted with permission from Ref (77). Copyright 2020 Dove Medical Press.

Yao et al.,[78] on the other hand, developed 3D PCL and PCL/PLA scaffolds through combination of three techniques: electrospinning, thermally induced nanofiber self-agglomeration and freeze drying. PCL/PLA 3D scaffolds exhibited higher mechanical properties and in vitro bioactivity compared to neat 3D PCL scaffolds, while in vivo studies revealed that PCL/PLA 3D scaffolds supported new bone formation in a cranial critical-sized bone defect in a mouse model.

The multi-technique approach was also adapted by Ye et al.[79] who manufactured 3D nHA/PLLA/gelatin scaffolds by combining electrospinning, homogenising, freeze-drying, and thermal crosslinking techniques (Figure 12). Electrospun 2D mats were first cut into small pieces and dispersed in a beaker containing tert-butanol for homogenising via a homogeniser for the duration of 20 minutes. Uniform nanofiber dispersions were subsequently freeze-dried for 24 h followed by thermal treatment at 180°C for 2 h. Following the thermal treatment, scaffolds were immersed into a crosslinking solution containing N-[3-(dimethylamino) propyl]-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) at 4°C for 24 h and then washed with deionised water and freeze-dried for another 48h. Finally, the 3D scaffolds were coated with BMP-2-derived peptides using a polydopamine (pDA)-assisted coating strategy to obtain a sustained release. In vitro studies have shown that the 3D scaffolds increased the alkaline phosphatase levels in bone mesenchymal stem cells (BMSCs) and gene expression related to osteogenic differentiation, while in vivo studies performed in a rat cranial bone defect model indicated that the scaffolds facilitated bone formation in the defects.

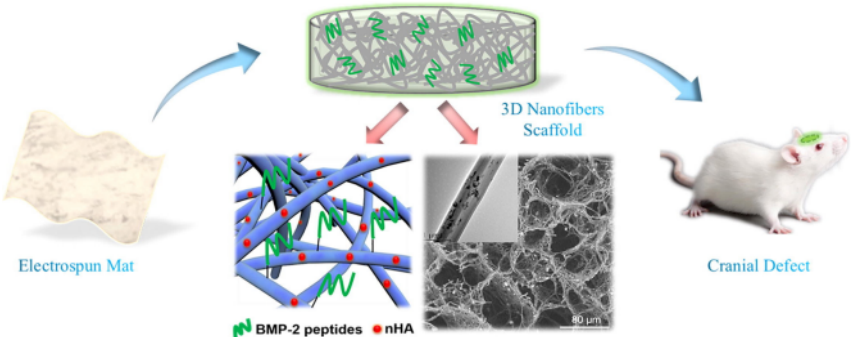

Figure 12 3D electrospun nanofibrous scaffold for rat cranial bone regeneration using electrospinning, homogenising, freeze drying, and thermal crosslinking. Reprinted with permission from Ref (79). Copyright 2019 Elsevier

CONCLUSIONS

Mimicking the morphology of the natural extracellular matrix, high porosity, surface functionalization, and the ability of encapsulating drugs and proteins makes either conventional or coaxial electrospun scaffolds ideal biomaterials for bone tissue engineering applications. However, traditional electrospinning techniques produce 2D mats which lack the 3D macro-porous structures that are crucial for cell infiltration and tissue regeneration. Therefore, new methods to fabricate 3D fibrous scaffolds based on electrospinning have been developed more recently.

In this review, various strategies for producing three dimensional electrospun/co-electrospun scaffolds have been discussed. The strategies included stacking or rolling 2D electrospun mats to produce 3D structures, using modified collectors beyond the traditional stationary plate collector, or combining conventional or coaxial electrospinning with other scaffolds fabrications techniques such as solvent casting, 3D printing, freeze drying and thermally induced self-agglomeration.

While the results of the techniques discussed seem to be very promising so far, further preclinical studies are needed before adopting any of these approaches in clinical applications.

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