Nanocomposites and Their Applications in Dentistry, Orthopaedic and Drug Delivery systems

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Chapter 2 Electrospun Hydrogels Composites for Bone Tissue Engineering

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Abstract

Electrospinning is one of the foremost nanotechnology applications to fabricate bone tissue engineering scaffolds with sizes in the nanometre range. It is regarded as a versatile, inexpensive and relatively simple methodology with potential for mimicking the nano-architecture of bones. In this chapter, the fabrication of three types of nanocomposites based on the biocompatible electrospun hydrogels, namely:(i) electrospun nanofibre-reinforced hydrogels, (ii) electrospun hydrogels with biological electrospray cells and (iii) electrospun hydrogels with antimicrobial activity, exclusively for the field of bone tissue regeneration are presented. Hydrogels have been used widely in biomaterial applications, mainly due to their low interfacial tension, useful swelling properties and high lubricity. In addition to their promising biocompatibility characteristics, certain hydrogels are desirable in the biomedical field due to their sensitivity to the physiological or biological environment where these are used. There are many current applications for hydrogels including 8,000 different kinds of medical devices and 40,000 different pharmaceutical preparations. The addition of electrospinning technology to hydrogels is an ideal combination as it facilitates the production of nanoscale hydrogel to allow the properties of the materials to be tailored. Indeed, the remarkable tunability of nanofibres morphology and diameter can be performed through appropriate adjustment of different variables from processing parameters, polymer solution parameters and ambient parameters during electrospinning, in order to control scaffolds mechanical properties and consequently affect cell behaviour. Therefore, the incorporation of the electrospinning technology with hydrogels have allowed novel perspectives in the research of electrospun hydrogel composites for successful osteoconductive scaffolds with better cell adhesion, proliferation and differentiation.

Key Words

Electrospinning; Biomaterials; Electrospun hydrogels; scaffolds; Nanofibres; Bone tissue engineering; Osteoconduction

Chapter starts here

2.1 Introduction

Nanotechnology has the power to transform society. It is the science of changing properties of materials at the molecular and atomic level [1]. The drive for materials with specific sizes and geometry has made an enormous impact on biomedical applications in terms of nanotechnology. The integration of nanotechnology into biomedical applications is called

"nanomedicine". Electrospinning is one of the foremost nanotechnology applications to fabricate materials with sizes in the nanometre range. It has widespread applications in nanomedicine and the potential to solve unmet medical needs in the future. In addition, the incorporation of the electrospinning technology with the biocompatibility and controlled biodegradable rate of hydrogels have driven the research of electrospun hydrogel composites for successful tissue engineering. These electrospun hydrogels with hydrophilic polymeric network are beneficial because they are capable of trapping a large amount of water or biological fluid without dissolving in the polymer matrix and consequently giving a moist environment for cell seeding.

The core objective of this book chapter is to examine the utilisation of different polymer-based high-functional and high-performance electrospun nanomaterials for tissue engineering, particularly in the field of bone tissue regeneration. Bone tissue engineering is a combination of biology and engineering that involves the use of cells (e.g. osteoblasts), bioactive molecules (e.g. bone morphogenetic protein 2) and biomaterials (e.g. electrospun hydrogels composites) under an *in vitro* culture system. Bone tissue engineering aims to develop substitute bone tissue, to either replace or restore the function of impaired bone tissues. Bone tissue is considered hard tissue, it is a type of mineralized tissue or calcified tissue. Traditionally, electrospun hydrogels have been merely used for soft tissue engineering, however successful efforts have been made to render hydrogels appropriate for hard tissue engineering [2].

Bone tissue is a nanostructure composed of tough and yet flexible collagen fibres (1-10μm) reinforced with calcium phosphate nanocrystals as well as minor proteins and growth factors. Numerous methods have been employed to generate biomaterials in nanoscales. One such method is electrospinning which is regarded as a versatile, inexpensive and simple methodology with positive outcomes for mimicking the nano-architecture of bones. It allows the construction of very compact and high-performance nanomaterials. This chapter captures an in-depth overview of the current state of research and knowledge related to electrospun composites in terms of theory, electrospinning processing conditions and mechanisms for bone tissue engineering applications. The electrospinning technology outlined in this chapter includes (i) electrospun nanofibre-reinforced hydrogels, (ii) electrospun hydrogels with biological electrospray cells and (iii) electrospun hydrogels with antimicrobial activity. The effects of the attachment, migration and proliferation of cells and bioactive molecules for *in vivo* bone regeneration have also been investigated among these three primary-hydrogel nanocomposites.

When discussing how to produce biomaterials via electrospinning, it is not possible to omit the parameters (i.e. polymer solution, processing and ambient parameters) for tuning fibres morphology and size. Through appropriate adjustment of different variables from the parameters, the variations in properties, morphologies, structures and diameters of electrospun nanofibres can be generated. This chapter also reviews some of the recent patents issued in the field of electrospinning and bone tissue engineering. As seen in the slow growth in the number of patents for the electrospun hydrogels for bone tissue engineering applications in the twenty-first century, it is expected that there would be a significant increase in granted patents for bone tissue repair and regeneration in the future. Finally, the chapter concludes with the future applications and possible research opportunities of electrospun hydrogels.

2.1.1 General principles of electrospinning

There is an increasing recognition that a nano-sized fibre is very likely to be more bioactive than a micro-sized one [3]. Undeniably, the most applicable and controllable nanofibre production method is electrospinning. This method allows the fabrication of filament-forming, super lightweight polymers as well as electrospun nanofibres from solutions and melts (polymer or polymer mixed) in the presence of an electric field. It is one of the few techniques used to create composite materials made up from two or more different materials[4]. The first development of electrostatic attraction of a fluid was investigated in the seventeenth century by the physician, William Gilbert [5]. After a duration of time of dramatic growth, the first patent for electrospun nanofibres was issued in the year 1902 by William James Morton titled 'Method of Dispersing Fluid' [6].

An electrospinning apparatus setup (Figure 1) comprises of three main parts: a high voltage generator, a syringe pump and a collector plate. Initially, a syringe pump containing polymer solution with a metal needle tip is connected to the positive electrode of the power supply generator, while the grounded electrode is connected to a collector plate on the opposite end. Subsequently, a high voltage is applied in the system to create an electric field between the tip of the needle and the collector plate, which helps to cross-link the polymer chains during the electrospinning process. The polymer solution acts as a charged carrier transferring the electricity over to the side of the collector with no charge and this can result in a potential voltage difference between the polymer solution and the collection plate [7]. When the relatively weak surface tension of the charged solution droplet is overwhelmed by a strong electrostatic force, the droplet is distorted and forms a conical shape known as a Taylor cone. The distortion is continued and leads to an electrically charged jet ejection which draws the aligned thin polymer fibres to accelerate toward the collector [8]–[12]. In the electrospinning process, the solvent evaporates and leaves the dry nanofibres deposited on the collector [7].

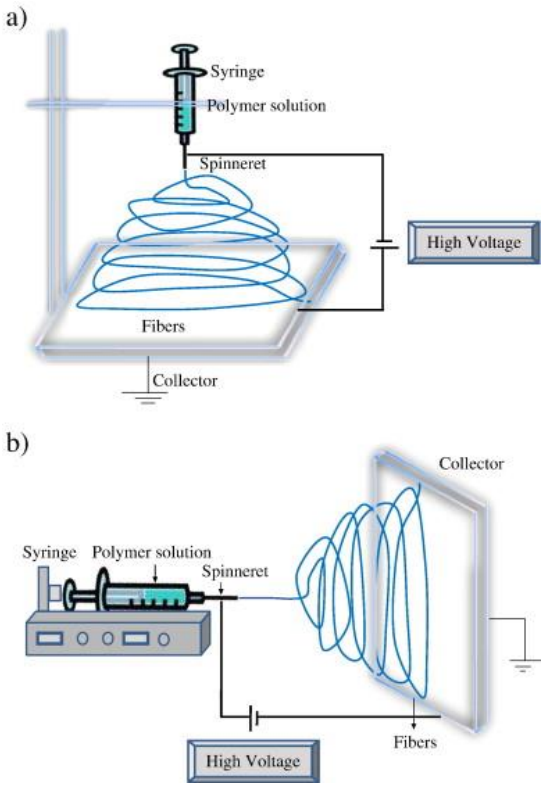

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Figure 1 Schematic diagram of setting up of electrospinning apparatus (a) typical vertical set up and (b) horizontal set up of electrospinning apparatus [13].

There are a number of conventional techniques available for the fabrication of nanofibres for use in bone tissue engineering, including electrospinning [14], rotary jet spinning [15], self-assembly [16], sol–gel methods [17], phase separation [18], melt-blow [19], melt spinning [20] and template synthesis [21]. Out of all of these methods, electrospinning tends to have the most remarkable effects in relation to the size and shape of fibres, which is similar to the extracellular matrix (ECM). Unlike the use of mechanical forces to draw nanofibres via conventional spinning processes (i.e. melt spinning) [8], the electrostatic force acts as a driving mechanism in electrospinning to produce nanofibres which have uniform, non-beaded and ultrafine morphologies (Figure 2) [10], [22].

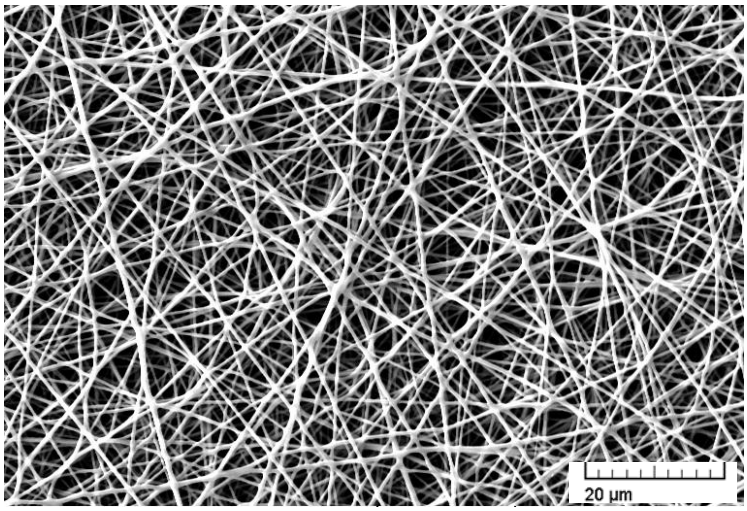

Figure 2 Scanning Electron Microscopy (SEM) photograph of PVA electrospun nanofibres at 2.00 KX magnification [23].

Electrospun nanofibres tend to contribute excellent control over nanofibre dimensions and alignment with lengths measuring up to kilometres as well as having an average diameter of submicrometres to tens of nanometres [8], [24], [25]. However, electrospun materials are not very cost-effective and economical, due to the slow production rate. In order to increase the reproducible nanofibres production rate for scale-up commercialisation, the sluggish single jet spinning has been substituted by several methods such as multiple jets (Figure 3) [26]or needleless electrospinning from multiple ring [27].

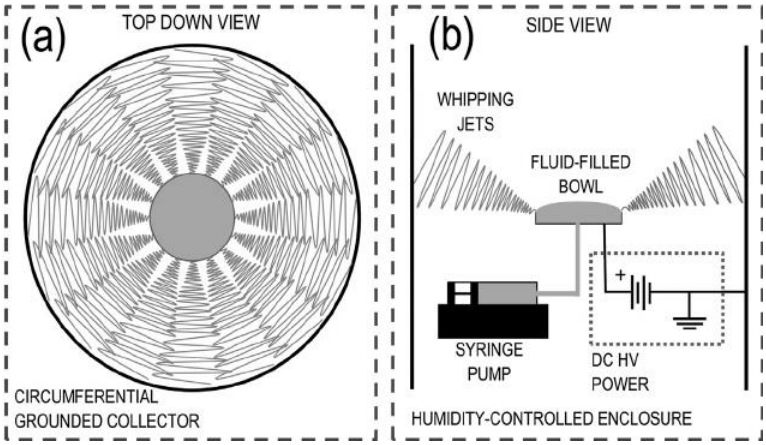

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Figure 3 Schematic diagram of the bowl edge electrospinning apparatus. (a) Top-looking down view of jets spinning directly from the bowl-lip to the collector. (b) A side view of the refill system and power supply [28].

2.2 Electrospun nanocomposites for medical applications

Electrospun nanomaterials have some outstanding properties, such as high surface area-to-volume ratio compared to film [25], [29], light weight [22], high porosity [30], controllable membrane thickness [10] and bioavailability [31]. Due to the unique structure of these biomaterials, these are ideal candidates for use on biomedical structured elements such as wound dressings [22], drug delivery systems and scaffolding used in tissue engineering [8], and are not limited to use for filtration operation [32], textiles field [33] and electronic component coating [8] in industrial applications.

Research activities in electrospinning became more prevalent when novel polymer/drug nanofibres were successfully fabricated [29]. The initial patent describing this new drug delivery system was granted in the year 2010. In the invention, Kim and Yun have demonstrated a drug delivery system using electrospinning of biodegradable polymers: poly (ε -caprolactone) (PCL) and polyethylene oxide (PEO) [34]. In addition, according to research papers, Li et al. and Bahrainian et al. have studied the fast-dissolving drug delivery systems. They reported that the introduction of drug ingredients in an amorphous or in a nanocrystal state into the electrospinning polymer aqueous solutions can give the medicines a fast-wetting surface property, enhancing both drug solubility and dissolution rates, especially for poorly water soluble components [11], [31]. If there are multiple nano-formulated fibres processed together to form a mesh, the surface area-to-volume ratio of the polymer increases into numerous folds, making them ideal for the active ingredients entrapment inside and for the rapid release from the polymer membranes when these are swallowed by the patients.

In addition, drug delivery systems can also be applied via an electrospun nanofibre implant in dentistry, orthopaedic and tissue engineering applications etc.[7]. The effectiveness in drug delivery has the potential for implantation of electrospun biomaterials into the human body as an alternative for the use of injections or tablets every day, providing a long-term drug release systems from the electrospun mesh into the body over a period time in a controlled way [11].

The human body has many functions and almost every part of our body can be replaced. Indeed Professor Seeram predicted that “In the future, maybe 20-30 years from now, every human

would have 20% of their body replaced with these devices at the International Conference on Nanofibres, Applications and Related Technologies (NART) on 31 Aug 2015.

2.2.1 Electrospun nanocomposite for bone tissues regeneration via osteoconduction, osteoinduction and osteogenesis

Bone has an innate ability to heal well after mild fractures without the need for surgical intervention. Nevertheless, patients do not have the potential for self-healing with large bone defects because the bone lacks an orchestrated regeneration. This normally can lead to the need for an autologous bone transplant procedure called autografting [35] and there are estimated 2.2 million of such surgeries happening worldwide every year [36]. Despite, its widespread use, complications have been reported due to the short supply of bone stock, especially in patients who suffered fractures from osteoporosis or patients who already underwent a similar procedure that requires a second surgery. This may result in a significant donor site morbidity [13] and increase the risk of infection at the defect site [37]. Therefore, the search for new bone regeneration strategies to substitute bone-grafting procedure is desirable for bone tissue engineering.

Extracellular matrix (ECM) is an essential part of the natural habitat of cells and tissues. An electrospun scaffold is one of the strategies recommended to produce structurally and functionally similar ECMs for bone implant [25]. Nanofibrous scaffolds are found to match both mechanical and biological contexts of real bone tissue matrix because these are able to resemble the architecture of the native ECM at a nanometre scale to assist the migration, organisation and survival of cells in bone regeneration. Moreover, the extraordinarily high porosity of scaffolds provides a substrate for better cell adhesion, proliferation and differentiation to achieve better cell growth for postsurgical implantations [11], [38]. In order to recreate bio-functional nanoscale scaffolds to repair damaged bone tissues, electrospinning technique is emerging as an interesting candidate for biomimetic materials [35], [39].

There are three main elements as depicted in Figure 4 that support the promotion of bone formation process in bone tissue engineering: osteoconduction (electrospun scaffolds), osteoinduction (bioactive molecules) and osteogenesis (stem cells) [40], [41]. The current challenge for the scientific community is the engineering of biocompatible and bioactive nanomaterials for the creation of osteoconductive three-dimensional extracellular matrix scaffolds that promote osteoinduction and osteogenesis of bone tissue [41]. Osteoconduction is the capability to allow the growth and rearrangement of bone tissue on the surface of implanted nanomaterials. These materials act as an adjunct for both the occurring of osteoinduction and osteogenesis. Without the presence of stem cells and bioactive molecules, the osteoconductive scaffolds cannot form any new bone tissues [42].

Osteoinduction is the capability to induce new bone formation by signalling molecules and growth factors. Appropriate osteoinductive agents, essentially the bone morphogenic proteins (BMPs) involved in electrospun scaffolds, can help to attract the recipients' own mesenchymal stem cells. The stem cells are consequently stimulated to develop into pre-osteoblasts for ultimate bone formation [35], [42]. However, osteogenesis is the capability to produce new bone tissue by cells called osteoblasts. The introduction of osteogenesis into osteoconductive scaffolds mimics the composition and structure of human trabecular bone for early-stage bone colonisation by osteoblasts [43]. It simply means electrospun scaffolds act as a substrate for *in*

vitro cell growth. The osteogenetic and osteoinductive potential of electrospun scaffolds have shown strong effects to facilitate bone regeneration.

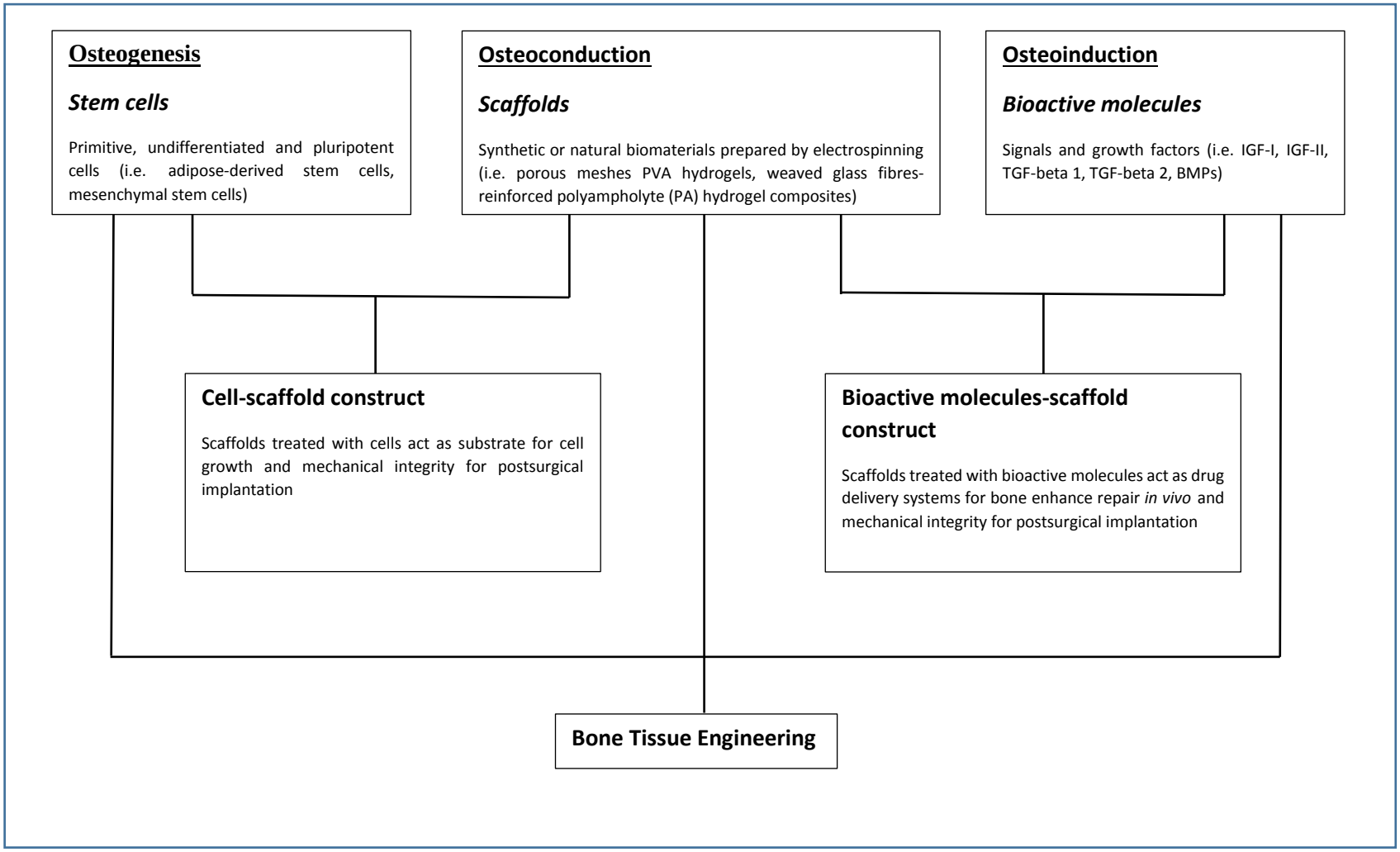

Figure 4: Osteoconductive scaffolds promote osteoinduction and osteogenesis of bone tissue

2.2.1.1 The effect of osteogenesis and osteoinduction on osteoconductive electrospun scaffolds

An electrospun scaffold with osteoconductive, osteoinductive, and osteogenic properties is regularly seen in the bone healing process. The porous nature of bone tissue engineering scaffold offers a place to accommodate cells and to direct the cells to grow in the correct physical form [1]. Initially, the cells that need to be multiplied are predominantly from pre-cultured pre-osteoblasts/osteoblasts that are stimulated by trauma or generated from cells collected from primitive mesenchymal cells via osteoinduction [44], [45]. These cells are pre-treated with media containing serum and bioactive molecules (e.g. signalling molecules), then employed within the specific region of engineered 3D electrospun biomaterial sector. The cell-treated nanocomposite is then cultured at a condition which normally exists in the human body (*in vivo* conditions) for one to two weeks before re-implantation to allow cells differentiate into functioning tissues [46]. The nanocomposite serves as a mechanical integrity and a shape-determining biomaterial. This whole process is called tissue engineering which involves cells, bioactive molecules and biomaterials under an *in vitro* culture system (Figure 5).

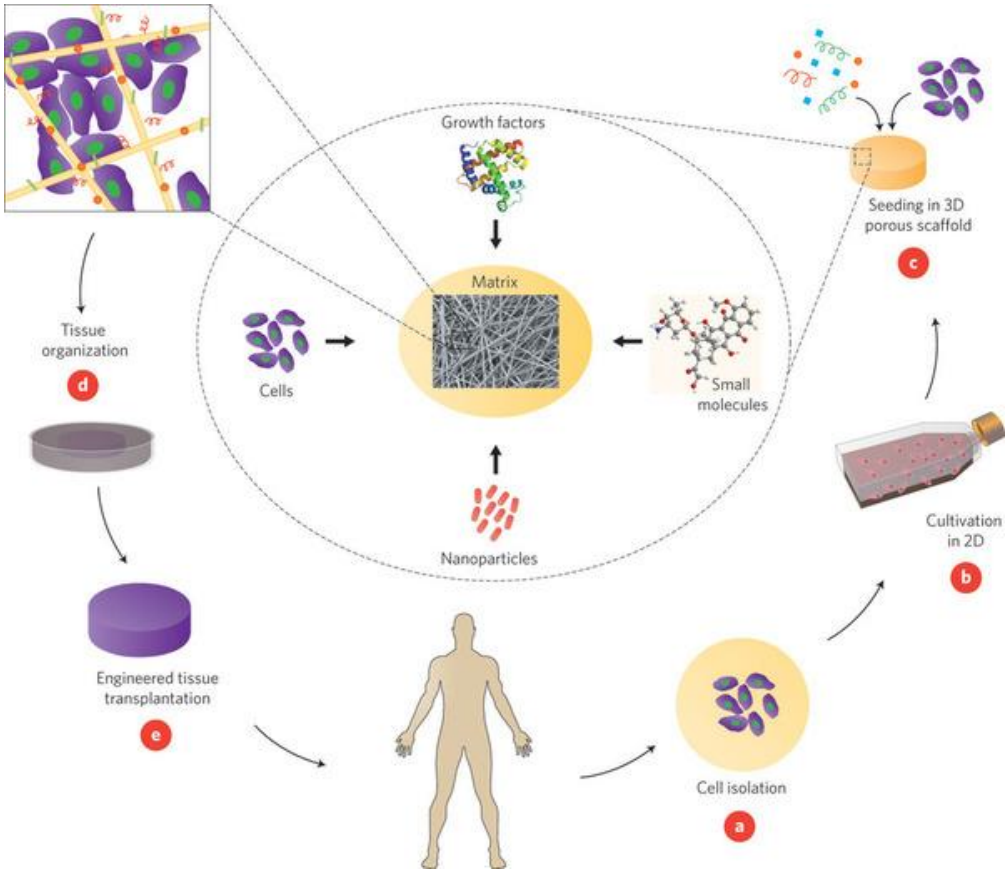

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Figure 5 Schematic diagram of a tissue engineering concept that involves seeding cells within electrospun scaffolds

(a) Cells are isolated from the patient and may be cultivated (b) in vitro on two-dimensional surfaces for efficient expansion. (c) Next, the cells are seeded in porous scaffolds together with growth factors, small molecules, and micro- and/or nanoparticles. The scaffolds serve as a mechanical support and a shape-determining material, and their porous nature provides

high mass transfer and waste removal. (d) The cell constructs are further cultivated in bioreactors to provide optimal conditions for organisation into a functioning tissue. (e) Once a functioning tissue has been successfully engineered, the construct is transplanted on the defect to restore function [47].

There are a multitude of techniques illustrating the process of delivering bioactive molecules (e.g. growth factors) into bones. For example, i) chemical immobilisation of the growth factor bound to the scaffold via chemical binding or affinity interactions [48], and ii) physical encapsulation of growth factors into polymeric microspheres via water/oil/water emulsion [49], [50], oil/water emulsion or spray-drying and subsequently incorporation of the microspheres within scaffolds [51]. However, some of those synthetic biomaterials implanted into bone defects lack the capability to contribute to load bearing. These are mostly encapsulated by a fibrous tissue and do not interact with bones due to a lack of bioactivity. Consequently, the scaffold remains as a foreign body, resulting in the isolation of the adjacent bone which can possibly lead to severe complication of the fractures [52]. Thus, the load bearing of the scaffold may improve by employing nanocomposite technology that mimics natural bone architecture.

For instance, Li *et al.* fabricated a nanofibrous scaffold seeded with growth factor, BMP-2 using the electrospinning method, and examined its biological properties with human bone marrow-derived mesenchymal stem cells (hMSCs). The researchers concluded that the co-processed BMP-2 in electrospun scaffold had sustained a high level of calcium deposition and boosted transcript levels of bone-specific markers (i.e. osteocalcin, bone-specific alkaline phosphatase) than in controls [53]. This provides a positive result for further development, the nanofibrous scaffolds that are treated with cell and bioactive molecules can act as an ideal drug delivery systems to enhance bone healing *in vivo* and thus immediately treat the patient in a single surgery [54], [55].

2.3 Electrospun biomaterials for bone tissue engineering

As a biomaterial, the biocompatibility and functionality of hydrogels play an important role in biomedical application due to hydrogels closely resembling the extracellular matrix, making them the best choice of materials as tissue engineering scaffolds [56], [57]. These materials are able to assist cell proliferation when new bone tissues are assembled [58]. A recently published Allied Market Research report predicted the global hydrogel market to be of approximately 27.2 billion USD by the year 2022, with a compound annual growth rate (CAGR) of 6.3% from the year 2016 to 2022.

In the case of biomimetic scaffold implants, bone conduction depends on the biomaterial used and its reactions to the site of injury on the bone. A wide range of materials are recommended for bone tissue engineering as listed in Table 1, most of these biocompatible and biodegradable polymers. The major advantage of biodegradable scaffolds is the controlled therapeutic release with slow degradation occurring on implantation and gradually substituted by the growth extracellular matrix proteins secreted from the adhered cells [59], [60]. The polymeric materials are classified into (i) natural biopolymers, such as chitosan, collagen, gelatine (ii)

synthetic polymers, such as PLGA[1], PLA[2], PCL[3], PVA[4] (iii) Inorganic and metals, such as ceramics, bioactive glasses, MgO[5] and (iv) polymer composites, such as PCL/collagen [61], [62]. The synthetic polymers exhibit excellent chemical and physical properties, while biopolymers produced from animal- or plant-derived proteins or carbohydrates are biocompatible and are of great importance in shaping cells [25]. Hence, the blends of synthetic polymers and natural biopolymers are of particular significance for improving or modifying the physicochemical properties of constructed polymer materials [63].

PVA is an interesting as a biomaterial, widely used in practical applications such as implantation [40] because of its outstanding chemical properties such as good chemical resistance, non-toxicity, high hydrophilicity, biocompatibility; and physical properties such as good film formation capacity, processability, thermal stability, complete biodegradability and high crystal modulus. PVA has been identified as the suitable candidate to be electrospun as hydrogel nanofibres and to implant on the human beings [63]–[65]. The abundant hydroxyl groups present on the backbone of electrospun PVA ease the attachment of drugs or cell signalling molecules [66] and also helps to release drugs or biological materials in a controlled manner [67]. In an ongoing study by De Lima et al. electrospinning of PVA with a ceramics component plays an important role for successful tissue regeneration [23].

2.3.1 Electrospun nanofibre-reinforced hydrogels

An electrospun nanofibre-reinforced hydrogel is a blend of two or more dissimilar polymer constituents, which provide an environment similar to extracellular matrix. Hydrogels allow free cell movement through the matrix, inertness to interact with the body and resistance to protein adsorption. For instance, PEO is bioinert and highly resistant to non-specific protein adsorption [68], [69]. However, hydrogels have some disadvantages including poor mechanical integrity when the water content is high and to provide for encapsulated cells. Hence, the addition of meshes of nanofibres embedded in hydrogel forms a complex structure that can solve problems that are unattainable by any monolithic material. The nanofibres infiltration with hydrogel matrix can enhance the biological activity of new tissue by fixing the gel to living tissues (Figure 6C) [58].

The electrospun fibres can be cut into shorter strands and physically mixed with a hydrogel. In this mixture, the electrospun fibres networks act similar to fibrous proteins of natural bone tissue to provide stiffness and strength in tension as well as promoting directional cell growth, while the hydrogel acts as a gelatinous ground substance to provide a highly hydrated three-dimensional environment with comparable complex mechanical behaviour and nutrient transport [70]. The biocompatibility of electrospun fibres is inversely proportional to the fibre diameter, as the decrease in fibre diameter leads to an increase in biocompatibility due to the larger surface area, which offers more cell adhesion sites (Figure 6B). As evident from Table 2, PCL has been widely selected to manipulate into a large range of orthopaedic implants because it was approved by the US Food and Drug Administration (FDA) as the biological inert fibre material for direct contact with biological fluid among the synthetic polymers [71].

[1] Poly(lactic-co-glycolic acid)
[2] Poly(lactic acid)
[3] Polycaprolactone
[4] Poly(vinyl) alcohol
[5] Magnesium oxide

Moreover, PCL is hydrolysable in the human body, it is more attractive for long-term implants and drug delivery systems due to PCL degrades at a much slower rate than PLA, PGA, and PLGA *in vivo* [72].

More biomimetic environments can be created as the binding sites provide cells with contact guidance and directionality that are vital for cellular differentiation. According to Sakai's research group, "Eelectrospun nanofibre-reinforced hydrogels provide a constant volume and surface area to the adhered cells, allowing more extensive cell proliferation compared to the hydrogel alone" [73]. This is partially due to the resistance of contractions among fibrous components during the growth of new tissue by seeded cells (Figure 6A) and the pore size of the mesh nanofibres. The lack of vascular supply in bone defects is likely to cause high cell morbidity immediately after implantation of a cell-seeded scaffold [35]. Hence, the pores formed by electrospun fibre meshes are highly interconnected which prohibit cell penetration along the structure's thickness, dimishing integration between the electrospun scaffolds with cells and extracellular matrix, as well as aiding the diffusion of oxygen and nutrients. The increased rate of cells growth then results in greater cellular expression of osteogenic markers and more distinct cell mineralization of osteoblasts [58], [74].

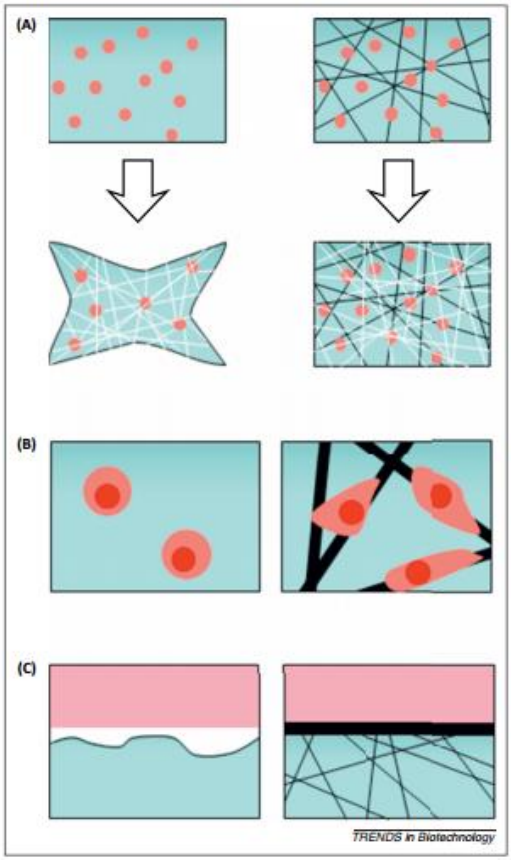

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Figure 6 Electrospun nanofibres embedded in hydrogels provide enhanced biological activity by (A) resistance to contraction during the development of new tissue, (B) provision of attachment sites and contact directionality to cells, and (C) improved binding to body tissues [58].

In addition, the nanofibres have a high strength to weight ratios. The electrospun nanofibre-reinforced hydrogels can be much more mechanically robust to failure than pure hydrogels, even at a very low volume fraction of nanofibers [39], [58]. In a recent patent, Koh et al. have demonstrated a novel multi-layered electrospun fibre incorporated hydrogel that consists of at least two bioactive ingredients for independently controlled release *in vivo* [75]. For example, Keller et al. had studied the bi-layered nanoactive implant with the incorporation of two bioactive molecules: hyaluronic acid and BMP-7 for osteoarticular repair (Table 2). The upper and lower layers were composed of electrospun PCL membrane with BMP-7 and PCL electrospun nanofibres reinforced alginate hydrogel with hyaluronic acid respectively [76].

2.3.2 Electrospun hydrogels with biological electrospray cells

The continuous techniques of scaffold fabrication and subsequent cell seeding or the fabrication of blend scaffold/cell have optimised by the micro integration of two devices, electrospinning and electrospraying to produce one biofunctional scaffold [4]. The micro integration system tends to directly integrate the cells into the hydrogels during their production stages [77]. 'Bio-electrospraying' or 'cell- electrospinning' is the term specifically describes the process of producing fine micrometre-sized cell-bearing droplets and delivering the cell suspensions on a scaffold via electrospraying. Table 3 presents the combination of nanofibres and nanobeads scaffolds. Braghirolli et al. studied the electrospun PLGA with bioelctrospraying of MSCs elicited appropriate physicochemical characteristics and the scaffold offered a favourable response towards MSC differentiation into osteogenic lineages [77], [78]. This has demonstrated the viability of electrospun hydrogel/cell construct in tissue repair and functional restoration.

The combination of bioelectrospraying and electrospinning has been recommended as more suitable for cell growth and proliferation than a single electrospinning technique. This method can increase the osteoconductive and osteoinductive effect, with particular regards to improved cell–scaffold interaction [61]. The incorporation of the bio-electrospray method has promoted cell evenly 3-D distributed along the fine-tuned hydrogel nanofibres scaffolds, especially at the beginning of the cultivation stage without causing any momentous deleterious effects on engineered cell construction at a molecular level [14]. In addition, the cells can interact well with the surface and pore inner walls of the scaffolds, resulting in a favourable surface topography and an osteophilic condition for the cells stay under a balanced material and energy exchange environment to promote cell penetration [61], [79].

2.3.3 Electrospun hydrogels with antimicrobial activity

Infection is the biggest global challenge in medicine complications. *E. coli, S. Typhi, V. cholera, P. aeruginosa, R. rhodochrous, P. vulgaris, A. hydrophila,* and *B. cereus* are human pathogenic bacteria that can cause bone infections (osteomyelitis) [80]. These bacteria are resistant to various antibiotics which can probably cause the extension of hospitalisation associated with high morbidity. Therefore, the goal of finding the right antibiotics to kill the antibiotic-resistant bacteria *in vivo* has driven the development of biodegradable, infection-resistant bone repair implant scaffolds [81]. These scaffolds should not only be tailored for biodegradable applications to eradicate the need for surgical removal of the implant but also possess natural characteristics of being an antimicrobial agent for long-term release of

antibiotics and guarantee biocompatibility with native cells. Besides, an immune modulatory effect of electrospun hydrogels with antimicrobial activities encourages both tissue growth and differentiation in tissue culture [15], [82].

The increased awareness of antimicrobial activity for medical applications has promoted the growing trend of using antimicrobial polymers for implantation to reduce the outbreak of infectious diseases. In the year 2007, Townsend Polymer Services stated in their global plastics polymer additives market report that there was a 15,500 tons worldwide consumption of polymer/biocides formulation [83]. That is the major reason why biocide additives, either inorganic (metallic nanoparticles: silver and copper) or organic (natural and synthetic compounds) are incorporated into polymers [84]. However, certain medical practitioners tend to avoid inorganic and synthetic biocides for bone conduction. So, new tissue engineering approach is addressed for infection control by using electrospinning method to crosslink natural biocides with polymers, which in many cases do not inhibit the biocompatibility of scaffolds. These engineered scaffolds can give limited cytotoxicity and a much longer duration of the antimicrobial activity as well as providing diverse pharmacological and therapeutic activities such as anti-inflammatory and anti-oxidant activity, while simultaneously initiating bone regeneration [85].

The natural biocides are originated from animals (e.g. chitosan, propolis) and plants (e.g. *Aloe vera*) [86]–[88]. For example, Paipitak et al. had characterised the synthetic PVA polymer with chitosan (animal-derived polysaccharides) nanofibres prepared by electrospinning [87]. Chitosan has been considered as an excellent material for hydrogel production in bone tissue engineering because chitosan has cellular binding capability [89] and is able to induce migration of cells [90], as well as it is being able to confer considerable antibacterial activity against a broad spectrum of bacteria [63]. All these characteristics can accelerate the bone regeneration process after a surgical intervention of PVA/chitosan. In addition, Selvakumar et al. produced a promising scaffold which has antimicrobial activity against various human pathogens while retaining the function of enhancing cell proliferation for bone regeneration using segmented polyurethane (SPU) and *Aloe vera* wrapped mesoporous hydroxyapatite (Al-mHA) nanorods [88]. Both studies have proved that the use of natural-derived synergistic compounds can tackle biofilms of multidrug-resistant microorganisms, such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended Spectrum Beta-Lactamases (ESBL) *E. coli* and *K. pneumonia*.

Table 1 Electrospun hydrogel for bone tissue engineering

Type of electrospun hydrogel	***Cells or bioactive molecules seeded on hydrogel***	***Electrospinning conditions***				***Reference***
		Voltage (kV)	***Tip-to-collector distance (cm)***	***Flow rate (µL/min)***	***Feed rate (mL/h)***	
Chitosan/Alginate/ Poly(ethylene oxide) (CTS/Alg/PEO)	*MC3T3 cells*	13	10	20.0	-	[91]
Medical grade poly(ε-caprolactone)/ Collagen (mPCL/Col)	*Mesenchymal stem cells* (MSCs)	12.5	12.5	25	-	[78]
Poly(ε-caprolactone) (PCL)	*Human primary osteoblasts* (HOB)	15	17	20	-	[92]
Medical grade poly(ε-caprolactone)/ Collagen/ Poly(ethylene oxide) (mPCL/Col/PEO) *Medical grade poly(ε-caprolactone)/ Collagen/Gelatine* (mPCL/Col/Gel)	*Human fetal osteoblasts* (hFOBs)	mPCL-col = -9 PEO= 7 Gel= 7	6	-	mPCL-col = 0.75 PEO= 1.25 Gel= 1.25	[93]
Polycaprolactone/poly(methyl methacrylate) (PCL/PMMA)	*MG-63 osteoblast cells*	25	-	25	-	[94]
Polycaprolactone /hydroxyapatite/Gelatine (PCL/HAp/Gel)	*Human fetal osteoblast cells* (hFOB)	13	12	16.7	-	[95]

Poly(L-lactic acid) (PLLA)	*Bone morphogenetic protein 2* (BMP-2)	20-30	15	14.0	-	[57]
Poly(L-lactic acid)/Collagen/ Hydroxyapatite (PLLA/Col/HAp)	*Human fetal osteoblasts* (hFOB)	12	15	-	-	[96]
Poly(L-lactic acid)/Collagen I (PLLA/Col I)	*Human mesenchymal stem* cells (hMSC)	10–18	15	0.2–0.3	-	[97]
Gelatine/ Polycaprolactone (Gel/PCL) *Gelatine/ Polycaprolactone / nanohydroxyapatite* (Gel/PCL/nHAp) *Gelatine/ Polycaprolactone /Bone powder* (Gel/PCL/Bone powder)	*Human adipose-derived stem cells* (hASCs)	17	13	16.7	-	[98]
Gelatine/Siloxane (GS)	*Bone marrow-derived mesenchymal stem cells* (BMSCs)	15-25	15-30	-	-	[71]
Poly(vinyl alcohol)/ Gelatine (PVA/Gel)	*MG-63 cells*	22	10	-	0.5–1.0	[99]
Poly(vinyl alcohol)/Chitosan (PVA/CTS)	*Umbilical cord blood (UCB) -*	50	15	-	-	[100]

	derived mesenchymal stem cells (MSCs)					

Table 2 Electrospun fibres coupled with hydrogels for bone tissue engineering

Type of hydrogel	**Type of electrospun fibre**	***Cells or bioactive molecules seeded on hydrogel***	***Electrospinning conditions***				**Reference**
			Voltage (kV)	***Tip-to-collector distance (cm)***	***Flow rate (μL/min)***	***Feed rate (mL/h)***	
Alginate	*Polycaprolactone* (PCL)	*Recombinant bone morphogenetic protein-2* (rhBMP-2)	13-20	20-23	12.5	-	[101]
Alginate/ Hyaluronic Acid	*Polycaprolactone* (PCL)	*Mesenchymal stem cells* (hMSCs) *and human chondrocytes* (hCHs)	15	17	20	-	[76]
Polyethylene glycol (PEG)	*Polycaprolactone* (PCL)	*PC12 cells*	30	20	50	-	[102]
Poly (lactide-co-ethylene oxide fumarate) (PLEOF)	*Poly-L-lactic acid* (PLLA)	*Bone marrow stromal cells* (BMS)	25	7	16.7	-	[103]
Heprasil	*Medical grade poly(ε-caprolactone)/*	*Human fetal osteoblasts* (hFOBs)	9	6	-	4	[93]

	Collagen (mPCL/Col)						

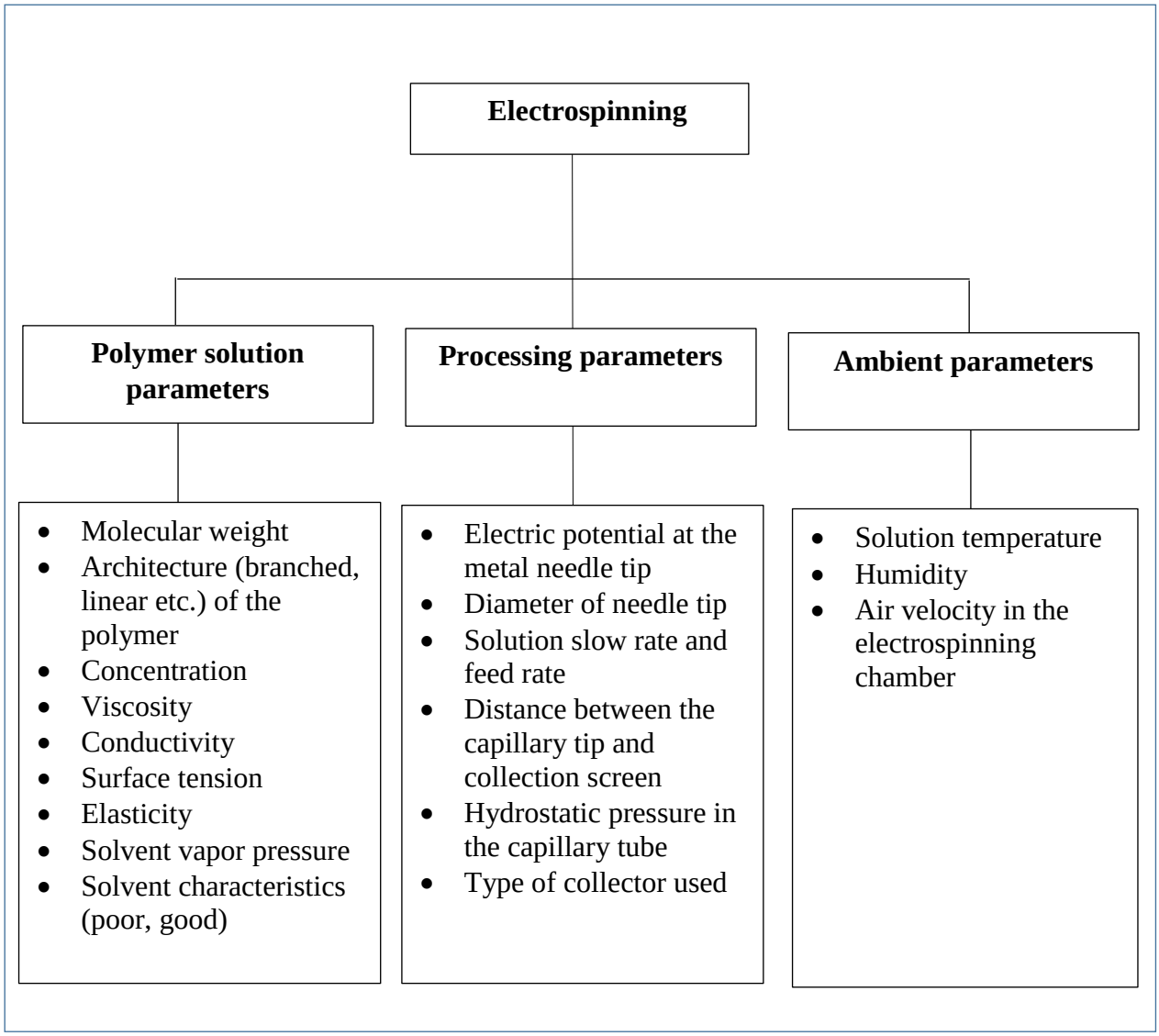
Table 3 Electrospun hydrogel with electrospray nanoparticles or bio-electrospray cells for bone tissue engineering

Type of electrospun hydrogel	**Type of bio-electrospray cell**	***Electrospinning conditions***			***Electrospraying conditions***			**Reference**
		Voltage (kV)	***Flow rate (µL/min)***	***Tip-to-collector distance (cm)***	***Voltage (kV)***	***Flow rate (µL/min)***	***Tip-to-collector distance (cm)***	
Poly(lactic-co-glycolic acid) (PLGA)	*Mesenchymal stem cells* (MSCs)	15	9	7.5	15	44	4	[77]
Poly(etherurethane urea) (PEUU)	*Vascular smooth muscle cells* (SMCs)	10	25	23	5	250	5	[104]

2.4 The impact of various parameters on the electrospinning process for nanofibre morphology

Many experiments have been conducted to determine the effects of various parameters on morphological structures and diameters of electrospun nanofibres. Through appropriate adjustment of different variables from processing parameters, polymer solution parameters and ambient parameters (Table 4), different properties of nanofibres can be generated [8], [22], [64], [105]. However, not all the variables listed in Table 4 are fundamental control parameters nor are they independent of each other. For example, applied voltage, target distance and electric field are all interconnected [106]. The fact that one variable can influence one or more parameters, it is ideal to change the setting of one variable at a time.

Table 4 Polymer solution, electrospinning process and ambient parameters that affect the characteristics of electrospun nanofibres



2.4.1 Polymer solution parameters

Chain entanglement is considered to be one of many parameters that can significantly influence fibre formation during polymer electrospinning [106] and it can be varied depending on both

fundamental variables: the polymer molecular weight and concentration. The chain entanglement plays a major role in stabilising the fibrous structure. If the chain entanglements in the solution are insufficient to stabilise the solution jet, only beads will form during electrospinning [80]. The establishment of the optimum ranges for concentration and molecular weight is desirable to ensure stable nanofibre formation. It is well known for a given molecular weight (M), the entanglement density varies exponentially with concentration [107]. Alternatively, the same result is achieved at a fixed polymer concentration by increasing molecular weight [106]. It is also known that the polymer concentration and molecular weight may have a significant effect on electrical conductivity and on fibres diameter [64].

Furthermore, both the increase of polymer concentration and molecular weight also result in a corresponding increase in solution viscosity, [η] [108]The Mark-Houwink-Sakurada equation relating the intrinsic viscosity to the molecular weight of a linear polymer is,

$$[\eta] = KM^{a}$$

where the empirical constants K and α depend on the nature of polymer, solvent and temperature [109]. For example, Tacx et al. have obtained the Mark–Houwink relationship for PVA in different solvents and temperatures as: [η] = 1.51 × 1024 M $^{0.804}$ for PVA in DMSO at 65°C, [η] =3.54 × 1024 M $^{0.692}$ for PVA in ethyleneglycol at 140°C and [η] =6.51 × 1024 M$^{0.628}$ for PVA in water at 30°C [110]. This equation has provided a simple approach for characterising the intrinsic viscosity of a polymer and can be used for determining the Berry number (Be). The relationship between intrinsic viscosity and the concentration can be normalized with respect to the Berry number as:

$$Be = [\eta]C$$

where [η] is the intrinsic viscosity and C is the solution concentration [111]. The Berry number is generally used by the researchers as a processing index for controlling the diameter of electrospun nanofibers [112].

At a constant concentration, the morphological transition is defined from beads, to beaded fibres, to complete fibres and to flat fibres with the increase of molecular weight. A fibrous structure cannot be stabilised when the Berry number is [η]C<4. As a minimum degree of chain entanglement is needed for producing fibrous structures, it required a [η]C between 5 and 12 for producing a stable fibrous structure. Therefore, when [η]C>4, where the solution is under a semi-dilute entangled regime, the polymer chains in the solution begin to entangle with each other and the solution viscosity increases significantly [64]. While, the gradual shift from circular fibres to flat fibres starts at a Berry number of 12 ([η]C≥12) [114].

2.4.2 Processing parameters

By changing the distance between the capillary tip and collection screen, the applied electric field between the tips and collector can be altered and it affects the formation of the fibrous membranes. Owing to the distance increase, the fibres are continually stretched and thinned within the whipping region, resulting in smaller fibre diameters [114]. Bead generation will appeared when the distance is too large or too small [32]. Moreover, there is a direct impact on the fibre diameter of injection needle tip diameter. Wang et al. reported that the fibre diameter becomes smaller with a smaller injection needle tip diameter and increased working distance

[115]. Another process parameter is the type of collector used. It can vary the arrangement of electrospun fibres as random nonwoven fibrous mats, or as uniaxially aligned arrays [74].

2.4.3 Ambient parameters

In addition to the effects of controlled processing and solution parameters on fibre morphology and properties, the influence of ambient parameters such as humidity, temperatures and air velocity in the electrospinning chamber were investigated. Nezarati et al. reported that fibre breakage occurs at low humidity due to decreased electrostatic discharge from the jet. However, a high humidity level did not guarantee the fibres formation, it was dependent on the polymer hydrophobicity, solvent volatility and miscibility with water. Furthermore, the humidity directly influences the number of pores on the fibre. The surface pores formed via vapour-induced phase separation increased with a high evaporation rate of the highly volatile solvent at a high humidity level [32], [116].

2.5 Inventions related to electrospun hydrogels for bone tissue engineering

There are numerous patents on hydrogels for producing contact lenses, hygiene products and wound dressings and these have well-established roles in the markets. However, the commercial hydrogel-based products (e.g. coating of scaffolds) in tissue engineering market are still limited. Many hydrogel-based scaffolds have designed, studied and published in research papers or in some cases, these are even patented, but not many have been commercialised [117]. Therefore, there will be even less granted patents specifically for electrospun hydrogels in bone tissue engineering. Limited patents and commercial products with electrospun hydrogels in tissue engineering are related to some extent to their low mass-production (further discussion to follow in section 6). Further research is required to elucidate the influence of production rate on the electrospun hydrogels.

It is generally supposed that the interest in electrospinning started with the significant contributions of Anton Formhals in the 1930s who filed made 22 patents on differents aspects of electrospinning processes carried out in several countries, such as America, France, the United Kingdom and Germany between 1931 and 1944 [5]. Although this technology was invented in the early twentieth century, there are still many opportunities to use electrospinning in various forms of organic and inorganic materials for different types of applications. Their potential in bone tissue engineering can be widely explored. According to the European Patent Office, the patents describing these electrospun hydrogel scaffolds exclusively for bone tissue engineering were first granted in the twenty-first century (Table 5). The electrospun hydrogels scaffolds have recently experienced an increasing global interest during last few years. It is expected that there would be a significant increase in granted patents for bone tissue repair and regeneration in order to increase of entrepreneurial activities and investments, consequently expanding the size of the market opportunity for new uses in the future [118].

Table 5 Patents applications of electrospun hydrogels, exclusively for the use of bone tissue engineering

Patent #	Subject	Applicant (s)	Inventor(s)	Priority application date	Publication date	Reference
WO 2012048188	Electrospun Mineralized Chitosan Nanofibres Crosslinked with Genipin for Bone Tissue Engineering	Lelkes Peter I, ; Frohbergh Michael, ; Drexel University	Lelkes Peter I, ; Frohbergh Michael	07-10-2011	12-04-2012	[119]
KR 20130091824 A	The Fabrication Method of Porous Hyaluronic Acid-Gelatin Hydrogel Scaffolds for Bone Tissue Engineering and The Hydrogel Scaffolds Fabricated Thereby	Soonchunhyang University Industry Academy Cooperation Foundation	Lee, Byong Taek, ; Jang, Dong Woo, ; Nguyen Thuy Ba Linh	09-02-2012	20-08-2013	[120]
KR 20130091822	A Manufacturing Method of Novel Fibrous Scaffold Composed of Electrospun Porous Poly([epsilon]-Caprolactone) (PCL) Fibres For Bone Tissue Engineering	Soonchunhyang University Industry Academy Cooperation Foundation	Lee, Byong Taek, ; Jang, Dong Woo, ;Son, So Ra	09-02-2012	20-08-2013	[121]
KR 101428514	A Hybrid Manufacturing Method of Tissue Scaffolds for Bone Regeneration Using Electrospinning and Freeze Drying	Yonsei University Industry-Academic Cooperation Foundation	Ryu, Won Hyoung, ; Yang, Sung Yeun, ; Hwang, Tae Heon, ; Kim, Hyun Ryung	31-05-2013	12-08-2014	[122]
KR 20140147916	Method for Fabricating Composite Bone Hemostatic Material Composed of Chitosan Hydrogel and Electrospun Gelatin/BCP	Soonchunhyang University Industry Academy Cooperation Foundation	Lee, Byong Taek, ; Lee, Byoung Youl, ; Jang, Dong Woo, ; Kim, Bo Ram, ; Padalhin Andrew	19-06-2013	31-12-2014	[123]
KR 20170023636	Method of Fabricating Hydrogel Scaffold Using Electrospinning and Hydrogel Scaffold Manufactured By The Method	Gwangju Institute of Science and Technology	Yoon, Myung Han,; Kim, Dong Yoon	24-08-2015	06-03-2017	[124]

2.6 Future applications of electrospun hydrogels

The work in the past regarding electrospinning has mainly focused on determining suitable settings for electrospinning of various polymers and on understanding the important features of the preparation processes with the purpose of gaining control of electrospun nanofibre morphology, configuration, porosity and etc. [8]. In contrast, the recent investigations have focused on introducing different types of organic materials (i.e. plant-based materials, biologics, nanoformulated vitamins) into hydrogels via electrospinning. In addition, it is also worth to focus on investigating surface functionalisation with aminoalkyl groups, aligned electrospun nanofibres and the methods to scale up nanofibres production.

Animal-based, plant-based and natural nanofibres have been used for therapeutic approaches, yet plant-based materials are rarely used for electrospinning. Zhang et al. reported that there were only around a hundred publications on plant extract among all the journal articles (i.e. *Cissus quadrangularis* and Asian Panax Ginseng root) from the year 2008-2016 when compared with the total amount of papers published annually on electrospinning. The plant extracts have been found to give a positive effect on the hydrophilicity and mechanical properties of nanofibres. Besides, these are important for inducing osteogenic differentiation of MSCs, giving a significant osteocalcin gene expression in human osteoblast cells. The osteocalcin is fundamental for bone formation, as it involves in bone mineralization and calcium ion homeostasis. It is expected that additional research in the field of plant-based nanomaterials would be advantageous with respect to cost, accessibility, and other commercial issues [80].

Biopharmaceuticals are also known as biologics, which include not only recombinant therapeutic proteins but also naturally sourced proteins and peptides, live virus vaccines and blood components [125]. In the pharmaceutical industry, biopharmaceuticals have become one of the nascent and rapid growing sectors for drug delivery due to ongoing technological advancements and multi-disciplinary efforts of researchers in different fields, such as tissue engineering, molecular medicines and therapeutic field. As for 2014, there were more than 300 biopharmaceutical molecules have been approved for marketing with monoclonal antibodies (mAbs) leading the market growth, followed by recombinant proteins [126]. To date, there are currently more than 900 biologics in the development stage for the treatment and prevention of more than 100 diseases [127]. However, the major drawback in the development of biologics is in their formulation, as difficult to formulate into a suitable drug delivery system. Hence, the researchers have shown increased interest in pursuing methodologies that can shorten the window for both process development and manufacturing of biologics [128].

One of the successful examples is the invention of alginate slow-release antibacterial peptides microspheres for bone tissue engineering which patented by Fei and Yu in the year 2009. The peptides tend to have more effective potential to confront drug-resistant bacteria and biological membranes than any other antimicrobial agents. In detail, the peptides, beta-alexin and lactoferrin are attached on micropores of a bone grafting material to improve the anti-infection capability and maintaining antibiotic concentration for a long time in the scaffold [129]. Despite the micro-scale biologics interact well in bone, the development of nanostructure-mediated transport of biologics with specific controlled diameter and physiochemical properties is recently one aspect of particular interest in the biopharmaceutical field. Electrospraying is emerging as the most efficient technique for the preparation of nanoparticles/nanospheres biologics, which allows specific tailoring for drug delivery

applications. Nanoparticles are ideal candidates for these advanced requirements, and one of the easiest technique that can produce such nanostructured materials for delivering the biologics is the electrospinning process.

Furthermore, a potential approach to expand the effectiveness of electrospun hydrogels to bone tissue engineering is nanoformulated vitamins which are commonly used for delivery applications via oral, pulmonary, transdermal, and ocular routes of administration. However, vitamins have not yet been developed to undergo electrospinning or electrospraying, which might provoke a surge in research efforts to optimise nanoparticulate vitamin formulations in the coming future for both tissue regeneration and pharmaceutical delivery [25]. A recent work from Li et al. had incorporated vitamins A and E to electrospun gelatine nanofibres and cross-linked through a glutaraldehyde treatment as antibacterial wound dressing materials. It was found that both vitamins A and E can continually be released over more than 60 hours and greatly inhibited the growth of microorganisms, *E. coli* and *S. aureus* as well as promoting the adhesion and proliferation of L929 fibroblasts cells [130].

Despite the success of incorporation of antimicrobial agents in electrospun hydrogels (discussed in section 2.3.3), significant efforts have been made in the development of antibacterial electrospun hydrogels without the use of antibacterial agents to enhance the antimicrobial effect of scaffolds. The antibacterial activities of scaffolds have been attempted to obtain by surface functionalisation with aminoalkyl groups [131]. Roemhild et al. and Fernandes et al. had studied the electrospun PVA nanofibres containing amino-modified cellulose nanofibrils and nanostructured amino-modified bacterial cellulose membranes respectively. Both studies have reported the chemical grafting of aminoalkyl groups onto the surface of nanomaterial scaffolds can mimic the antimicrobial property of antimicrobial agents, exhibiting a high antimicrobial activity against *S. aureus, K. pneumonia, E.coli* and etc. [132], [133].

The natural compact bone tissue consists of highly oriented osteons which are aligned parallel to the long axis of the bone. Therefore, some researchers have started to investigate the effect of cells organise along the aligned electrospun nanofibres in a directional manner typified by the orientation of the osteons, suggesting that nanofibres orientation can impart a functional development on the cells [134]. Doustgani et al. and Jose et al. have reported that the mechanical response of uniaxially aligned electrospun nanofibres was significant, providing higher tensile strength and elastic modulus than randomly-oriented electrospun nanofibres. In the biological site, the alignment orientation of nanofibers provide a higher surface-to-volume ratio, which enhances the osteogenic differentiation of mesenchymal stem cells and drug release rate [135], [136]. Hence, the aligned electrospun nanofibres worked effectively than randomly oriented electrospun nanofibres for tissue-engineered scaffolds, especially in the field of artificial bone implant.

Electrospinning is one of the nanotechnology applications with the potential of industrial processing. The ability of electrospinning to process up to several litres of polymer solution under continuous operations led electrospinning research towards commercialisation. The efforts to scale up electrospinning from a laboratory scale to an industrial production level are needed. The researchers at North Carolina State University in the United States, have modified the traditional electrospinning setup, called “bowl edge electrospinning”. This apparatus can trigger multiple jets to deposit nanofibres onto a collector placed around the outside of the bowl.

The results of this study demonstrated the orders of magnitude to be approximately 40 times higher than that of traditional needle electrospinning (TNE) [26].Due to the advancement in the scaling-up technologies on electrospinning, it is projected that the existing nanofibre market worldwide may be around 400 million USD and will increase up to 1 billion USD by the year 2020 [137].

2.7 Conclusion

Nanomedicine and nanotechnology are transforming society's approach to medicine and biomedical science. However, as in every material science technology, there are three distinct interrelated aspects: the applications, the process and the material. The applications are diverse for electrospinning and include wound healing, bone regeneration and tissue engineering. Electrospun materials have remarkable properties in terms of biocompatibility and this combined with the developments in the electrospinning process will lead to a rapidly growing area. In particular, the reconstruction of defected bone tissues is supported by the polymer composites formed by the electrospinning process. As carriers for bone tissue regeneration, these nanofibrous hydrogel composites are made by ionic complexation in the absence of toxic crosslinking agents. The scaffolds and the by-products of these composites degradation are non-toxic to tissues and cells *in vivo*. Therefore, electrospinning has emerged as a favourable nanostructuring methodology.

The polymer blends are of particular significance for improving or modifying the physicochemical properties of constructed scaffolds. The current scientific approaches in developing electrospun nanofibre-reinforced hydrogels, electrospun hydrogels with biological electrospray cells and electrospun hydrogels with antimicrobial activity are likely to enable even closer matching of scaffolds to the *in vivo* environment. The electrospun nanofibre-reinforced hydrogels have the ability to resist the contractile force during the expansion process of newly grown tissues; the electrospun hydrogels with biological electrospray cells can promote cells evenly distributed along the nanofibres at the beginning of cultivation stage of the scaffolds to remain higher cell viability; and the electrospun hydrogels with antimicrobial activity can offer anti-inflammatory and anti-oxidant activity for implanted scaffolds.

However, further development cannot be carried out unless the challenges of electrospinning dealing with different parameters (discussed in section 4) for different types of polymers are resolved because different types of polymer blends have different kinds of requirements during electrospinning. It has been projected that the majority of the issues will be addressed in the near future. In addition, the ability to mass-produce hydrogels nanofibres with the bowl edge electrospinning method could possibly bring electrospun hydrogels into a new era.

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