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REVIEW

Electrospun scaffolds for bone tissue engineering

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Abstract Tissue engineering aims to regenerate native tissues and will represent the alternative choice of standard surgery for different kind of tissue damages. The fundamental basis of tissue engineering is the appropriate selection of scaffolds and their morphological, mechanical, chemical, and biomimetic properties, closely related to cell lines that will be seeded therein. The aim of this review is to summarize and report the innovative scientific contributions published in the field of orthopedic tissue engineering, in particular about bone tissue engineering. We have focused our attention on the electrospinning technique, as a scaffold fabrication method. Electrospun materials are being evaluated as scaffolds for bone tissue engineering, and the results of all these studies clearly indicate that they represent suitable potential substrates for cell-based technologies.

Keywords Bone tissue engineering · Electrospinning · Nanofibers · Scaffold · Stem cells

Introduction

Tissue engineering is a field that encompasses many disciplines, combining principles of life sciences and engineering, having the purpose to develop biological substitutes for restoring, maintaining, or improving tissue functions. The basis for new tissue formation is given by the appropriate choice and evaluation of the interactions between suitable biomaterials for scaffolds fabrication and cells seeded therein [1, 2]. Scaffolds are generally regarded as basic elements of engineering of living tissues. In fact, these allow cell adhesion, growth, and differentiation in order to promote the regeneration of extracellular matrix (ECM) and eventually biologically functional tissue. Electrospinning has been used for the fabrication of nanofibrous scaffolds, and electrospun biomaterials ease and support cell–matrix and cell–cell interactions [3]. Moreover, to simulate the natural cellular environment, the diameter of electrospun fibers can be modulated in size to mimic fibrils of the native ECM [4].

At present, autograft represents the gold standard for bone repair after injuries or diseases. Despite surgical techniques may provide enough bone for grafting procedures, there are limitations in its use related to the availability of sufficient supply and patient morbidity. A major effort has been then produced to find alternatives to autologous bone grafts, such as allografts (either as fresh frozen, freeze-dried, or demineralized bone matrix), growth factors, degradable and non-degradable polymers, bioactive glass, and calcium phosphates [5].

In this contest, bone tissue engineering has become a rapidly expanding research field: the practices of this research area usually involve the use of biomaterials for scaffolds fabrication, in combination with biological cues and tissue cells [6].

The aim of this review is to underline the relevance of using electrospun scaffolds for tissue engineering in the orthopedic field, particularly for bone tissue regeneration. In particular, an introductive section reports details

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regarding the electrospinning technique and a brief description of the biology of bone. Then, fundamentals of bone tissue engineering are presented, reporting scaffold characteristics and cells sources for bone tissue engineering applications. At last, a summary of the electrospun scaffolds fabricated for bone tissue engineering applications is reported.

Electrospinning technique

Electrospinning is a widespread technique for obtaining microfibers and nanofibers from polymeric solutions or melts, by the application of a high electric field between a syringe tip charged positively and a grounded or negatively charged collector. The fibrous structures (*meshes*) produced by this technique present good mechanical properties and a very high specific surface area, which are some of the ideal properties for biomaterials in tissue engineering applications [7, 8]. Electrospun polymeric fibrous meshes provide a suitable environment for cell attachment, and their fabrication and scaling-up is easy and low-cost. Systems based on synthetic and natural polymers provide additional benefits because of their adjustable mechanical properties and the possibility to perform surface functionalization, protein coatings or chemical grafting of specific signaling molecules. In the field of tissue engineering, these electrospun fibers can be used alone or in combination with other materials and particles in order to improve cells hostage [2].

Even though the theory behind electrospinning or electrospraying has been known for over a century—it was actually first noticed by Rayleigh in 1882—electrospun polymeric nanofibers have become a subject of great interest only in the last years, and actually, the word "electrospinning" was introduced in 1994 [9].

Typically, the main elements of an electrospinning setup are a syringe that contains the polymeric solution, having the function of a reservoir; a volumetric pump that regulates the flow rate at which the solution is supplied; a power supply with a voltage range of several tens of kV, with its positive pole connected to the syringe tip; and lastly, a target for the collection of fibers that can be grounded or negatively charged (Fig. 1).

A number of alternative configurations and evolutions of the standard setup have been developed recently [2, 10–21], but we can summarize that the electrospinning process is affected by three classes of parameters that consequently influence the final shape of the fibers. The first class consists of solution properties, like volatility; in fact, depending on solvent volatility, fibers could be characterized by pores on the surface (Fig. 2). Other parameters are polymer concentration, solvent polarity, solution conductivity, and viscosity.

The second class is constituted by processing parameters as applied voltage, feed rate of polymeric solution, temperature, distance between the tip and the collector, the type of collector, needle diameter, and the configuration of the nozzle. An example of the influence of these parameters on fiber morphology is reported in Fig. 3.

Lastly, the third class includes ambient parameters such as temperature, humidity, pressure, and type of atmosphere [2, 22, 23].

One of the advantages related to the use of electrospinning technique is the capability to adjust and control the size of the produced fibers, since nanofibers closely simulate the scale of fibrous proteins, such as collagen, found in the natural ECM. This property is fundamental, as it has been proven by previous studies that electrospun constructs topography has an important function in cell attachment and proliferation [24, 25]. Also, nanofibrous non-woven meshes are ideal for cell adhesion, as a greater part of the surface is available for cell interaction [26, 27]. Moreover, the porosity of these biomaterials eases nutrient transport. Also, newer techniques allow to engineer nanofiber meshes to achieve openings of a desired size to allow cell infiltration and angiogenesis, thus promoting the incorporation of the nanofiber scaffold with the surrounding bone.

Usually, polymers processed by electrospinning are described by three distinct categories of characterizations: physical, chemical and mechanical (Table 1).

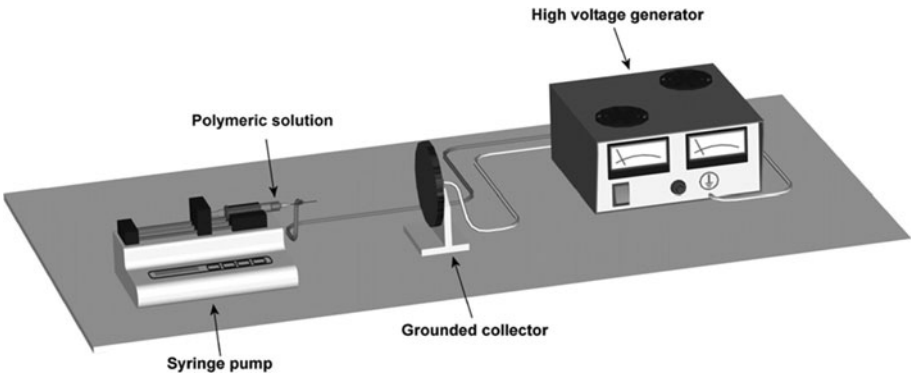

Fig. 1 A scheme of the standard electrospinning setup, having a planar target for fiber collection

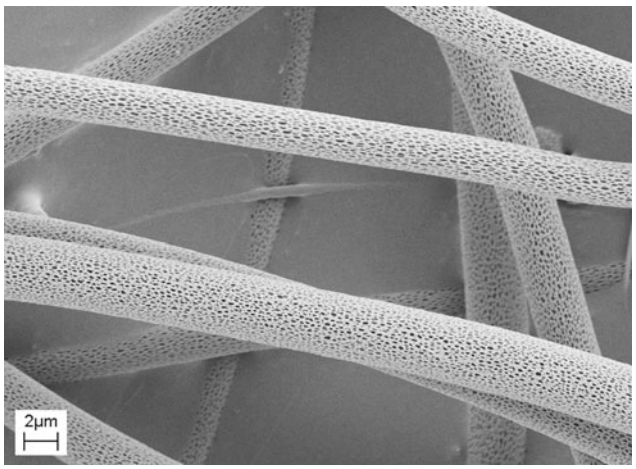

Fig. 2 Electrospun poly(L-Lactide) (PLLA) microfibers, fabricated in our laboratory, characterized by pores on the fibers surface

Bone tissue engineering

In order to make the best choice among different type of biomaterials who best fit the original bone properties, it is essential to briefly summarize the biological and biomechanical characteristics of bone, which is a highly organized and specialized connective tissue [28].

Bone is constituted of several arrangements of material structures at different length scales, whose synergic work is essential to perform different mechanical, biological, and chemical functions, such as structural support, protection and storage of healing cells, and mineral ions homeostasis. In order to describe the hierarchical architecture of bone and understanding relationships between structures at various levels of hierarchy, it is possible to identify three levels of structures: (1) the nanostructure, such as non-collagenous

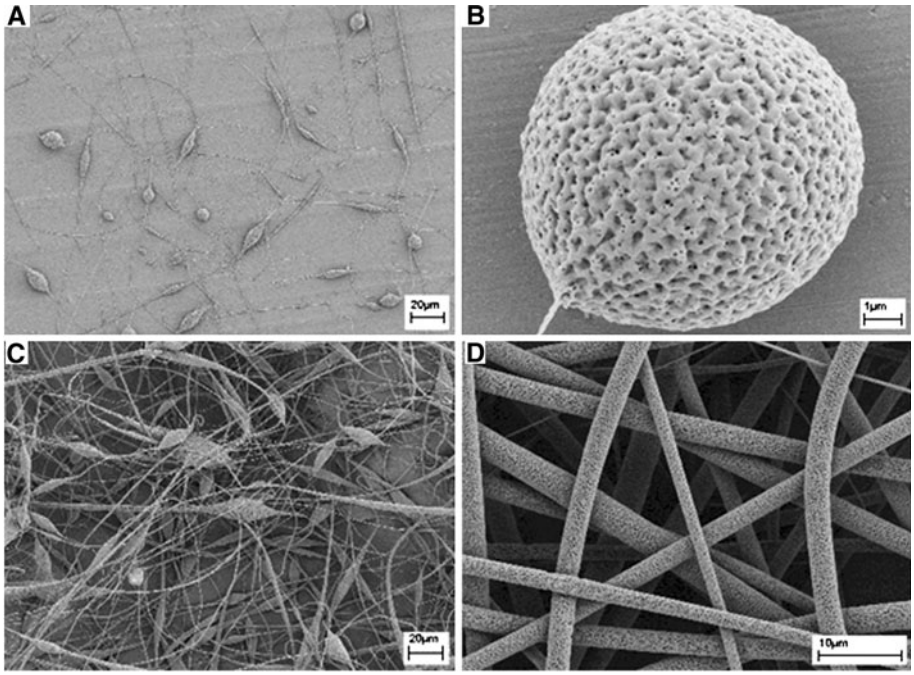

Fig. 3 Variation in electrospun PLLA fiber morphology, from beads without fibers (**a** and zoom in **b**), fibers with beads (**c**), and fibers without beads, obtained by varying only polymeric solution feed rate

Table 1 Characterization of polymeric electrospun fibers [2]

Physical properties	Chemical properties	Mechanical properties
Fiber diameter	Fiber composition at the molecular level	Fiber resistance to loads by application of tensile strength
Diameter distribution		
Fiber orientation		
Fiber morphology		
Analysis	**Analysis**	**Analysis**
Electron Microscopy (SEM)	Fourier Transform Infrared (FT-IR)	Tensile test
Field Emission Scanning Electron Microscopy (FE-SEM)	Nuclear Magnetic Resonance (NMR)	Nanoindentation
Transmission Electron Microscopy (TEM)		Bending tests
Atomic Force Microscopy (AFM)		Resonance frequency measurements
		Microscale tensile tests

organic proteins, fibrillar collagen, and embedded mineral crystals; (2) the microstructure, such as lamellae, osteons, and Haversian systems; (3) the macrostructure, such as cancellous and cortical bone. The assembly of these three levels of oriented structures forms the heterogeneous and anisotropic features typical of bone tissue. Natural bone is a composite material composed of organic compounds (consisting for approximately 90% by type I collagen, and in small quantities type V collagen, and the remaining 10% consisting of non-collagenous proteins, like osteocalcin, osteopontin, osteonectin and fibronectin, and other substances) reinforced with inorganic compounds [constituted by minerals, primarily crystalline hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$]. Collagen fibers, surrounded and infiltrated by minerals, are the most prevalent structures presented at nanoscale level. At a macroscopic level, bone shows the capability of self-repairing and remodeling under excessive mechanical stresses. The remodeling process is based on the synergic action of three type of bone cells: osteoblasts (bone-forming cells), osteocytes (bone-maintaining cells), and osteoclasts (bone-resorbing cells) [29].

Osteoblasts are situated on the surfaces of bone and regulate the formation, the organization, and the subsequent mineralization of bone extracellular matrix. Osteoblasts are activated by growth factors such as insulin-like growth factors I and II secreted by osteoclasts and/or osteocytes to deposit calcium-containing minerals. Osteoclasts adhere to the bone surface via integrins, which are specialized cell surface receptors [30]. They are activated by growth factors, cytokines, and proteins present in the bone matrix in order to reabsorb old bone. Osteocytes regulate new bone formation by modulating osteoblasts differentiation from non-calcium-depositing to calcium-depositing cells through the secretion of growth factors such as insulin-like growth factor I and transforming growth factor β [31].

Scaffold characteristics for bone tissue engineering applications

Besides the choice of biomaterials among polymers (synthetic or natural), ceramics (in particular minerals having a biomimetic aim) or hybrid (mixing the two previous types), another essential factor to fabricate a successful engineered construct is the evaluation of natural bone tissue structure in the scaffold design. For what concerns an ideal scaffold for bone tissue engineering applications, it is possible to list some fundamental requirements as biocompatibility, osteoconductivity, osteoinductivity, osteogenicity, and mechanical match between implanted scaffold and surrounding tissues; in fact, the scaffold would have similar mechanical properties to the neighboring tissue at the implant site to prevent mechanical mismatch that can lead to stress shielding and bone resorption [32].

Scaffolds are considered as a fundamental part for the success of engineered constructs. In fact, dependently upon its composition, properties, and particular functionalization, a scaffold will be more suitable for in vitro culture and in vivo tissue formation. The main characteristics for scaffolds intended for use in bone tissue engineering are an adequate porosity that allows cell adhesion, migration and proliferation within the biomaterial, and good mechanical properties for temporarily supporting and guiding new tissue formation.

Three-dimensional (3D) scaffolds have a variety of functions: they provide the necessary support for cell adhesion, growth, and differentiation and define the overall shape of a bone tissue-engineered transplant [33]. For what concerns bone regeneration, two essential topics, as osteoconduction and osteoinduction, are at the basis of the fabrication of constructs for bone tissue engineering. Osteoconduction supports ingrowth of capillaries and cells from the host into a three-dimensional structure to form bone. An osteoconductive material guides repair in a location where normal healing will occur if left untreated. Osteoinduction is defined as the ability to cause pluripotential cells, from a non-osseous environment to differentiate into chondrocytes and osteoblasts, culminating in bone formation. An osteoinductive material allows repair in a location that would normally not heal if left untreated [34]. The fabrication of a variety of scaffolds has been facilitated by recent technological advances and has allowed us to modulate pore configuration and nanostructure. The approach of mimicking the properties of the native bone ECM among which the composition, morphological traits, and mechanical function has promoted the design of matrices suitable for the recruitment of osteoprogenitor/stem cells. The advantages of electrospun nanofibrous structures were first achieved with degradable polymers that stimulate cells into osteogenic pathways aided by well-controlled differentiation signals.

Cells for bone tissue engineering

Another fundamental step for the success of tissue engineering approaches is represented by the choice of the cell line for seeding on the scaffolds. The success of the final construct, comprising both scaffold and cells, is dependently from both these elements and their interactions, as reported in the next paragraph.

Several types of cells are used for scaffold seeding in this field, comprising bone cells (like osteoblasts) and, recently, stem cells. The use of stem cells represents a recent strategy in the field of bone tissue engineering, the innovation is represented by the evaluation of cell adhesion, proliferation, and mainly differentiation on the scaffold, having also the function to stimulate cell differentiation toward desired phenotype. The main cell line used for bone tissue

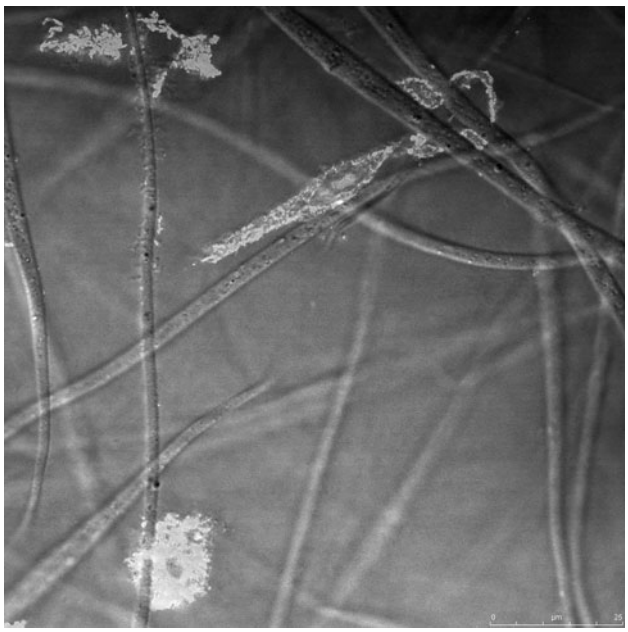

Fig. 4 Confocal microscopy. hMSCs seeded on PLLA fibrous scaffold

engineering applications are mesenchymal stem cells (MSCs). In Fig. 4 are reported, as example, human MSCs seeded on PLLA fibrous scaffolds. The harvesting of mesenchymal stem cells from a donor can be achieved through tissue particularly from selected tissues that have the function of stem cells reservoirs. Adult bone marrow contains MSCs that contribute to the regeneration of mesenchymal tissues, such as bone, cartilage, muscle, ligament, tendon, adipose tissue, and stroma [35]. In fact, under appropriate stimulation, given by the scaffold, MSCs undergo osteogenic differentiation through a well-defined pathway, acquiring osteoblastic markers and secreting extracellular matrix and calcium crystals [36]. In vitro and animal implantation studies have suggested that the population is either multipotent MSCs or mixtures of committed progenitor cells, each with a restricted potential [37]. However, clinical translation, in particular in older patients often affected by fractures and non-union problems, is prevented by the low population of MSCs in bone marrow.

Another cell type is represented by blood mesenchymal precursor cells (BMPCs), used in tissue engineering for bone regeneration, after the discovery of the presence of this kind of cells in the circulation of healthy patients, reporting also that they are easy to culture and expand in vitro [38]. It is reported that BMPCs are able to differentiate toward bone cells, in fact, after adding osteogenic supplements into the culture, fibroblast formation is inhibited, and the BMPCs then assume the more cuboidal shape of osteoblasts, as confirmed by alkaline phosphatase (ALP) and osteocalcin staining [39, 40]. More recently, MSCs with osteogenic potential have been isolated from a wide variety of tissue types, including adipose tissue, umbilical cord blood, amniotic fluid, and fetal blood [41, 42].

Cell-scaffold interactions

A greater understanding of the complex interactions between cells and their environment has been acquired due to numerous studies in the last decades. Typically in the range of 10–100 μm in diameter, cells react to *stimuli* induced from the macroenvironment down to the molecular level. These cell–environment interactions are attained through an array of receptor systems situated on its outer membrane, these respond to adjacent cells, secreted signaling molecules and ligands in the ECM that surrounds the cells. The extracellular matrix plays an important role in these interactions, as it can influence the cells by both the physical arrangement of fibers and the chemical cues. Each tissue often has its own physical structure, composition, and arrangement of the ECM. For example, the fibrils found on the skin are randomly oriented, while those that make up the ECM of tendons are parallel and aligned. Therefore, the goal of mimicking the natural ECM is a challenging task for researchers. Recently, the development of new processing methods has allowed to synthesize scaffolds matching some of the properties of tissue-specific ECMs.

The ECM is composed mainly of two classes of macromolecules: fibrous proteins and polysaccharide chains known as glycosaminoglycans (GAG). In order to mimic this arrangement, the electrospinning process has been used to produce nanofibrous scaffolds that simulate ECM structure. The wide range of materials that can be electrospun to form nanofibers is another key advantage of the electrospinning process; in fact, synthetic non-biodegradable polymers, biodegradable polymers, natural polymers, composites, and even ceramic precursors have been so far processed by electrospinning [43].

After these introductive considerations, it is important to underline that the main aim of tissue engineering is the replacing of the damaged or diseased tissue or the facilitating of tissue regeneration. This is achieved by applying a combination of bioactive molecules, cells, and biomaterials; however, these materials must be safe, non-immunogenic, non-toxic, and biodegradable. Moreover, they must display a high surface area with porous structure as well as other appropriate mechanical properties [44]. Tissues are made of cells with insoluble materials present between the cells known as ECM, which is composed of various biomacromolecules secreted by the cells themselves. These ECM provide structural support, tensile strength for tissues, and substrate for cell adhesion, migration, and finally regulates cellular differentiation [45]. Among the

properties of the ECM, cell adhesion plays a crucial role in the displacement of individual cells into three-dimensional tissues. Three types of proteins are responsible for cell adhesion: (1) cell adhesion molecules/adhesion receptors, that usually consist of transmembrane glycoproteins, such as cadherin at cell–cell contact and integrin at matrix contact, (2) ECM proteins, large fibrillar glycoproteins, such as collagen, that are situated in the ECM and that are linked tightly to adhesion receptors, and (3) cytoplasmatic plaque/peripheral membrane proteins, such as catenins of the cell–cell contact sites, that are associated with adhesion receptors at the membrane intracellular surface to form a link between the adhesion systems and the actin cytoskeleton. Other than regulating cell adhesion, these proteins also have a key role in the transduction of signals from the cell surface.

Receptors of cell adhesion are usually transmembrane glycoproteins, which task is to mediate binding interactions at the extracellular surface, in order to determine the specificity of cell–cell and the cell–ECM type recognition. These receptors include members of the cadherin, integrin, selectin, immunoglobulin, and proteoglycan (syndecans) superfamilies. Typically, the ECM proteins are large glycoproteins: these include several types of collagens, fibronectins, laminins, and proteoglycans. These proteins assemble into fibrils or other complex macromolecular arrays [46]. Cell interactions with nanofibers are similar to cells/ECM interactions in vivo. Fibronectin, laminin, and vitronectin, as well as other adhesion proteins, are soluble in body fluids such as blood plasma; they can also be found in the cell culture medium, in which fetal bovine serum is frequently used. The same adhesion proteins act as ligands for cell integrins, once spontaneously adsorbed on the nanofibrous surface, upon contacting the nanofibers. The fabrication and the design of tissue-engineered substrates will in future require surfaces that either naturally adhere to ECM molecules or surfaces that have high affinity binding sites for these cell-associated receptors, for the reason that this is the natural tissue organization displayed by bone, cartilage, skeletal muscle, tendons, and ligaments [47].

A great amount of physical properties, besides fiber size, could be analyzed and optimized, gaining a critical role in tissue engineering and drug delivery applications. These properties include the development of beaded and core–shell structures, which can potentially act as drug reservoirs with the capability to provide sustained release, inhibiting the initial burst release. These nanofibrous mats can be loaded with a greater quantity of drugs compared with other scaffold typologies and have the potential to overcome mass transfer limitations presented by other polymer drug delivery systems, because of their high surface/volume ratio. Moreover, when optimizing electrospinning processes, chemical properties such as degradation rate and mechanical properties should also be taken into consideration in addition to the above-described physical properties [22].

Electrospun biomaterials for bone tissue engineering

Electrospun polymeric scaffolds have been produced using homopolymers, copolymers, polymeric blends, and, more recently, developing organic-inorganic hybrid materials (Table 2).

Electrospinning of polymers

Among available biopolymers, poly(ε-caprolactone) (PCL), poly-L-lactide (PLA), poly-glycolide (PGA), and their copolymers, have been the most extensively studied nanofiber systems for the regeneration of tissues, including bone [48].

PCL was first suggested to be a degradable nanofiber matrix for bone regeneration [49] that demonstrated good support of rat bone marrow stromal cells and in vitro bone matrix formation (synthesis of collagen I and deposition of calcium phosphate mineral) at 4 weeks. Moreover, a cell–nanofiber construct implanted in rat omenta for 4 weeks revealed the formation of collagen I and bone-like mineralization, highlighting its potential in bone tissue engineering [50]. This ability could be influenced by fibers diameter, since hMSCs attached and spread rapidly on nanofibrous scaffolds in comparison with microfibrous PCL, and the cells on nanofibrous PCL were found to differentiate into osteoblast lineage and subsequently mineralize upon addition of in vivo osteogenic regulators [51]. Ruckh et al. reported and investigated the capability of PCL electrospun scaffold to enhance the osteoblastic behavior of marrow stromal cells (MSCs) in osteogenic media compared with smooth PCL electrospun substrates. Results indicated that nanofiber scaffolds supported greater cell adhesion and viability compared with control surfaces. Also, in osteogenic conditions, MSCs cultured on nanofiber scaffolds displayed higher levels of alkaline phosphatase activity and calcium phosphate mineralization compared with controls [52].

Badami et al. [53] observed that PLA electrospun nanofibers with variable sizes affected osteoblast-like cells (MC3T3-E1) response in vitro. Interestingly, when an osteogenic medium was used, a higher cell density was observed on PLA nanofibers than on flat PLA.

Natural polymers like collagen have also been used for scaffold fabrication by electrospinning technique. Since collagen type I is the major organic component of bone ECM, Shih et al. developed nanofibrous electrospun mats

Table 2 Electrospun polymeric scaffolds for bone tissue engineering

Biomaterial	Solvent	Average fiber diameter	Cells seeded on the scaffold	Ref
PCL	Chloroform	400 ± 200 nm	MSCs derived from the bone marrow of neonatal rats	[49, 50]
PCL	Chloroform/methanol	7.1 ± 0.38 μm 277 ± 54 nm	hMSCs	[51]
PCL	Chloroform/methanol	372 ± 179 nm	MSCs were isolated from male Wistar rats	[52]
PLA/PEG	Chloroform/methanol	0.171 ± 0.066 μm	MC3T3-E1 mouse calvaria-derived	[53]
Collagen type I	1,1,3,3-hexafluoro-2-propanol	50–200 nm 200–500 nm 500–1,000 nm	Bone marrow hMSCs	[54]
Collagen type I	1,1,1,3,3,3-hexafluoro-2-propanol/ 2,2,2-trifluoroethanol	Nanometer range (value not specified)		[55]
Collagen type I	1,1,3,3-hexafluoro-2-propanol	495 ± 78 nm		[56]
Collagen type II	1,1,1,3,3,3-hexafluoro-2-propanol	180 ± 69 nm		[57]
Silk fibroin	Aqueous solution of 0.02 M Na_2CO_3, rinsed with ultrapurified water (UPW), and dissolved in 9 M LiBr. (addition of PEO)	530 ± 100 nm	Human mesenchymal stem cells (hMSCs)	[58]
Silk fibroin	Aqueous solution of 0.02 M Na_2CO_3, rinsed with ultrapurified water (UPW), and dissolved in 9.3 M LiBr. (addition of PEO)	700 ± 50 nm	Human bone marrow stromal cells (BMSCs)	[59]
Silk fibroin	$CaCl_2$/ethanol/H_2O for silk fibroin sponge, then formic acid	From 183 ± 13 to 810 ± 67 nm depending on solution concentration	Mouse osteoblast-like cells MC3T3-E1	[60]
Silk fibroin	$CaCl_2$/ethanol/H_2O for silk fibroin sponge, then formic acid	Range: 200–400 nm	MC3T3-E1	[61]
Chitosan	Trifluoroacetic acid	390 nm		[63]
Chitosan	1,1,1,3,3,3-hexafluoroisopropanol and methylene chloride	200 nm	Human osteosarcoma cell line MG63	[64]
PCL/collagen	1,1,1,3,3,3-hexafluoroisopropanol/ chloroform	513 ± 83 nm	Pig bone marrow mesenchymal cells (pBMMCs)	[65]
PLGA/collagen	1,1,1,3,3,3-hexafluoro-2-propanol	Range from 240 to 386 nm		[66]
PLGA/collagen/CaP	Hexafluoro-2-propanol; acetic acid, $CaCl_2$, H_3PO_4	Range from 97.3 to 353.8 nm		[67]
PLA/DBP	2,2,2-trifluoroethanol	Range from 300 to 700 nm	Human mandible-derived mesenchymal stem cells (hMSCs)	[70]
PLLA/HA	Dichloromethane/tetrahydrofuran	Range from 3.5 to 2.3 μm	Mouse fibroblasts (L929) for cytotoxicity and mouse pre-osteoblastic cells MC3T3-E1 for attachment and proliferation tests	[71]
PLGA/MWNTs/HA	Dimethyl formamide/ trichloromethane	Range from 600 to 1,400 nm	Rat Bone Mesenchymal Stem Cells (BMSCs)	[72]
HA/chitosan (addition of UHMWPEO)	Acetic acid/dimethyl sulfoxide	214 ± 25 nm	Human fetal osteoblast cells (hFOB)	[73]
Collagen/HA/chitosan (addition of UHMWPEO)	Acetic acid/dimethyl sulfoxide	180 ± 31 nm	Human fetal osteoblasts (hFOB)	[74]

Table 2 continued

Biomaterial	Solvent	Average fiber diameter	Cells seeded on the scaffold	Ref
PLLA/collagen/HA	1,1,1,3,3,3-hexafluoro-2-propanol	860 ± 110 nm (PLLA), 845 ± 140 nm (PLLA/HA), 310 ± 125 nm (PLLA/coll/HA)	Human fetal osteoblasts (hFOB)	[75]
Silicate ($58SiO_2{\cdot}38CaO{\cdot}4P_2O_5$)/collagen type I (mixed with poly-vinyl-butyral)		320 ± 87 nm	Human osteoblastic cells	[76]
Bioglass ($58SiO_2 38CaO4P_2O_5$)/PLLA	Tetrahydrofuran	320 ± 87 nm	Murine-derived pre-osteoblast MC3T3-E1	[77]
Gelatin-siloxane (3-glycidoxypropyl trimethoxysilane)	Formic acid and $Ca(NO_3)_2$	Nanometric range dependently to solution viscosity	Bone marrow-derived mesenchymal stem cells (BMSCs)	[78]
PCL/silica sol (one step acid-catalyzed hydrolysis of tetramethylorthosilane (TMOS)) (pure PCL as control)	1,1,1,3,3,3-hexafluoro-2-propanol (HFP)	350 ± 189 nm (pure PCL: 552 ± 295 nm)	MC3T3-E1pre-osteoblast cells	[80]
Silk/PEO/BMP-2, Silk/PEO/nHAP, Silk/PEO/nHAP/BMP-2 (control: silk/PEO)	Aqueous solution of 0.02 M Na_2CO_3, rinsed with ultrapurified water (UPW) and dissolved in 9.3 M LiBr. (addition of PEO)	Range from 510 to 590 nm	Human bone marrow-derived mesenchymal stem cells (hMSCs)	[79]

of collagen type I, having different diameters and providing good substrate conditions for BMSCs adhesion and growth [54]. Zeugolis et al., conversely proposed the method of collagen coating on electrospun nanofibers [55, 56]. Nevertheless, cross-linked electrospun collagen is believed to have a stronger potential as a nanofibrous substrate for cells to anchor and replicate, as well as in differentiation toward the osteogenic lineage with consistent mineral deposition [57].

Silk fibroin has been selected to be processed by electrospinning for scaffolds creation, due to its useful properties for tissue engineering, as cell compatibility, biodegradability, and minimal inflammatory reaction [58]. In fact, electrospun nanofibers of silk with sizes ranging from 500 nm to 1 μm were able to support the initial attachment and growth of BMSCs [59] and osteoblastic cells [60]. In a recent study, Park et al. developed three-dimensional electrospun silk fibroin (ESF) scaffolds with controllable pore size. The goal of this study was to evaluate ESF scaffolds with pores for bone regeneration via in vitro and in vivo studies, with a comparison to a commercially available porous three-dimensional polylactic acid (PLA) scaffold. Results highlighted the suitability of ESF scaffold as bone substitute for bone regeneration [61].

Chitosan, a deacetylated derivative of chitin, largely used in orthopedic applications [62], is considered relatively difficult to be processed by electrospinning, in comparison with other natural polymers, mainly due to limited solvents and high viscosity at low concentrations [63]. Electrospun chitosan nanofibrous meshes have been developed as a dental barrier membrane to selectively guide hard tissues within the periodontal pocket. The in vivo result at 4 weeks of implantation using the membrane within a critical-sized defect of a rabbit calvarium demonstrated almost full coverage of the defect and new bone formation [64].

Synthetic polymers can also be blended with natural ones for scaffolds fabrication with the aim to improve cell compatibility. Ekaputra et al. [65] reported the fabrication of electrospun PCL and collagen composite blend scaffolds that resulted in modulation of the attachment and proliferation of bone marrow mesenchymal cells of pigs. Moreover, they reported the development of long bone analogs, by wrapping osteogenic cell sheets around the PCL/collagen meshes to form hollow cylindrical cell–scaffold constructs. Culturing these constructs under dynamic conditions enhanced bone-like tissue formation and mechanical strength of the construct.

Aligned nanofibrous blends of PLGA and collagen with various PLGA to collagen ratios (80/20, 65/35 and 50/50) were fabricated by electrospinning and characterized for bone tissue engineering. Morphological characterization showed that the addition of collagen to PLGA resulted in narrowing of the diameter distribution and a reduction in

average fiber diameter [66]. The addition of collagen to PLGA also improved the mineralization process, making this kind of scaffold more suitable for bone regeneration [67].

Hybrid scaffolds with synthetic polymers

For the most part, bone ECM contains calcium phosphates mineral phases that require a mineralization step that is essential in the bone regeneration process. The existence of bone bioactive inorganic components within polymeric scaffold generally favors calcium phosphate mineralization followed by an osteogenic differentiation process. Therefore, several studies have focused on introducing a range of inorganic phases within the polymeric nanofibers with the ultimate aim of achieving both bone-specific bioactivity and improved mechanical properties [68]. Moreover, the combination of degradable polymers with bioactive inorganic materials during electrospinning is considered an interesting and smart way to produce nanofibers with the appropriate properties targeted for bone regeneration. In fact, the inorganic phase may act to improve the biological properties of polymeric nanofibers, such as cell compatibility, osteogenic differentiation, and bone matrix calcification, and the introduction of a polymeric phase should provide some degree of mechanical flexibility. In addition, the fact that there is no need for thermal treatment because of the binding polymer matrix is another attractive point for its use in drug delivery systems [69].

Ko et al. developed nanofibrous organic and inorganic composite scaffolds containing nano-sized demineralized bone powders (DBPs) with PLA by using an electrospinning process for engineering bone. In order to assess their biocompatibility, in vitro osteogenic differentiation of human mandible-derived mesenchymal stem cells (hMSCs) cultured on PLA or PLA/DBP composite nanofibrous scaffolds was examined. Results showed better early mineralization of hMSCs cultured with osteogenic supplements on PLA/DBP nanofibrous scaffolds compared with PLA alone. Then, in vivo osteoconductive effect of PLA/DBP nanofibrous scaffolds was further investigated using rats with critical-sized skull defects. Microcomputerized tomography revealed that a greater amount of newly formed bone extended across the defect area when PLA/DBP scaffolds were used [70].

Another recent study dealt with the fabrication of electrospun PLLA fibrous mats with and without the addition of hydroxyapatite (HA) particles in amounts of 0.25 or 0.50% w/v. The presence of HA particles at 0.50% w/v concentration not only promoted the attachment and the proliferation of mouse pre-osteoblastic cells (MC3T3-E1) but also increased the expression of osteocalcin mRNA, and the extent of mineralization after the cells had been cultured on the scaffolds for 14 and 21 days, respectively [71].

A recent interesting study reported the fabrication, by electrospinning technique, of biocomposite scaffolds of PLGA, multiwalled carbon nanotubes (MWNTs), and hydroxyapatite (HA) nanoparticles. The structure, surface morphology, and some properties of these PLGA/MWNTs/HA composites were evaluated. Cultured bone marrow-derived mesenchymal stem cells (BMSCs) were seeded on PLGA/MWNTs/HA scaffolds, and their attachment and proliferation were investigated. The average diameter of PLGA/MWNTs/HA fibers increased with increasing HA content from 0.5 to 1.5% in the composites. Results indicated good biocompatibility of the scaffolds, and that the attachment and proliferation of BMSCs were significantly increased in PLGA/MWNTs/1.0%HA and PLGA/MWNTs/1.5%HA scaffolds compared with the sham PLGA control [72].

Natural polymers have been also used in combination with mineral compounds in order to obtain biomimetic electrospun constructs. In particular, Zhang et al. [73, 74] reported the fabrication of nanofibrous hydroxyapatite/chitosan scaffold with the addition of poly(ethylene oxide) (PEO); in vitro experiments, performed by seeding human fetal osteoblast (hFOB) cells on these scaffolds, showed that after 15 days of culture, the incorporation of hydroxyapatite nanoparticles into chitosan nanofibrous scaffolds led to significant bone formation compared with pure electrospun chitosan scaffolds.

Prabhakaran et al. fabricated composite electrospun nanofibrous scaffolds, and in particular poly-L-lactide (PLLA), PLLA/HA and PLLA/collagen/HA scaffolds. In vitro analysis showed osteoblasts adhering and growing actively on PLLA/collagen/HA nanofibers with enhanced mineral deposition of 57% compared with bare PLLA/HA nanofibers, suggesting a synergistic effect of the presence of collagen and HA in the PLLA/collagen/HA scaffold [75].

Recently, bone bioactive inorganic materials (i.e., calcium phosphates and bioactive glasses/glass ceramics) have been used to obtain biomimetic electrospun constructs.

Nanofibrous inorganic materials have been usually investigated in the role of nanofillers for the production of nanocomposite scaffolds with degradable polymers [76, 77]. In particular, electrospun nanofibrous bioactive glass was well homogenized with collagen or a PLLA solution with the aim to produce uniform and homogenous scaffolds, for improving bone-bioactivity of the organic phase, osteogenic differentiation, and cell mineralization. Positive results, related to coupling bone-bioactivity of the inorganic component with shape-formability of the organic phase, led to the consideration that this kind of approach is very promising for further applications [76, 77].

The matching of sol–gel processing and electrospinning technique was also used by Ren et al. [78] for gelatin/siloxane hybrids fabrication. Bone marrow-derived mesenchymal stem cells (BMSCs) were seeded on these hybrid constructs. Results indicated that, by varying the viscosity of the gelatin/siloxane precursor solution, it was possible to regulate the porous structure and fiber size of the gelatin/siloxane fibrous mats. Additionally, gelatin/siloxane fibrous mats biomimetically deposited apatite in a simulated body fluid (SBF) and stimulated BMSCs proliferation and differentiation in vitro.

Another innovative approach to design suitable electrospun fibrous scaffolds for tissue regeneration is to add biofunctionality onto the surface of nanofibers, because cells first recognize the surface of the material, which mostly regulates their response. Moreover, nanofibers, which are surface-conjugated or internally incorporated with proteins, genes, and growth factors, are an elegant way of using nanofibrous matrices in drug delivery systems. An example of a combination of different types of polymers, organic and inorganic biomaterials, and growth factors to obtain an electrospun scaffold suitable for bone tissue regeneration was presented by Li et al. [79]. Scaffolds were composed of silk, PEO, nanoparticles of hydroxyapatite (HA), and bone morphogenetic protein 2 (BMP-2). Scaffolds were seeded with human bone marrow-derived mesenchymal stem cells (BMSCs), and the experiment was led on silk/PEO/BMP-2, silk/PEO/HA, silk/PEO/HA/BMP-2 scaffolds. Electrospun silk/PEO and solvent-cast silk/PEO were used as controls. Results showed that silk-based scaffolds supported BMSC growth and differentiation toward osteogenic outcomes. The scaffolds with co-processed BMP-2 supported higher calcium deposition and enhanced transcription levels of bone-specific markers with respect to the controls, indicating that these nanofibrous electrospun silk scaffolds were an efficient delivery system for BMP-2. The coexistence of BMP-2 and HA in the silk fibers resulted in the highest calcium deposition and upregulation of BMP-2 transcription levels when compared with the other systems.

Conclusions

Tissue engineering approaches, aiming to regenerate native tissues, will represent the alternative choice of standard surgical intervention for several kinds of tissue damages and injuries. In particular, the fundamental basis of tissue engineering is the appropriate choice of the scaffold, in virtue of its morphological, mechanical, chemical, and biomimetic properties closely related to cell lines to be used.

The aim of this review is to summarize and report the innovative scientific contributions published in the field of tissue engineering in orthopedics, in particular for what concerns bone tissue engineering. We have chosen to focus on the electrospinning technique, since electrospun scaffolds are able to mimic extracellular matrix components much closely when compared with the conventional techniques. Moreover, electrospun fibrous mats present several advantages, such as high surface-area-to-volume ratio, the possibility to regulate mats porosity, and the ability to manipulate nanofibers composition in order to get desired properties and function. For what concerns tissue engineering applications, it is proved that nanofibers have a positive effect on cell cultures, in particular they affect cell adhesion, proliferation, and differentiation, mainly due to the high surface area and enhanced porosity.

In conclusion, at present time, electrospun nano- or microfibrous matrices are being evaluated as scaffolds for bone tissue engineering, and the results of all these studies clearly indicate that electrospun scaffolds represent suitable potential substrates for bone regeneration.

Conflict of interest It is certified that there is no actual or potential conflict of interest in relation to this article by any of the authors.

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