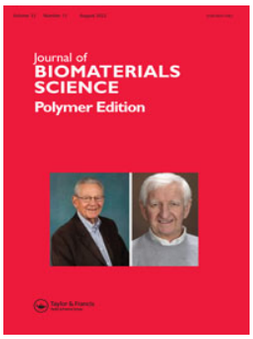

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REVIEW ARTICLE

Synthetic electrospun nanofibers as a supportive matrix in osteogenic differentiation of induced pluripotent stem cells

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ABSTRACT

Continuous remodeling is not able to repair large bone defects. Bone tissue engineering is aimed to repair these defects by creating bone grafts. To do this, several technologies and biomaterials have been employed to fabricate an *in vivo*-like supportive matrix. Electrospinning is a versatile technique to fabricate porous matrices with interconnected pores and high surface area, replicating *in vivo* microenvironment. Electrospun scaffolds have been used in a large number of studies to provide a matrix for bone regeneration and osteogenic differentiation of stem cells such as induced pluripotent stem cells (iPSCs). Electrospinning uses both natural and synthetic polymers, either alone or in combination, to fabricate scaffolds. Among them, synthetic polymers have had a great promise in bone regeneration and repair. They allow the fabrication of biocompatible and biodegradable scaffolds with high mechanical properties, suitable for bone engineering. Furthermore, several attempts have done to increase the osteogenic properties of these scaffolds. This paper reviewed the potential of synthetic electrospun scaffolds in osteogenic differentiation of iPSCs. In addition, the approaches to improve the osteogenic differentiation of these scaffolds are addressed.

Abbreviations: IPSCs: induced pluripotent stem cells; ECM: extracellular matrix; ESCs: embryonic stem cells; ICM: inner cell mass; MSCs: mesenchymal stem cells; BM-MSCs: bone marrow mesenchymal stem cells; SCPL: solvent casting and particulate leaching; PCL: poly ε-caprolactone; PLLA: poly(l-lactic acid); PLCL: poly(lactide-co-ε-caprolactone); SF: Silk fibroin; PES: polyethersulfone;

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PVDF: poly(vinylidene fluoride); HA: hydroxyapatite; PRP: platelet-rich plasma; GO: graphene oxide; bFGF: basic fibroblast growth factor; CTGF: Connective tissue growth factor; PHBV: poly(3-hydroxybutyrate-co-3-hydroxyvalerate); DEX: dexamethasone; βGP: β-glycerophosphate; OC: osteocalcin; BSP: bone sialoprotein; MAPK: mitogen-activated protein kinase; ERK: extracellular related kinase; BMP: bone morphogenetic proteins; PEMF: pulsed electromagnetic field; FDA: Food and Drug Administration; FGF-2: fibroblast growth factor 2; BCM: bone conditioned media; IGFs: insulin-like growth factors; VEGF: vascular endothelial growth factor

1. Introduction

Bone is a dynamic organ with self-healing properties. The healing is mediated through naturally continuous bone remodeling however, this remodeling is not able to repair large bone defects [1]. The first-line treatment strategy is to use autologous graft or allograft bone substitutes. The use of these natural bone grafts requires intensive surgical interventions. In addition, this strategy is limited due to the infection transmission and graft rejection (following allograft transplantation) as well as severe pain experience. As a result, researchers have tried to develop new bone substitutes [2]. Fabricating polymeric scaffolds through tissue engineering may be a solution [3]. Bone tissue engineering is aimed to induce bone repair or generate alternative bone grafts. To do this, several technologies, biomaterials, and stem cells have been used to fabricate an *in vivo*-like bone grafts. By introducing induced pluripotent stem cells (iPSCs) by Yamanaka and Takahashi in 2006 [4], many researchers have used this type of stem cells for bone and other organs engineering. Among the technologies, electrospinning is considered as a versatile technique to fabricate matrices with interconnected pores and high surface area, replicating *in vivo* extracellular matrix (ECM) [5]. Various natural and synthetic polymers have been used as a building block for these matrices [6]. As many people suffer from osteoporosis worldwide, fabricating bone grafts *via* tissue engineering may decrease the rising concern of the aging population.

Tissue repair and regeneration are the main goals of tissue engineering for degenerative diseases. To do this, there is a need to replace damaged or lost cells in the affected tissue. In this regard, stem cells have a central role in tissue engineering. They can proliferate and differentiate into desired functional somatic cells. Several types of stem cells have been used in bone tissue engineering. Table 1 summarizes the main characteristics of stem cells from various sources. Embryonic stem cells (ESCs) are known for their high proliferation rate and pluripotency. They

Table 1. A comparison look at iPSCs, ESCs, and MSCs.

iPSCs	ESCs	MSCs
Pluripotent	Pluripotent	Multipotent
High proliferation rate	High proliferation rate	Lower proliferation and differentiation capacity
Generated from many types of cells	Requires an embryo destruction	Immune-modulatory function
Enables autologous transplantation	Elicit immune responses due to allograft transplantation	Enables autologous transplantation
May develop teratoma	May develop teratoma	Requires invasive technique for stem cell isolation

differentiate into almost all somatic cells from mesodermal, ectodermal, and endodermal lineages. ESCs are derived from the inner cell mass (ICM) of blastocysts. Therefore, there is a need for embryo destruction to obtain ESCs which is the major limitation of these stem cells [7,8]. After transplantation, they may also elicit immune responses as they are considered as an allogenic graft [9]. In addition, they may cause teratoma formation as they have highly active c-myc and oct-4 proto-oncogenes expression [10]. Mesenchymal stem cells (MSCs) are multipotent stem cells that are found in almost all organs. They are first isolated from bone marrow mesenchymal stem cells (BM-MSCs). Their intrinsic immune-modulatory property makes them appropriate stem cells in tissue engineering [11]. However, their proliferation and differentiation capacity are not as high as pluripotent stem cells [12,13]. Therefore, they may hardly be expanded and trans-differentiated into somatic cells. IPSCs have advantages of a high proliferation rate and differentiation capacity. They are obtained through somatic cell reprogramming and have a great function in disease remodeling, drug delivery, and cell-based therapy [14]. They are produced by the introduction of pluripotent reprogramming factors including oct-4, nanog, or other alternative factors. They could be either introduced through a viral vector/plasmid or used as recombinant proteins [15,16]. IPSCs have been used in basic research to evaluate the cellular and molecular biology of stem cells. IPSCs seem to be an acceptable tool for drug screening and toxicity assay. It is also widely used for disease modeling [17]. Finally, they could be used as a therapeutic option in cell-based therapy. In the field of tissue engineering, iPSCs have been differentiated into many cell types including cardiomyocytes [18], insulin-producing cells [19], chondrocytes [20], osteo-like cells [21], and so on. Following iPSCs generation, stem cell characterization should be performed to validate their stemness (pluripotency) and integrity (by karyotyping, DNA fingerprinting, gene expression analysis, epigenetic evaluation, etc.) [14, 22,23]. IPSCs have an advantage over ESCs as they could be obtained from patients, reprogrammed *in vitro*, and used as autologous grafts in cell-based therapies. Many studies have used iPSCs in bone tissue engineering [24,25]. They could be differentiated *in vitro* and transplanted into the affected tissue [26]. Furthermore, iPSCs could be used in combination with scaffolds as bone substitutes for bone repair and regeneration (Figure 1) [27]. The potential use of iPSCs in tissue engineering has been reviewed in many studies [28–30]. Studies by Rana et al. [25] and Fliefel et al. [31] have reviewed the potential use of iPSCs in bon regeneration as a cell-based therapy. A recent study by Hashemi et al. [32] has reviewed some of the scaffold properties in differentiation of stem cells into osteo-like cells. Here, we focused on the synthetic polymers, their properties as well as their potential modifications prior to iPSCs seeding and osteogenic differentiation.

2. Preparation of a 3D microenvironment for stem cells

Fabricating a biomimicking scaffold is a challenging step in tissue engineering. Several techniques have been developed to fabricate such scaffolds [33]. The properties of these techniques are compared in Table 2 [34]. In the self-assembly process, non-covalent intermolecular forces are formed between the components in a reaction.

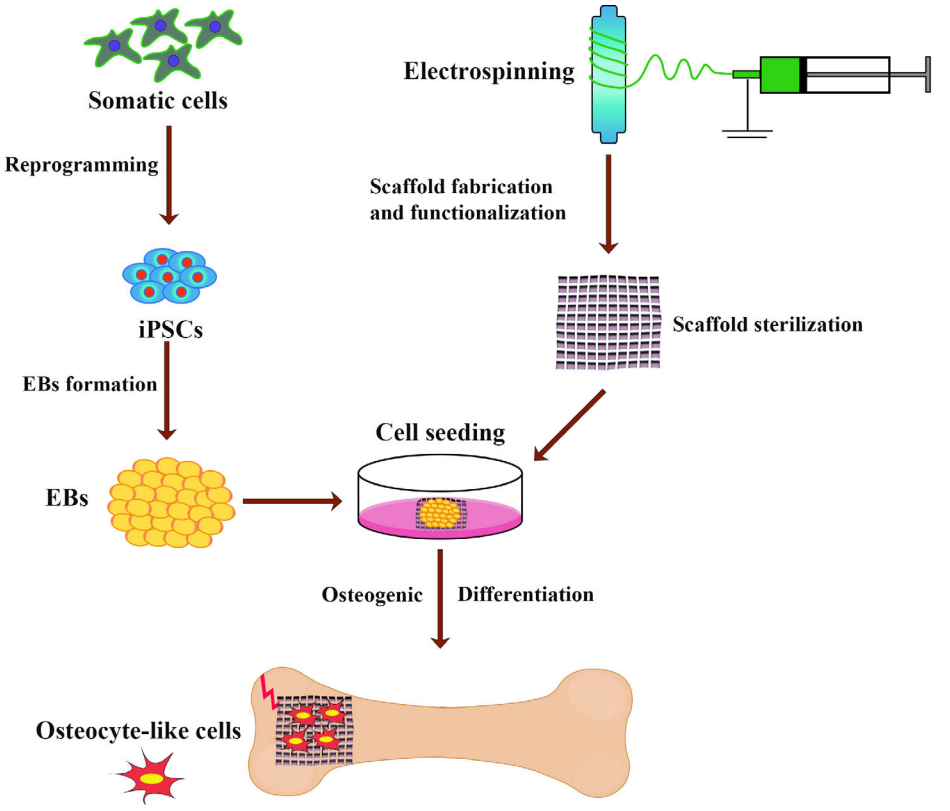

Figure 1. Fabricating bone grafts using electrospinning of a synthetic polymer and iPSCs, a schematic view.

Table 2. The properties of nanofiber fabricating techniques.

Method of fabrication	Advantages	Drawbacks
Self-assembly	Simple, non-expensive, high porosity	Poor control of parameters, not scalable
Phase separation	Simple, non-expensive, up to 95% porosity	Toxic solvent, poor control of parameters, restricted range of pore size, not scalable
Gas foaming	Simple, solvent-free	Lack of porosity, high-pressure requirement, presence of a skimming film on the scaffold
Solvent casting	Simple, high mechanical stability	Poor control of parameters, no reproducibility, toxic solvent
Freeze drying	Simple, high cell *via*bility	Only small pore size, special equipment, time-consuming
Electrospinning	Simple, high porosity, uniform, high cell *via*bility, ECM-like structure, high surface area, high mechanical properties	Toxic solvent, high voltage, special equipment
3D printing	Uniform, controllable parameters	Lack of mechanical properties, special equipment, toxic solvent

Although this method is simple, cheap, and frequently occurs in nature, it can only be performed on certain polymers such as co-polymers, peptides, and dendrimers. In addition, it yields only a low level of fibers, limiting its use in bone tissue engineering [35]. Phase separation is another simple and effective tool, however; it cannot be

Table 3. The physicochemical properties of synthetic polymers in comparison to PAN and PPy.

Polymer	Chemical formula or systemic name	Melting point (T_m)	Molecular weight (g/mol)	Solvent	Tensile strength
PCL	$(C_6H_{10}O_2)_n$	60 °C	114.143	Inorganic solvents	10.5–16.1 MPa
PLLA	$(C_3H_4O_2)_n$	150–160 °C	2×10^4	Inorganic solvents	0.5–1.0 GPa
PES	Poly(oxy-1,4-phenylsulfonyl-1,4-phenyl)	227–238 °C	42–65	Inorganic solvents	6.76e7–9.52e7 Pa
PVDF	$-(C_2H_2F_2)_n-$	177 °C	64.03	Inorganic solvents	9–120 MPa
PLGA	C5H8O5	150–160 °C	148.11	Inorganic solvents	16.7 ± 1.9a MPa
PAN	C6H7N	>330 °C	Highly variable	Organic solvent	170–366 MPa
PPy	H(C4H2NH)nH	>300 °C	3–630	Organic solvent, low soluable	136 MPa

employed for a large number of polymers [36]. Solvent casting and particulate leaching (SCPL) is another technique. In this method, polymer, organic solvent, and salts are used in a reaction and a composite is fabricated after evaporating the solvent and dissolving the salt. Organic solvents and salts limit the use of growth factors, proteins, and other therapeutic agents during scaffold fabrication [37].

Electrospinning provides the fabrication of submicron to micrometers fibers from any synthetic or natural polymers. By the use of a syringe pump, a polymer is dissolved in a solvent and the solution is ejected toward a collector by passing through a high voltage electric field. At a controllable flow rate, a liquid jet is formed from a polymeric solution in the high voltage electric field. As a result, dried nanofibers with different diameters are generated on collector [5]. The properties of nanofibers are affected by several factors. To generate thinner fibers, lower flow rate, higher voltage, lower solution viscosity, higher temperature, and lower concentration are suggested. However, too much increase of these parameters causes the generation of beads. The optimum range of other parameters such as humidity, the distance between the needle and collector, and dielectric constant is also required for the generation of successful beads-free fibers [38]. Electrospinning allows the formation of nanofibers with various topography and structures [39]. The morphology of the fibers could be solid, porous, or core-shell. The fibers could be assembled randomly, aligned, or layered [40]. Electrospinning has been widely used in the engineering of tissues including bone [41], cartilage [42], liver [43], and so on. Due to its application in the field of bone tissue engineering, here we chose it to review its potential application in guiding iPSCs into osteo-like cells. The physicochemical properties of synthetic polymers that have been used to differentiate iPSCs into osteo-like cells are listed in Table 3. Also, Table 4 summarizes the advantages and drawbacks of some of these polymers. Tissue engineering uses synthetic and/or natural biomaterials, helps to simulate the natural three-dimensional structure of the ECM, and provides the proper environment and favorable conditions for cells to proliferate and differentiate. Polymers, cells, and growth factors are the three basic elements in tissue engineering [44]. The compatibility of the mechanical properties of polymers with the natural ECM is of great importance; as it ensures the progress of the tissue repair process *in vivo* [45]. In addition, the mechanical properties of the polymer are effective on morphology, migration, and cell differentiation. Polymers selected for bone tissue engineering must not only be biocompatible but also have high mechanical strength. Most natural polymers have low mechanical properties. Hence, attention is focused on

Table 4. The most frequent synthetic polymers have been used to differentiate iPSCs into osteo-like cells.

Polymer	Advantages	Drawbacks
PCL	Biocompatibility, biodegradability, non-toxic, high mechanical properties, low cost	Surface hydrophobicity
PLLA	Biocompatibility, biodegradability	Poor toughness and thermal resistance
PES	Biocompatibility, non-toxic, good chemical and mechanical properties	Surface hydrophobicity
PVDF	Biocompatibility, low cost, high flexibility, piezoelectric property	Surface hydrophobicity
PLGA	Biodegradability, non-toxic, good mechanical properties	Surface hydrophobicity

synthetic polymers [32, 46]. Many synthetic polymers have been used for bone regeneration and engineering [6]. In the following section, the characteristics and functions of these polymers are discussed.

3. Synthetic electrospun scaffolds in bone regeneration

3.1. Poly ε-caprolactone (PCL)

PCL (chemical formula: $(C_6H_{10}O_2)_n$) has been widely used in tissue engineering. This synthetic polymer is a low-cost, biocompatible, biodegradable, and non-toxic polyester [47]. However, due to the presence of several hydrophobic $-CH_2$ moieties, the degradation rate of PCL is slow and takes 2–3 years which is lower than other polyesters such as PGA, PLGA, and poly(L-lactic acid) (PLLA) [48]. The polymer is degraded by microorganisms or the hydrolysis of the ester linkage [49]. The melting temperature of the PCL semi-crystalline polymer is above the body temperature (T_m: 59–64 °C). Under the physiological temperature, PCL has high mechanical properties (strength, toughness, and elasticity); making it suitable for bone tissue engineering [47]. Several studies have used electrospun PCL nanofiber to induce the osteogenic differentiation of iPSCs [50–52]. It is also feasible to blend PCL with other polymers as Soleimanifar et al. [52] have used a blend of PCL-poly(vinylidene fluoride) (PCL-PVDF) as a matrix to promote osteogenic differentiation of iPSCs. Moreover, it is possible to introduce active molecules such as growth factors, peptides, and miRNAs into the fiber without affecting their structure and properties [21, 53]. In this regard, Kang et al. [50] have incorporated hydroxyapatite (HA) into PCL nanofibers and showed that the nanofiber is able to induce osteogenic differentiation of iPSCs. In addition, Tahmasebi et al. [21] incorporated miRNAs into PCL nanofibers. They showed that miRNA-incorporated electrospun PCL nanofibers improve the osteogenic differentiation of iPSCs.

3.2. Poly(L-lactic acid) (PLLA)

PLLA with a chemical formula of $(C_3H_4O_2)_n$ is another synthetic polymer that is biocompatible and biodegradable. Its tensile strength and melting temperature are 0.5 and 1.0 GPa and 150–160 °C, respectively [54,55]. With high mechanical properties, PLLA has been widely used in the engineering of tissues such as bone [56]. D'Angelo

et al. [57] were used PLLA electrospun scaffolds to induce the osteogenic differentiation of iPSCs. This study showed that the activity of ALP and the expression of bone markers such as *osteocalcin*, *osteonectin*, and *runx-2* mRNAs increased in iPSCs differentiated on the PLLA scaffold. However, PLLA has poor thermal resistance and toughness which may limit its application. Hosseini et al. [58] showed that a blend of PLLA-PCL promotes the osteogenic differentiation of iPSCs. Hu et al. [59] showed that PCL improves the toughness and thermal resistance of PLLA. Moreover, it is possible to fabricate a co-polymer from both PLLA and PCL which is called poly(lactide-co-ε-caprolactone) (PLCL). PLCL has advantages of both PCL and PLLA. Xu et al. [60] showed that iPSCs more efficiently differentiate on the PLCL/SF (silk fibroin) electrospun scaffold into osteo-like cells.

3.3. Polyethersulfone (PES)

PES is a synthetic biocompatible non-toxic polymer with a wide range of applications. The polymer has high thermal and chemical resistance as its melting temperature is above 200 °C. The fabricated polymer has a high surface area, allowing a large number of cells to be attached [61]. It has good mechanical properties, provides a favorable matrix for tissue engineering purposes. However, a relatively low molecular weight (MW: 42–65) and tensile strength limit its use in bone tissue engineering [62–64]. Ardeshirylajimi et al. [65] used PES electrospun scaffold to enhance osteogenic differentiation of iPSCs and showed higher bone markers expressions. However, hydrophobicity is a major drawback of PES. Therefore, increasing the hydrophilicity of the polymer by plasma treatment and other approaches is recommended to improve the tissue engineering applications [66]. Moreover, in some studies, PES has been used in combination with other polymers and biomaterials including graphene oxide (GO) [67], chitosan, poly (amide-imide) (PAI) [68], cellulose acetate phthalate (CAP) [69], and so on.

3.4. Poly(vinylidene fluoride) (PVDF)

PVDF (chemical formula: $(C_2H_2F_2)_n$) is a biocompatible and flexible material that has good thermal and mechanical properties (melting point: 177 °C, tensile strength: 9–120 MPa) [70,71]. It is also known as a piezoelectric polymer. This property allows the polymer to transform mechanical signals into electrical signals [72,73]. PVDF is a favorable polymer for bone regeneration and tissue engineering. Several studies have shown that PVDF enhances the osteogenic differentiation of iPSCs [74,75]. In this regard, Mirzaei et al. [76] have shown that PVDF scaffold could promote the osteogenic differentiation of iPSCs *in vitro*. It is also shown that the composite of PVDF with other synthetic polymers such as PCL [77] and PVA [78] or natural polymers such as collagen [79] enhances the osteogenic differentiation of iPSCs. Recently, Abazari et al. [80] showed that piezoelectric PVDF has higher potential in osteogenic differentiation of stem cells rather than PCL and PLLA.

3.5. Poly(lactic-co-glycolic acid) (PLGA)

Recently, food and drug administration (FDA) has approved PLGA based devices for biomedical applications. Also, there are some commercial products based on PLGA and among them, OsteoScaf^{TM} is used as a bone substitute in orthopedics. PLGA is a biodegradable polymer that formed during the polymerization of lactic acid (LA) and glycolic acid (GA) monomers. The physicochemical properties of PLGA mainly depend on the polymer molecular weight. PLGA is fabricated in various molecular weights from several thousands to hundreds of thousands of daltons (Da). The biodegradability of PLGA polymers also depends on the monomer contents and their order. A faster degradation is observed in polymers with higher GA content and polymers with random sequences. Finally, microenvironmental conditions such as pH and temperature also affect the polymer biodegradation [81,82]. Therefore, controlling the parameters allows the fabrication of PLGA polymers with suitable mechanical properties for bone regeneration. PLGA has been used in several studies as a supportive matrix for osteogenic differentiation of MSCs [83]. Recently, Abazari et al. [84] have used electrospun PLGA nanofibers to induce osteogenic differentiation of iPSCs and showed that PLGA provides a suitable artificial ECM with a positive impact on osteogenic differentiation of stem cells.

4. Modifying electrospun scaffolds

Some studies have tried to increase the osteogenesis potential of the electrospun scaffolds. Increasing the hydrophilicity of the scaffold, the use of bone-like materials such as HA, and the use of growth factors to enhance the osteogenic differentiation of stem cells are some of these attempts. In the following section, the modifications that have been used to enhance the osteogenesis of the synthetic electrospun scaffold are reviewed. These strategies are listed in Table 5.

4.1. Plasma treating

Most of the electrospun scaffolds have a hydrophobic surface and therefore, decrease the possibility of cell adhesion on the surface of the scaffold. Plasma treatment is one of the most common strategies to increase the hydrophilicity of the electrospun nanofibers [85]. It is shown that plasma treatment enhances the osteogenic differentiation of iPSCs and other types of stem cells [52, 66, 75, 86, 87]. During plasma treatment, new oxygen-containing groups such as O_2 (by O_2 plasma) or nitrogen-containing groups such as NH_3 (by NH_3 plasma), are generated on the surface of the scaffold. Plasma includes ionized gas containing active atoms, electrons, ions, radicals, and photons. These active ingredients induce chemical and physical modification on the surface of the scaffold (Figure 2) [88]. Peptides and proteins that are located on the cell surface and are responsible for cell adhesion reach faster on the surface of pretreated scaffolds because of the presence of new active groups [89]. These active groups allow stem cells to spread over the electrospun fibers during the initial stage of cell adhesion [90, 91].

Table 5. Strategies to enhance osteoinductivity of electrospun scaffolds.

Strategy	Functions	Mechanisms	Ref.
Plasma treating	Increasing stem cell adhesion and proliferation	Increases the hydrophilicity of the scaffold	[52, 66, 75]
The use of PRP	Increasing stem cell adhesion and differentiation	Increase the adsorption of cultured medium proteins	[79]
Incorporation of inorganic polyphosphate (poly-P)	Inducing osteogenic differentiation	Activates the Wnt/β-catenin signaling pathway	[58]
Incorporation of HA	Inducing osteogenic differentiation	Provides a bone-like environments	[50, 57]
Incorporation of GO	Increasing stem cell adhesion and proliferation and matrix formation	Enhances scaffold mechanical properties, Increasing the hydrophilicity of the fiber, Inducing the apatite and calcium phosphate formation	[75, 78]
Coating with bioceramic nanoparticles	Enhancing osteogenesis and matrix formation	Increase the mechanical properties of the fibers; Provide the *in vivo*-like microenvironment.	[87]
Incorporation of bFGF	Directing stem cell proliferation and, differentiation and scaffold angiogenesis	Acting as a paracrine effect and activating several signaling pathways	[60]
Anchorage of Vitronectin peptide	Increasing stem cell adhesion and proliferation	Increase the hydrophilicity of the scaffold	[53]
Incorporation of peptide H1	Increasing stem cell adhesion and proliferation	Increase the hydrophilicity of the scaffold	[62]
Incorporation of miRNA	Directing osteoblast differentiation, Increasing the angiogenesis	Facilitates the osteogenesis of stem cells and angiogenesis of the scaffold	[21]
Using MSC-derived CM	Directing stem cell proliferation and, differentiation and scaffold angiogenesis	Acting as a paracrine effect and activating several signaling pathways	[53]
Applying PEMF	Inducing osteogenesis	Regulates the bone formation through the Wnt/β-catenin pathway	[52]
The use of herbal extracts	Increasing stem cell adhesion and proliferation	Increasing the surface hydrophilicity of the scaffold	[86]

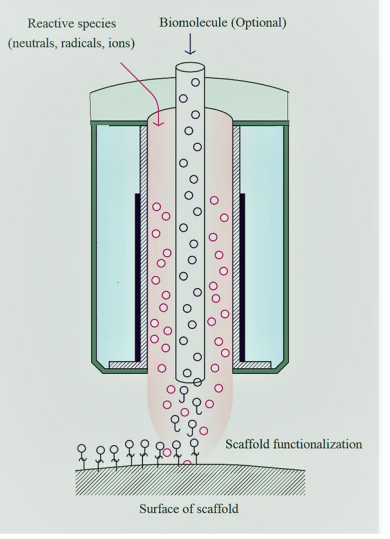

Figure 2. Surface plasma treating.

4.2. The use of platelet-rich plasma (PRP)

Studies have shown that PRP increases bone repair and regeneration *in vivo*. In a study by Abazari et al. [92], the use of PRP enhanced the osteogenesis of the PVDF/col composite scaffold. PRP-incorporated scaffold directed osteogenic differentiation of iPSCs more efficiently. It is shown that the use of PRP increases the adsorption of culture medium growth factors by the scaffold [93,94]. PRP includes several platelet growth factors including various isomers of platelet-derived growth factor (PDGF), numerous transforming growth factors-β (TNF-β1 and TNF-β2), vascular endothelial growth factor (VEGF), and epithelial growth factor (EGF). In addition, PRP includes blood-related proteins including fibrin, bronectin, and vitronectin. These growth factors and blood-related proteins are believed to mediate cell adhesion [95].

4.3. Doping of inorganic materials

Bioceramic nanoparticles: Bioceramics have been largely used as a bone substitute. Al_2O_3 and ZrO_2, as inert non-absorbable ceramics, were the first generation of bioceramics used for bone repair. However, recently, the focus is more on active ceramics such as bioglass and organic–inorganic hybrids [96]. These materials also could be used to enhance osteogenesis. When Zn_2SiO_4 was introduced into the electrospun scaffold, the osteogenic differentiation of iPSCs increased [87]. It is shown that $Ca_2ZnSi_2O_7$ enhances the osteogenic differentiation of stem cells [97]. Bioceramics enhance the rigidity and stiffness of nanofibers, making the fibers suitable for bone engineering [98,99]. The chemical structures of Zn_2SiO_4 and other bioceramics are similar to minerals presented in native bone. Therefore, the structure and topography of bioceramics mediate their osteoinductive properties [100]. It is shown that doping of inorganic bioceramic materials into electrospun polymers enhances the mechanical properties and functionality (osteogenesis) of polymers. In this regard, Wang et al. [101] showed that doping of laponite (LAP) nanodisks, a synthetic aluminosilicate clay material, into electrospun PLGA induces the osteogenic differentiation of MSCs. Another study showed that doping of calcium magnesium silicate (akermanite) and attapulgite (ATT), a magnesium aluminum phyllosilicate, also could induce the osteogenic differentiation of MSCs [102,103]. Therefore, doping of such biocermaics into electrospun scaffold may also induce the osteogenic differentiation of iPSCs.

Hydroxyapatite (HA): HA constitutes the main components of natural bone. HA has been widely used to fabricate bone grafts [104]. It is also used to coat implants and other dental materials [105,106]. The presence of HA on the surface of the scaffolds supports osteoblastic cell adhesion, growth, and differentiation, and induces the deposition of ECM by bone cells [107]. The microporosity, surface area, geometry, and topography of HA are important for its osteoinductivity [108]. Therefore, the use of HA in electrospun nanofibers could provide a suitable microenvironment for osteogenic differentiation of stem cells, as shown by Kang et al. [50], D'Angelo et al. [57], and Xu et al. [60].

Graphene oxide (GO): GO is composed of carbon, oxygen, and hydrogen and has been used to fabricate reinforced films, fibers, papers, etc. Because of its high mechanical properties, the use of GO is favorable for the fabrication of suitable scaffolds

for bone regeneration [109–111]. It is shown that electrospun nanofibers reinforced with GO increase the osteogenic differentiation of iPSCs and MSCs [75, 78, 112–114]. GO has also many hydroxyl, carboxyl, and carbonyl functional groups. These groups increase the hydrophilicity of the fiber, allowing cell adhesion and proliferation [115]. Due to the exposure to the body fluid, GO induces apatite and calcium phosphate formation, making it an interesting compound for bone tissue engineering [116–118]. GO is a biodegradable material and degrades into acidic molecules for up to several months. As bone healing takes about 40 days, GO helps to stabilize cells until bone repair [109].

Inorganic poly-P: It is shown that the incorporation of inorganic poly-P into synthetic electrospun nanofibers increases the osteogenic differentiation of stem cells [58]. Poly-P activates the Wnt/β-catenin signaling pathway by phosphorylating and subsequently inactivating inhibitors like GSK-3. Wnt/β-catenin signaling pathway plays a crucial role during osteogenesis [119].

4.4. Incorporation of growth factors and peptides

Growth factors have a great role in modulating cell fate and function. They are involved in cell survival, function, proliferation, and differentiation. It is shown that basic fibroblast growth factor (bFGF) enhances the osteogenic differentiation of iPSCs cultured on the synthetic PCL-PVDF electrospun scaffold [77]. The roles of bFGF in the proliferation and differentiation of progenitors have been shown in a study by Hanada et al. [120]. This factor is also involved in angiogenesis. Therefore, the use of bFGF not only enhances the proliferation and differentiation of cultured stem cells but also could increase the angiogenesis and survival of the implanted scaffold [121,122]. Xu et al. also evaluated the function of connective tissue growth factor (CTGF) in promoting the osteogenesis of the electrospun scaffold. In this study, a 15-aminoacid peptide of the CT domain of CTGF, called peptide H1, was introduced into the SF/PLCL composite fibers. The incorporation of peptide H1 increased the osteogenic differentiation of iPSCs [60]. The peptide has a hydrophilic nature due to the presence of lysine (Lys) and arginine (Arg) residues and promotes cell attachment and proliferation [60]. The same results were obtained when vitronectin peptide was incorporated into the PCL fibers. Vitronectin has also a hydrophilic nature and therefore facilitates cell attachment and proliferation [53].

4.5. Incorporation of miRNA

MicroRNAs are key regulators during development. They are involved in the regulation of many genes therefore, play important roles in cell hemostasis, differentiation, proliferation, and differentiation [123]. These small non-coding RNAs are also involved during skeletal development [124,125]. Several miRNAs have been identified during skeletal development. It is shown that miR-29 regulates osteoblast maturation. The microRNA miR-125b is involved in bone hemostasis. The osteoblasts are rich in miR-138, miR-204, miR-211, miR-101, miR-199, and miR-135. These miRNAs have a crucial role in osteoblast's differentiation and function. It is shown that the structure

and functions of miRNAs are preserved when they introduced into the nanofibers. Furthermore, the incorporation into nanofibers allows the miRNAs to control-release at cell microenvironment [126,127]. One study showed that the osteogenic potential of the electrospun scaffold increased when miR-22 and miR-126 were incorporated into the fibers [21]. The microRNA miR-22 and miR-126 are involved in bone regeneration and angiogenesis, respectively. The miR-22 targets histone deacetylase 6 (HDAC6) mRNA and suppresses its expression at a post-transcription level. It is known that HDAC6 modulates chromatin structure in a way that suppresses the transcription of many transcription factors that are involved in osteogenesis [128]. On the other hand, miR-126 is a potent inducer of angiogenesis. The miR-126 regulates the response of endothelial cells as well as cancer cells to VEGF. The miR-126 directly targets PI3KR2 (p85-b) and SPRED1 which are regulators of VEGF-dependent PI3 kinase and MAP kinase signaling pathways. Both of these targets negatively affect the VEGF signaling pathway [129]. Therefore, the use of miR-22 increases the osteogenic potential of the scaffold, and miR-126 guarantees long-term graft survival *in vivo* [130–133]. In another study by Abazari and coworkers, miR-2861 has been used to induce the osteogenic differentiation of iPSCs. However, they transducted iPSCs with miR-2861 and showed that miR-2861-overexpressing stem cells differentiate more efficiently on PLGA scaffold [82].

4.6. The use of herbal extracts

In several studies, herbal extracts have been used to induce bone regeneration at the defect sites in animal models [134–136]. It is shown that coating with herbal extracts increases the surface hydrophilicity of the scaffold, therefore, improving stem cell attachment, differentiation, and proliferation. In a study by Tahmasebi et al. [86], *Aloe vera* gel was used to coat the poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) electrospun scaffold. The results showed that the gel enhances the osteogenic potential of the PHBV scaffold. The results of Soares et al. [135] indicated that the use of *Aloe vera* extract along with MSCs increases the bone regeneration of non-critical defects in an animal model. *Aloe vera* compounds have anti-inflammatory, antimicrobial, immune-modulating, and wound-healing properties [137]. Furthermore, *Aloe vera* extract contains a secondary metabolite named acemannan. This polysaccharide is involved in ECM synthesis and mineralization [138,139]. Therefore, *Aloe vera* has great potential in bone tissue engineering by facilitating stem cell attachment, proliferation, differentiation, and mineralization.

5. The role of osteoinductive inducers

Dexamethasone (DEX), ascorbic acid, and β-glycerophosphate (βGP) are the common osteoinductive inducers. DEX is a synthetic glucocorticoid with anti-inflammatory effects. It is widely used for the osteogenic differentiation of stem cells from various sources. Osteogenic differentiation is characterized by three stages including proliferation, ECM maturation, and mineralization. It is shown that DEX involves in the early stage of osteogenic differentiation as well as osteoblast maturation and

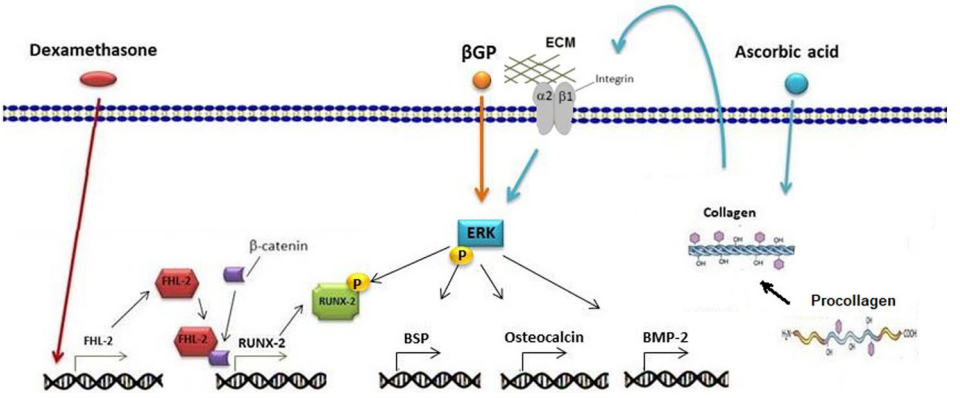

Figure 3. The possible role of dexamethasone, βGP, and ascorbic acid in osteogenic differentiation.

mineralization [140]. Studies have shown that DEX increases the expression of OC and bone sialoprotein (BSP) [141]. DEX induces the expression and activation of Runx2 by activating FHL2, β-catenin-like molecule TAZ, and mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) transcription factors (Figure 3) [142]. DEX increased the activity of ALP [143] however; it decreased the expression of collagen I [144]. Therefore, it is essential to use other inducers along with DEX for osteogenic differentiation. Ascorbic acid which is known as vitamin C is another osteogenic inducer. Unlike DEX, ascorbic acid is involved in the formation of collagen I from procollagen and its secretion into ECM. Subsequently, collagen I acts on integrin and activates MAPK/extracellular related kinase (ERK) pathway which enhances the Runx2 activity [142]. βGP is mainly involved in dystrophic mineralization. It serves as a phosphate source for bone minerals. It also induces the phosphorylation of ERK which activate the ERK signaling pathway, resulting in the induction of osteogenic genes including bone morphogenetic proteins (BMP) 2 and OC [142].

6. Enhancing osteogenesis

6.1. Applying pulsed electromagnetic field (PEMF)

For many years, PEMF, at low frequency, has been widely used for bone regeneration and healing. PEMF has been approved by the US food and drug administration (FDA) for nonunions bone healing. The clinical use of PEMF has shown promising bone healing [145]. Ardeshirylajimi and Khojasteh [51] showed that applying PEMF (1 mT, 50 Hz) increased the osteogenic differentiation of iPSCs cultured on PCL electrospun scaffold. It is also shown that PEMF improves the osteogenic differentiation of MSCs cultured on electrospun nanofibers [76]. These studies showed the synergistic effects of PEMF on cells cultured on the scaffolds [51, 76] as scaffolds are able to deliver the local mechanical stimulation of PEMF to osteogenic cells. In this regard, PEMF induces the mechanical strains which provide local mechanical stimulations to cultured cells [146]. Several mechanisms have been proposed for the osteogenic property of PEMF. Varani et al. [147] showed that PEMF acts through adenosine membrane receptors (ARs). PEMF could induce the signaling through A26 receptor that is

involved in bone regeneration in bone defects [148]. This receptor also regulates bone formation through Wnt/β-catenin pathway [148,149]. It is also demonstrated that PEMF increases the expression of BMP-2 and -4 [150]. BMPs are considered as osteoinductive factors that act through the Wnt/β-catenin signaling pathway [151]. PEMF has also increased angiogenesis by stimulating fibroblast growth factor 2 (FGF-2). Several studies have shown an increase in ECM synthesis following the use of PEMF. ECM synthesis is an important step during bone regeneration [150].

6.2. The use of MSC-derived conditioned media

DEX, Ascorbic acid, and βGP have been widely used as osteoinducers in experimental studies to differentiate stem cells into osteo-like cells. One study showed that the use of conditioned media derived from MSCs, in the absence of any osteoinducers, is able to differentiate iPSCs toward osto-like cells [52]. It is also shown that bone conditioned media (BCM) has an osteoinductive characteristic [152]. However, no study has used BCM for the osteoinduction of iPSCs cultured on an electrospun scaffold. The conditioned media derived from MSCs contains a variety of vesicles and growth factors (such as insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF), and BMPs) [27, 153]. These factors are involved in stem cell proliferation, differentiation, and angiogenesis [154]. Recently, conditioned media, as a cell-free strategy, has attracted attentions in tissue engineering and in regenerative medicine [155].

7. Scaffolds implantation

Limited studies have evaluated the potential of differentiated iPSCs – electrospun scaffolds *in vivo*. Xu et al. incorporated iPSCs, peptide H1, and HA into electrospun nanofibers. The scaffold was implanted at the site defect in the animal model. They showed that the scaffold has a higher potential to improve bone defects. Ardeshirylajimi et al. [66] also showed that a plasma-treated scaffold containing osteo-like cells derived from iPSCs improved the calvarial bone defects in rats. Scaffold acts as a supportive matrix for stem cells, helps the cells to differentiate and synthesize ECM components. The scaffold/cell structure integrates into the bone and therefore improves bone healing [156,157]. Further studies are required to evaluate the *in vivo* potential of electrospun scaffolds and determine the possible mechanisms in bone regeneration.

8. Characterization of bone cells differentiated on electrospun nanofibers

Natural bone in vertebrates consists of various types of cells including osteoprogenitors, osteoblasts, osteoclasts, and osteocytes. Osteoprogenitors are non-differentiated cells that are responsible for other bone cells formation. Osteoblasts promote osteoid calcification in bone structure while osteoclasts regulate calcium and phosphate balance. Osteocytes are important cells in bone formation and structure as they are involved in bone matrix decomposition by releasing hydrolysis enzymes. The aim of tissue engineering is to generate cells that are similar to bone cells. These cells are

called osteo-like cells. Several methods have been used to characterize the differentiated osteo-like cells. Gene expression analysis at both mRNA and protein levels, ALP activity assay, and analysis of calcium contents are used to characterize the differentiated cells. Analysis of osteonectin, OC, and osteopontin expression is important as they are highly expressed in bone tissue. These proteins are involved in bone remodeling as well as hydroxyapatite formation. In addition, evaluating the production of collagen I helps to characterize differentiated cells [158]. Mirzaei and colleagues have evaluated the expression of OC and osteopontin at both mRNA and protein levels. They showed the high expression of OC and osteopontin osteo-like cells (differentiated from iPSCs). Moreover, cells differentiated on electrospun PVA scaffold expressed higher levels of these genes [76]. Also, Abazari et al. [77] have reported the same results. RUNX-2 is another specific gene that is associated with bone tissue and is involved in osteoblastic differentiation and skeletal morphogenesis. The higher expression of RUNX-2 was observed by Abazari et al. [79] in cells differentiated on electrospun scaffold. ALP is highly active in bone tissue. The enzyme is involved in many cellular processes such as DNA synthesis, bone calcification, and reduction of inflammation [159]. Assessing ALP activity is a basic method to characterize osteo-like cells. Abazari et al. showed that iPSCs are efficiently differentiated into osteo-like cells on PVDF/collagen/platelet-rich plasma composite scaffold. The differentiated cells had higher ALP activity when compared to cells that are differentiated in 2D culture [79].

9. Conclusion and future directions

Tissue repair or creating a new tissue is the ultimate goal of tissue engineering. To do this, the essential and more challenging step is providing an *in vivo*-like microenvironment. Due to the unique structure of the bone, fabricated scaffolds have to meet some characteristics. In addition to biocompatibility and biodegradability, the scaffold must have high mechanical properties such as tensile strength, Young's modulus, and compressive strength [38, 160,161]. Some synthetic polymers have several of these properties. However, there is no perfect polymer that meets all of the bone characteristics [162]. The use of composite polymers is a step ahead [163]. However, there is a need for newer materials. In addition, our knowledge about the mechanisms of synthetic materials – host tissue interactions should be improved.

Electrospinning is considered as a versatile tool in tissue engineering. However, the control of the polymeric properties (including porosity, shape, surface topography, fiber diameter, etc.) during scaffold fabrication is difficult [164,165]. To overcome these difficulties in conventional fabricating techniques, new technologies have emerged. 3D-bioprinting technology has opened up a new window in the field of tissue engineering. This technology allows the fabrication of a personalized scaffold by applying natural or/and synthetic polymers, growth factors, and living stem cells [166].

The vascularization of the scaffold prior to implantation is a challenging task. It is possible to vascularize a scaffold by implanting it in an ectopic high vascular tissue such as muscle. Afterward, the scaffolds are harvested and implanted at the site of bone defect which requires a further surgical process [167]. A scaffold could be vascularized in a co-culture with angiogenetic cells [168]. Furthermore, it is possible to

use the angiogenetic cells or growth factors to vascularize the scaffold after implantation at the site of defect [169]. Recently, genetically modified stem cells transduced with VEGF have been used to vascularize the scaffold after implantation [170]. However, the control of angiogenesis is difficult [171]. Therefore, further improvements in vascularization strategies are needed to guarantee the clinical efficacy of the scaffolds.

Authors' contributions

SH, RT, and SS wrote the main manuscript text. KF and AA prepared tables and Figures 1 and 2. AM and RM helped in the revision stage of manuscript. ED and SP determined outlines of the manuscript and finalized the manuscript and supervised the overall study. All authors involved in preparing the manuscript. The corresponding author designed the manuscript and also involved in the writing and revisiting the text.

Data availability statement

The article includes all data from the study.

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