Electrospun ceramic and ceramic-polymer composite nanofibers for bone tissue engineering applications

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The electrospinning technique and the nanofibrous matrices have gained amazing popularity, mainly due to the structural similarity to the tissue extracellular matrix (ECM), the high porosities and surface area-to-volume ratios, the processing availability to a wide range of materials, as well as simple set-up and operation at low cost. Among many possible applications of electrospun nanofibers, such as multifunctional membranes, composite reinforcement and structures for nanoelectronic machines, biomedical applications, such as preparation of scaffolds used for bone tissue engineering have become one of the most interesting areas in electrospinning field. This review summarizes the electrospinning technique and provides a brief overview of current state-of-the-art research designing and using electrospun ceramic and ceramic–polymer composite nanofibers for bone tissue engineering.

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1. Introduction

The electrospinning technique, which was invented about 100 years ago, has attracted more attention in recent years due to its possible biomedical applications [1]. The process of electrospinning was first patented by Cooley [2] and Morton [3] in 1902, and its further developments toward commercialization were made by Formhals between 1934 and 1944 [4, 5].

Electrospinning is a method in which materials in solution are formed into nano- and micro-sized continuous fibers. The elements required for electrospinning include a polymer source, a high-voltage supply, and a collector [6].

The principle of electrospinning is that an electric field is used to overcome the surface tension of a polymer solution to shoot a jet of liquid out of a needle toward a conducting collector [7]. The volatile solvent evaporates in the air leaving behind, under the right conditions, a polymer fiber with a diameter that can range from tens of nanometers to microns [8]. Many parameters affect this process including polymer properties, solvent properties, distance from needle to collector, applied electric voltage, polymer solution or melt flow rate, needle-to-collector distance, solution concentration, and solvent type [9, 10].

A schematic description of the electrospinning process is shown in Fig. 1 [11]. As the metal capillary is charged by a high-voltage bias, polymer solution or melt is ejected from the tip forming the Taylor cone. The Taylor cone is the foundation for the jet of material that whips down toward a collection area. This motion is driven by bending instabilities in the jet as well as effects of evaporation and solidification of the solvent [12].

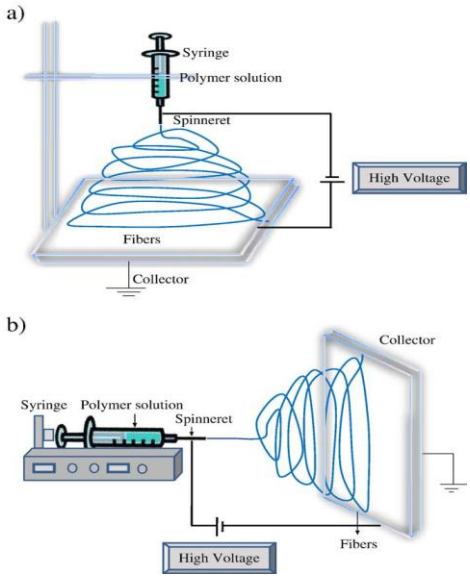

Fig. 1. Schematic diagram of set up of electrospinning apparatus (a) typical vertical set up and (b) horizontal set up of electrospinning apparatus. Reprinted with permission from [11]. @2013 Elsevier B.V.

The polymer jet spirals downward, while bending and stretching, creating ultrathin fibers. Processing conditions, such as working distance, voltage, and concentration, can be easily adapted to allow for the fabrication of different fiber diameters and orientation. Orientation is changed simply by using different collection devices such as dual rings, rapidly rotating drums, etc. [13, 14].

The collection area is either grounded or supplied with a negative charge to further attract the solution [12]. The collectors may have a range of shapes or configurations. The vast majority of electrospinning systems use a single collection plate to collect the fibers, while grounding a single rotating cylindrical collector can collect fibrous mats, orient the fibers in a thin sheet of fabric, or make a tubular (bilayered) construct. Oriented fibers can also be obtained using dual, grounded collection plates, or rings. Collection systems may also be a hydrogel, water, a confluent layer of cells, or part of a living body [14].

The electrospinning process has been extensively applied to create nanofiber scaffolds for cardiovascular [15], urologic [16] and bone tissue engineering applications [17], among others.

According to literature, bone tissue engineering approaches involve the use of scaffolding materials in combination with tissue cells and biological cues [18].

The ultimate aim in scaffold design involves the manufacture of a proper structure that can replace natural extracellular matrix until the host cells can resynthesize and repopulate a new natural matrix [19]. Currently, the application of nanotechnology in bone tissue regeneration is a challenge for the fabrication of novel bioartificial bone grafts. These nanostructures are capable of mimicking natural extracellular matrix with effective mineralization for successful regeneration of damaged tissues [20]. Nanofibrous scaffolds have proved to be effective and convenient in providing mechanical support and osteoconductivity to the growing cells in bone regeneration, thus being considered an alternative route to conventional autogenic and allogenic treatments for bone defects [21].

Many processing methods, such as drawing, self assembly, template directed synthesis, phase separation, and electrospinning are already developed to fabricate micro or nanoscale fibrous scaffolds [22].

The electrospinning process and the nanofibrous matrices thus fabricated have gained amazing interest, mainly due to the structural similarity to the tissue extracellular matrix (ECM), the high porosities and surface area-to-volume ratios, the processing availability to a wide range of materials, as well as simple set-up and operation at low cost [18, 14]. One known limitation on the electrospun scaffolds for bone tissue engineering is that scaffolds are sheets with thicknesses up to 500 μm [23].

In the bone reconstruction area, the electrospun nanofibers have also attracted remarkable attention aimed at identifying suitable material compositions and exploiting them into electrospinning [18]. As the bone-associated cells and their progenitor/stem cells show initial responses in a similar manner to those in other tissues, which are anchorage-dependent, the nanofibrous substratum may provide favorable conditions for cell anchorage and growth. In tandem with the initial cell responses, further osteoblastic differentiation and mineralization have also been reported to be regulated in a positive manner on nanofibrous surfaces compared to a dense substrate of polymers [24, 18].

Many types of materials have been proposed for bone tissue engineering, most of which are biocompatible and biodegradable polymers that follow previous applications in surgical procedures or other biomedical applications. Materials can be categorized as (i) natural polymers, (ii) synthetic polymers or (iii) ceramics, glasses and composites [20].

The following sections provide the current literature available on the development and applications of electrospun materials (ceramic and ceramic-polymer composite nanofibers) in bone tissue engineering.

2. Ceramic nanofibers

Tissue engineering scaffolds are often made of biodegradable polymeric materials, natural or synthetic [25-30]. However, the use of biodegradable polymer scaffolds is challenging for the regeneration of load-bearing bones, due to their low mechanical strength. Many efforts have been invested to reinforce the biodegradable polymers with a biocompatible inorganic phase e.g. hydroxyapatite. Although brittle, scaffolds fabricated from inorganic materials such as calcium phosphate based bioceramics and bioactive glass can provide higher mechanical strength than polymeric scaffolds [31].

The inorganic materials which have received most attention for bone repair applications are calcium phosphate-based bioceramics, such as HA, $Ca_{10}(PO_4)_6(OH)_2$, $Ca_3(PO_4)_2$, and biphasic calcium phosphate (BCP), b-tricalcium phosphate (b-TCP), a mixture of HA and b-TCP, composed of the same ions as bone, [31, 32-35]. These materials are biodegradable, osteoconductive, bioactive, biocompatible. Several studies have also reported that calcium phosphate ceramics have osteoinductive properties. In combination with bone marrow derived mesenchymal stem cells (MSC), calcium phosphate ceramics provide the required cues for stem-cell-induced bone tissue formation [36].

HA resorbs very slowly compared with b-TCP and undergoes little conversion to a bone-like material after implantation [31]. However, HA scaffolds often have higher strength than b-TCP scaffolds, for the same porosity. The use of BCP with different HA to b-TCP ratios allows manipulation of the degradation rate, as well as other properties [31].

As a major mineral component of human hard tissues, HA possesses excellent biocompatibility with bones, teeth, skin, and muscle, both *in vitro* and *in vivo*.

Recently, Franco et al. [37] reported the production of hydroxyapatite (HA) sub-micron fibers by combining a non-alkoxide sol–gel system and electrospinning, using cheap precursors. The HA fibers produced are viable to be used either in Biomedicine in the production of matrices for Tissue Engineering or in Biotechnology in membranes for ionic permutation [37]. Xiaoshu et al. electrospun hydroxyapatite fibrous networks with average fiber diameters between 200 nm and 800 nm. Varying the polymer molecular weight (polyvinyl alcohol (PVA)

polymer) and the sol volume fraction were obtained many structures including non-woven mats of solid or micro-porous hydroxyapatite fibers and highly porous scaffolds. These structures can have many potential uses in the repair and treatment of bone defects, drug delivery and tissue engineering [38]. Wu et al. have electrospun hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) fibers. They used a precursor mixture of $Ca(NO_3)_2 \cdot 4H_2O$ and $(C_2H_5O)_3PO$ with a polymer additive, followed by a thermal treatment. The randomly oriented HA fibers had an average diameter of 25 mm and they were up to 10 mm in length and the hydroxyapatite grain size was ~1 μm in the HA fibers [39].

Along with pure HA, its fluoridated form, fluor-hydroxyapatite [FHA; $Ca_{10}(PO_4)_6(OH,F)_2$] has gained its importance in the area of dental restoration because of its specific biological benefits, namely the protection against the formation of dental caries and the stimulation of bone cell responses and matrix synthesis. Moreover, FHA possesses the highest thermal and chemical stability among the apatite groups ever known, thus it has high potentials in the biomedical field [40].

Nanofibers of HA and its fluoridated form FHA were synthesized by Hae-Won Kim and colleagues [40] based on their sol-gel precursors using an electrospinning process. The fiber diameter was exploited within arange from several micrometers to hundreds of nanometers by controlling the processing parameters, particularly the concentration of the sol. The FHA nanofiber produced had higher chemical stability than the HA equivalent, and released fluorine efficiently following the dissolution profile. The HA and FHA nanofibers produced was considered to find potential applications in the biomaterials and tissue engineering fields [40].

According to literature cells are sensitive to microscale and nanoscale topography [41]. Osteoblasts or mesenchymal stem cells exhibit enhanced osteoblastic differentiation, when are cultured on titanium substrates, which have an inherent TiO_2 ceramic layer on the surface. Although not bioresorbable, TiO_2 could serve as an attractive substrate for bone tissue engineering due to its good biological performance. Whether surface structure also plays a role when cells are growing on TiO_2 nanofiber meshes is not known [41]. Recently, Wang et al. electrospun pure TiO_2 nanofiber meshes in order to obtain different surface microroughness and nanofiber diameters. The results showed that cells grew throughout the entire surfaces and with similar morphology in all groups. Cell number was sensitive to surface microroughness, whereas cell differentiation and local factor production was regulated by both surface roughness and nanofiber diameter. These results indicate that scaffold structural cues alone can be used to drive cell differentiation and create an osteogenic environment without the use of exogenous factors [41].

Glass–ceramics and bioactive glass are also used in bone repair applications and are being developed for tissue engineering applications. Glass–ceramics are crystallized glasses, whereas, bioactive glass has an amorphous structure, consisting of a composite of a crystalline phase and a residual glassy phase. Over the last two decades, there has been heightened interest in the science and biomedical application of bioactive glass [31].

Generally speaking, bioactive glasses and ceramics are able to promote a strong mechanical bond to bone as well as to soft tissues. This behaviour was first observed in a family of glasses belonging to the SiO_2–Na_2O–CaO–P_2O_5 system, investigated by Hench and co-workers since 1970s [42]. The glass called 45S5 Bioglass, available commercially in particulate form under the names Perioglass ® and Novabone® is the best-known and widely investigated glass of this group, due to its amazing bioactivity. Its clinical use includes bone grafting in orthopaedic, dental, maxillofacial and otolaryngological applications [43]. Nevertheless, the diffusion of BG in medical devices is severely compromised by its intrinsic brittleness, which limits the application of BG to non-load bearing situations. For this reason, BG is often applied as a coating on metal or plastic substrates, used as a ceramic scaffold (often coated with a biodegradable polymer, for example via impregnation methods), or introduced in polymer-based composites [44].

Kim et al. produced a bioactive glass in a nanofibrous form using an electrospinning process. The nanofiber possessed excellent bioactivity and osteogenic potential in vitro [45]. Shinji Sakai et al. prepared silicate fibers using the same technology and via the sol-gel process were evaluated as scaffolds for bone tissue engineering. They found that human osteoblastic MG63 cells successfully adhered on individual silicate fibers, and proliferated on them. In an apatite-formation ability study, spherical particles covered the fibers after soaking in simulated body fluid for 7 days. Furthermore, Energy dispersive X-ray analysis revealed that Ca/P atomic ratio of the particles was similar to that of human bone [46].

However, the clinical applications of ceramics (such as tricalcium phosphate and HA) have had limited use because of their brittleness and difficulty in shaping [36, 47, 48]. Moreover, post heat-treatment can limit their drug delivery potential. Thus, future knowledge and advanced technology need to be developed in order to overcome the disadvantages of bone-bioactive inorganic nanofibers as well as to identify appropriate uses as bone tissue engineering matrices [18].

3. Ceramic – polymer composite nanofibers

Natural bone is a highly complex composite, mainly constituted of molecules of type I collagen and biological apatite, where collagen serves as the matrix for cell growth and tissue repair, while apatite serves as the inorganic phase to improve mechanical strength and regeneration of bone [49].

The medical use of one synthetic or natural polymer is limited because degradation products of synthetic polymers may be harmful to newly grown tissue, whereas the poor stability of natural polymers precludes their use

alone. The main component (~60%) of natural bone is the mineral hydroxyapatite (HA) embedded in a collagen matrix; therefore, it is considered as an essential constituent for bone tissue engineering. However, using bioceramic nano-hydroxyapatite alone as scaffold material is not possible because of its brittle nature which has limited the scope of clinical applications and hence more research needs to be conducted to ameliorate the poor mechanical properties. To circumvent these limitations presented by bioceramics and biopolymers, ceramic/polymer composite materials could represent an alternative solution, since composite materials benefit from the distinct properties of the constituent phases [44, 50].

Thus, combining synthetic polymers or biopolymers with bioceramic materials such as $CaCO_3$, tricalcium phosphate (TCP), nanoparticles of hydroxyapatite (nHA), Fe_3O_4, carbon nanotubes, through electrospinning techniques is considered a fascinating and reasonable way of creating nanofibers with the suitable properties targeted for bone tissue engineering regeneration. The ceramic phase incorporation may enhance the biological properties of polymeric nanofibers, such as cell compatibility and bone forming process, involving the osteogenic differentiation and calcification of bone matrix. In addition, given that the brittleness of ceramic materials is a major limitation to their use as appropriate cell substrates, the introduction of a polymeric phase should provide some degree of mechanical flexibility [18, 51, 52]. However, only limited studies have been made on the electrospun fabrication of nanofibrous matrices composed of composite. This is because it is more easily to obtain a nanofibrous network from individual polymers than from the composites [53].

Until now, many natural and synthetic polymers for composite fibers have been used in the field of bone tissue regeneration. Some natural polymers were gelatin [54], chitosan [36], silk [55], collagen [56, 53, 48, 57] but the inferiority in mechanical properties has limited their use alone [58]. Apart from natural polymers, synthetic polymers have been also used in composite fibers during the course of electrospinning. The most frequently used synthetic polymers for composites are polycaprolactone (PCL), poly (L-lactic acid) (PLLA) and poly (lactic-glycolic acid) (PLGA) [36].

Poly (e-caprolactone) (PCL) biopolymer has been broadly used in biomedical applications, due to its mechanical strength, flexibility, biocompatibility and biodegradability [59]. Furthermore, the PCL small-sized fibers have been shown that favor cell anchorage and proliferation [51]. However, in the field of bone regeneration the application of PCL fibers is limited due to their low stiffness, hydrophobic nature and relatively low bioactivity.

The hybridization of PCL with inorganic substances is considered one of the most attractive methods in order to overcome some of the PCL's properties drawbacks [59]. Hybrid nanocomposite hard tissue scaffolds produced by electrospinning of a natural or a synthetic polymer along with nanoparticles of $CaCO_3$ (CC), hydroxyapatite (nHA), or tricalcium phosphate (TCP) have been shown to enhance the cell attachment kinetics. Such studies include those with PCL–CC nanocomposites and PCL–nHA [51]. For example, Fujihara et al. [60] fabricated polycaprolactone (PCL) nanofibers which contain $CaCO_3$ nanoparticles for guided bone regeneration application. It was found that increasing the $CaCO_3$ nanoparticles concentrations, greater mineralization occurred on the surface of attached cells cultured on nanofibrous composite membrane. In a previous work, Bioshop et al. and Wutticharoenmongkol et al. [61, 62] reported co-electrospinning of spherical HA particles with polycaprolactone (PCL) and polyvinyl pyrrolidone (PVP), respectively. In an attempt to devise a near-perfect scaffold for growing MSCs for bone tissue engineering, Doustgani's [63] group developed aligned electrospun PCL/nHA/PVA nanocomposite scaffold that is potentially a promising biomaterial for bone tissue engineering (Fig. 2).

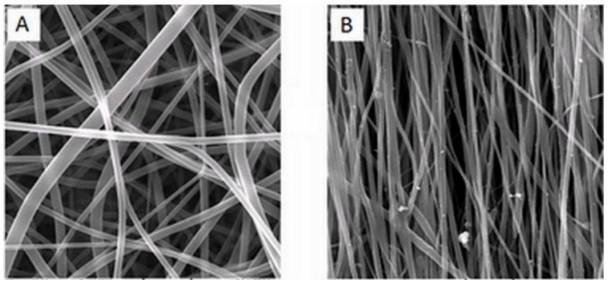

Fig. 2. SEM images of nanofibrous scaffolds (a) random, (b) aligned. Reprinted with permission from [62]. @2013 Nanomedicine Journal.

Bianco et al. [64] incorporated synthesized HA nanoparticles into PCL electrospun fibers with a diameter of ~1.5 μm. The content of Ca-deficient nanohydroxyapatite ranged between 2 and 55 wt. % and the results indicated that electrospun PCL scaffolds may provide adequate supports for murine embryonic stem cell proliferation in a pluripotent state, and that the presence of Ca-deficient nanohydroxyapatite within the mat does not interfere with their growth. Recently, Patlolla and co-workers [36] electrospun composite scaffolds of PCL and 20%HA/80% b-TCP in order to fabricate mechanically flexible composite scaffolds with uniform fiber morphologies, relatively large pore sizes for cell penetration and bone tissue in-growth, a maximum concentration of ceramic to achieve improved bioactivity and a homogeneous dispersion of the ceramic in the fibers for improved molecular interaction and mechanical properties. For electrospinning, PCL was dissolved in either methylene chloride (Composite–MC) or a combination of methylene chloride (80%) and dimethylformamide (20%). The results demonstrated that the solvent combination used plays a significant role in determining its properties. The results suggested that the

new Composite–MC scaffold obtained may be the more promising scaffold for bone tissue formation in vivo.

It has also been reported that the coating of polymers with a calcium phosphate layer ensure structures with adequate bone-bonding or osteoconductive properties, and help in tailoring the degradation and resorption of the polymer matrix and improving cell anchorage, proliferation and differentiation [51]. For instance, Mavis et al. [51] fabricated PCL–calcium phosphate composite nanofiber mats with high osteoinductivity by electrospinning of PCL, followed by the biomimetic coating of the electrospun mat in modified 10SBF-like solutions. It was found that biomimetic apatite-coated PCL nanofibers form a convenient physical and biological environment supporting and inducing functions of the preosteoblastic cells to form new bone [51].

Recently, Lee and co-workers illustrated another strategy to fabricate nano-fibrous PCL–silica xerogel hybrid membranes by combining the electrospinning technique and the sol–gel process [59]. The developed hybrids in the form of membrane showed great potential as bone regenerative materials.

Biodegradable poly-DL-lactide (PLA) is also considered a widely used polymer in bone tissue engineering because of its good biocompatibility, mechanical properties, adjustable degradation rate, and ease of processing [65, 52].

According to literature [66], electrospun fibrous nanocomposites of poly(DL-lactic acid) (PDLLA) and hydroxyapatite (HA) combine the osteoconductivity and bone-bonding ability of HA, possessing great potential for engineering functional bone-like substitutes. Thus, in order to mimic the biopolymer/HA composition as well as improve the mechanical properties of electrospun scaffolds, Li et al. and Kim et al. [55, 67] prepared spindle-shaped HA nanoparticles and co-electrospun them with silk fibrils and PLLA, respectively. Basing on the same concept, Chen et al. and Ito et al. electrospun poly(L-lactic acid) (PLLA) and poly(3-hydroxybutyrate–co-3-hydroxyvalerate) (PHBV) scaffolds and grew HA coatings on the scaffold surface using a biomimetic coating method [68, 69]. Prabhakaran and co-workers [48] fabricated poly-L-lactide(PLLA), poly-lactide/nanohydroxyapatite (PLLA/HA) and PLLA/collagen/ HA (PLLA/coll/HA) nanofibers by electrospinning and evaluated the potential of using these substrates for bone tissue regeneration. They concluded that electrospun biomimetic PLLA/coll/HA nanofibers have great potential for adhesion, proliferation and mineralization of osteoblasts and are promising biocomposite scaffolds suitable for bone tissue regeneration [48]. Recently, Peng et al. [70] successful prepared highly porous hydroxyapatite (HA)/poly(L-lactide) (PLLA) (20/80 wt.%) nanofibrous scaffolds by incorporating needle-shaped nano- or micro-sized HA particles into PLLA nanofibers using electrospinning. These HA/PLLA nanofibrous scaffolds were found to be good candidates for bone tissue engineering [70]. In another study done by Chen and co-workers [66], fibrous HA/PDLLA composites were formed from in situ growth of HA within ultrafine fibers with different HA inoculations. The non-stoichiometric HA particles existed on the fiber surface was able to maintain desirable cell substrate interactions, provide favorable conditions for cell proliferation and stimulate to allow osteogenic differentiation.

Compared with pure PLLA scaffolds, a greater density of viable cells was seen on the nanostructured biocomposite scaffolds of poly(l-lactide) (PLLA) blended with collagen (coll) or hydroxyapatite both fabricated by electrospinning (Fig. 3)[57].

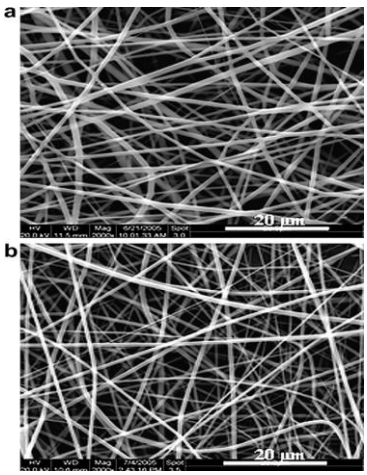

Fig. 3. SEM images of electrospun fibers PLA-F (a) and AHC-PLA-F (b). Reprinted with permission from [57]. @2013 Elsevier B.V.

Finally, composite nanofiber meshes which contain PLA polymers were successful produced by Obata's group [71] and Shao's group [52] through electrospinning techniques and were proposed for use in bone tissue engineering.

Poly(butylene succinate) (PBSU), as a novel biomaterial, has shown good biocompatibility and adequate biodegradability. However, some limitations such as the lack of bioactivity have been found considering the use of it in bone tissue engineering applications. In order to overcome these problems, a promising approach was proposed Zhang's group [72] for preparation of composite scaffolds including a biodegradable polymeric phase and a bioactive ceramic phase. Specifically, they electrospun pure poly(butylene succinate) (PBSU) fibers, PBSU/12.5% β-$CaSiO_3$, and PBSU/25% β-$CaSiO_3$ composite fibers [72].

As mentioned earlier, many researches have investigated Hap-polymer composite scaffolds for bone regeneration, due to the fact that the presence of Hap into nanofibrous polymer matrix not only mimics the natural bone structure but also can enhance the mechanical properties and biological response of the scaffolds. Thus, several different electrospun nanocomposite fibers, such as Hap/ chitosan, Hap/gelatine [54], silk/Hap [55], and

triphasic Hap/ collagen/PCL [56], PHBV/Hap [69] had been designed and explored for potential bone regeneration applications. Most recently, Gouma et al. [73] successfully manufactured cellulose acetate – nano-hydroxyapatite composite scaffolds for bone tissue engineering application by employing the electrospinning technique (Fig. 4).

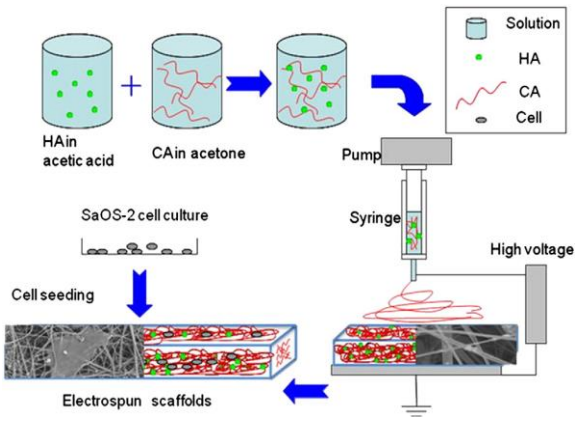

Fig. 4. Schematic of the electrospinning and cell culture process used to obtain 3D cell seeded scaffolds. Insets are SEM micrographs revealing cross sectional views of the scaffolds before and after the cell seeding process. Reprinted with permission from [73] @2013 Elsevier B.V.

The obtained nano scaffolds were proved to promote favorable adhesion and growth of osteoblasts as well as to stimulate the cells to exhibit functional activity of bone cells. Work done by Chen's group [74] fabricated HAp nanorods through a simple precipitation method and then incorporated into PVP nanofibers to form HAp nanorods/PVP composite nanofibers through electrospinning. Cytotoxicity experiments indicated that the HAp fabricated scaffold had good biocompatibility. In vitro experiments showed that mesenchymal stem cells could attach to the HAp fabricated scaffold after culture for 24 h. In a previous study, Madurantakam and co-workers [75] electrospun different amounts of nHA (0, 10, 25 and 50% by wt. of polymer) in combination with polydioxanone (PDO) or poly(glycolide: lactide) to generate composite scaffolds. Finally, Jegal et al. [53], illustrated the fabrication of functional nanofibrous materials composed of gelatine–apatite–poly(lactide–co-caprolactone) (PLCL) using an electrospinning process. Overall, the results demonstrate that the obtained nanofibrous matrix developed here could potentially be useful in the regeneration of hard tissues, such as a guided bone regeneration membrane in periodontology.

All these studies contributed to the field of bone tissue engineering with interesting concepts/ideas and impressive experimental results.

4. Conclusions

Electrospinning has gained popularity within the past years, due in large part to the rising interest in nanoscale properties and technologies. One attractive feature of electrospinning is the simplicity and relatively inexpensive nature of the setup.

The process is of interest for scaffold fabrication, as the resulting fibers have similar diameters to that of certain ECM microstructures, particularly the higher ordered collagen microfibrils. Using innovative collectors and spinning techniques, scaffolds with aligned fibers, different compositions, improved mechanical properties, varying degradation rates, or functional moieties can be produced. The flexibility of the electrospun fibers, due to the very high aspect ratio (length/diameter), is also beneficial as they allow the seeded cells to remodel their surroundings. The size of scale is important in this instance; instead of many cells adhering to one fiber, one cell may adhere to multiple fibers. It is evident that the response of many cells is significantly different when subjected to nano and microscale structures and topographies. One line of reasoning for the enhanced response of cells on nanoscale substrates is the dimensional similarities with some of the structural components found in the native ECM and or basement membranes. However, much of the underlying mechanisms for this enhanced response remain unknown and the interactions with nanostructured scaffolds have not yet been fully realized. However there is a clear need for further research on the subject to investigate the effects of nanofiber architecture and interfacial properties on cell behavior, which has just started to be realized.

In addition it remains difficult to create clinically relevant 3D constructs beyond a relatively 2D mat. For bone tissue engineering, a large 3D scaffold may be required. While new processing techniques have shown promise to increase the size and porosity of electrospun scaffolds, more work needs to be done to promote the architectural control. Having pores large enough for not only cell penetration, but vascular ingrowth is imperative for a vascularized tissue such as bone. It is also crucial to develop a strategy capable of producing fibers with a diameter identical to that of native ECM fibers (a diameter less than 100 nm, preferably in the range of 10–50 nm) while maintaining high porosity for cell infiltration and migration.

Therefore, for bone tissue engineering applications the selection of the raw material is crucial for the development of an ideal scaffold—the materials and manufacture technique are to be chosen in accordance with the specific tissue application.

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