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REVIEW ARTICLE

Artificial Bone via Bone Tissue Engineering: Current Scenario and Challenges

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Abstract Bone provides mechanical support, and flexibility to the body as a structural frame work along with mineral storage, homeostasis, and blood pH regulation. The repair and/or replacement of injured or defective bone with healthy bone or bone substitute is a critical problem in orthopedic treatment. Recent advances in tissue engineering have shown promising results in developing bone material capable of substituting the conventional autogenic or allogenic bone transplants. In the present review, we have discussed natural and synthetic scaffold materials such as metal and metal alloys, ceramics, polymers, etc. which are widely being used along with their cellular counterparts such as stem cells in bone tissue engineering with their pros and cons.

Keywords Bone · Bone tissue engineering · Scaffolds · Growth factors · Regenerative medicine

1 Introduction

Bone is a complex living connective tissue that provides structural frame work, mechanical support, and flexibility to the body along with mineral storage, homeostasis and blood pH regulation [1]. Bone structure typically comprises of cortical and cancellous bone [2] (Fig. 1). The unique organic and inorganic material constitution imparts its mechanical properties to the bone.

Bone defects and their repair is the most common problem worldwide [3] gaining bone as a second most transplanted tissue status followed by blood [4, 5]. In U.S. alone, more than 6.5 million bone defects [6] and more than 3 million facial injuries [7] are recorded every year. Annually, more than 2.2 million bone graft procedures are performed worldwide [8]. Tumor resection, congenital malformation, trauma, fractures, surgery, or diseases like osteoporosis, arthritis [8, 9] are the major cause of bone defects. Some clinical conditions like skeletal reconstruction of large bone defects or compromised regenerative processes such as avascular necrosis, atrophic non-unions and osteoporosis [10] also require bone related transplants. The repair or replacements of such damaged or traumatized bone tissue is achieved by standard approaches like distraction osteogenesis, bone transport [9] or different bone grafting methods like autografts, allografts, bone graft substitutes or by using growth factors [9]. The first commercial bone graft material was introduced in 1993 as Interpore's coral derived Pro-Osteon® [11]. Autografts have achieved various degrees of success in treating bone defects. However, the donor site morbidity, prolonged rehabilitation, increased risk of deep infection and restricted availability limits its potential applications [12]. Bone allografts have resolved transplantable bone samples limitations to some extents, but with potential risks of

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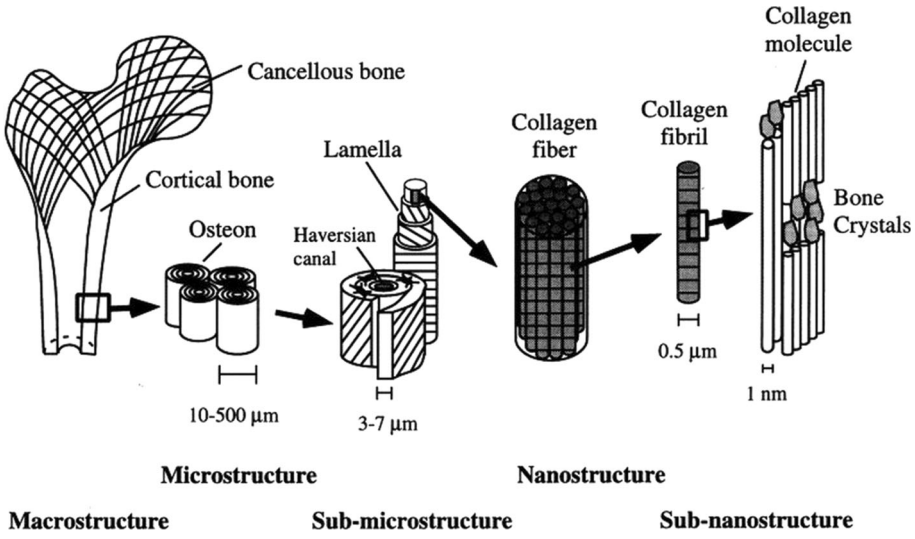

Fig. 1 Hierarchical structural organization of bone: **A** cortical and cancellous bone, **B** osteons with haversian systems, **C** lamellae, **D** collagen fibre assemblies of collagen fibris, **E** bone mineral crystals, collagen molecules, and non-collagenous proteins. Reproduced with permission from Rho et al. [2], ©1998 Elsevier Ltd

transmissible diseases, viral infection, immunological rejections, efficacy and cost effectiveness [13–15]. Due to avascular and porous nature of bone, osteocytes survive by diffusion of nutrients limits their application in case of bone defect size and host viability [16]. Furthermore, there are no heterologous or synthetic bone substitutes available at present which are superior or with similar biological or mechanical properties as of natural bone. Hence, an alternative and effective treatment method for bone regeneration is a necessity.

Recent bone substitute which can replace conventional bone grafts have shown a ray of hope [17]. Use of osteogenic growth factors like bone morphogenic proteins (BMPs), osteoinductive matrix, gene therapy, use of stem cells etc. [18] have demonstrated their potential in bone tissue engineering (Fig. 2). This review is an effort to summarize the different types of available scaffolds and/or biomaterials, stem cells and growth factors used for bone regeneration, either alone or in combination.

2 Cellular aspect of bone tissue engineering

Bone homeostasisis maintained by osteoblasts, osteocytes, and osteoclasts. Osteoblasts are originated from mesenchymal cells, while osteocytes are mature osteoblasts and osteoclasts are of hematopoietic origin [19]. Among all available cell sources *viz.* autogenic cells, allogenic cells, embryonic stem cells (ESCs) [20], induced pluripotent stem cells (iPSCs) [21], or mesenchymal stem cells (MSCs) [22]; ESCs are widely studied for bone tissue engineering including differentiation into osteoblasts [20, 23]. Co-culturing of ESCs with fetal fibroblast has showed enhanced formation of bone nodules [24]. However, teratoma formation limits ESCs clinical applications [21]. For example, transplantation of laminin coated 3D poly (L-lactide-co-glycolide) (PLGA) scaffolds with human ESCs into liver lobules of SCID mice resulted in teratoma formation [25].

MSCs are known to differentiate into maturated cells like osteoblasts, chondroblasts and chondrocytes on external chemical stimuli [26]. MSCs isolated from bone marrow [22], peripheral blood [27], adipose tissue [28] have been differentiated to osteoblasts, chondrocytes and healed critical sized bone defects *in vivo*. The study involving proliferative and osteogenic potential of MSCs from human fetal bone marrow (hfBMSCs), human adult adipose tissue (hADSCs) cultured in to poly(caprolactone) (PCL)-tricalcium phosphate (TCP) scaffolds revealed hfBMSCs possesses highest proliferation and osteogenesis with least immunogenicity [29]. The iPSCs have emerged as an alternative for MSCs and/or ESCs. There are reports available of differentiation of human iPSCs in to osteoblasts *in vitro* [30] and *in vivo* without teratoma formation [21, 31]. Murine iPSCs transduced with Special Adenosine-Thymine rich sequence binding protein 2 (SATB2) are known to express the osteoblastic genes [32]. Murine iPSCs overexpressing SATB2 seeded with silk scaffolds [32] and human iPSCs seeded with PCL scaffolds [33] transplanted in to mice model, showed increased mineralization and new bone formation.

Although BMSCs are gold standard in tissue engineering, its clinical use is restricted due to invasive procedures and decreased proliferation and differentiation with increasing age of donor [34]. Although morphologically and phenotypically similar to human umbilical cords, Wharton's jelly mesenchymal stem cells (hUCMSCs), human dental pulp stem cells (hDPSCs) have demonstrated greater proliferative properties than hBMSCs, hADSCs [35]. The study of hUCMSCs with non-rigid calcium phosphate cement scaffold revealed proliferation and differentiation of hUCMSCs into osteoblast and

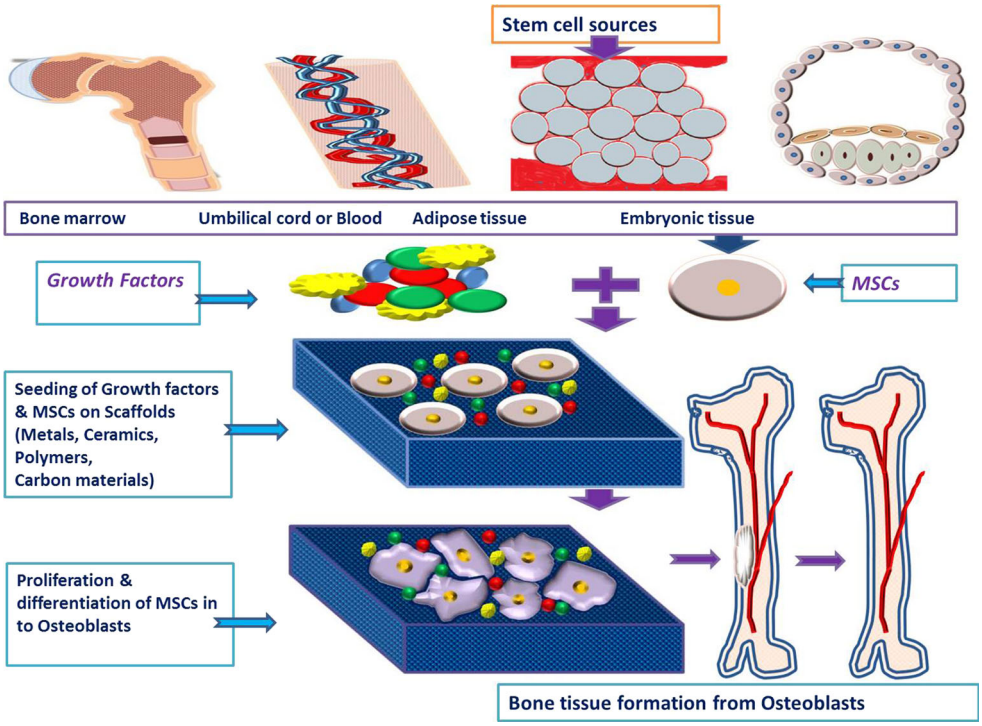

Fig. 2 Outline of bone tissue engineering: mesenchymal stem cells from bone marrow, umbilical cord, adipose tissue or embryonic tissue can be used along with growth factors on different biomaterials to repair or regenerate bone tissue

mineralization *in vitro* [36]. Cell origin and lineage differentiation conditions have significant effect on stem cells osteogenic differentiation pattern [37]. Many research groups have considered hUCMSCs as an alternative to BMSCs showing comparable expression of osteogenic phenotypes *in vitro* [34, 35] along with *in vivo* osteogenic differentiation when transplanted with scaffolds in nude mice model [38].

Human Amniotic fluid derived stem cells (hAFSCs) can be used as alternative for BMSCs in bone tissue engineering [37].The hAFSCs adhered to composite scaffolds of collagen matrix derived from porcine bladder submucosa matrix—PLGA differentiates into osteoblasts expressing osteogenic genes [39]. The hAFSCs and hDPSCs seeded on fibroin scaffolds [40] and on collagen scaffolds [41], support *in vivo* bone formation in a critical size cranial bone defects in rats. The hDPSCs seeded on collagen-hydroxyapatite (HA)-poly(L-lactide-co-ε-caprolactone) showed cell adhesion, growth, expression of osteogenic genes with mineralization and nodule formation [42]. The hDPSCs with HA-TCP paste transplanted into immunodefecient parietal region cranial defect rats revealed bone formation with increased mineralization and density of bone [43].

3 Bone tissue engineering using growth factors

Growth factors *viz.* Bone morphogenetic proteins (BMPs) [18], Fibroblast growth factors (FGFs) [44], Platelet derived growth factors (PDGFs) [45], Transforming growth factors (TGF-β) [46], Vesicular endothelial growth factors (VEGFs) [47], Insulin like growth factors (IGFs) [46]; alone or in combination are known to play important role in regulation of bone formation at different level. BMP is involved in skeletal development, adult bone homeostasis, and fracture healing along with differentiation of MSCs in to the cartilage, bone, tendon/ligament [18] with highest *in vitro* and *in vivo* osteogenic potential [48]. Three dimensional (3D) bio-printing of BMP-2 in DermaMatrix^{TM} human allograft revealed differentiation of mouse C2C12 progenitor cells *in vitro* and tissue formation in calvarial defect *in vivo* [49]. The high doses requirement of BMPs limits its direct use in regenerative medicine [18], but BMPs with combinations of growth factors have been used in bone regeneration. For example, adenovirus based expression of BMP2 in the C3H10T1/2 cell line, osteoblastic differentiations increased 10 fold [50]. The BMP-2 loaded nanoparticles with fibrin scaffolds showed more bone formation *in vitro* than BMP-2 alone [51]. Silica xerogel-chitosan hybrid coated BMP-2 with porous HA showed *in vitro* osteoblastic cell response and *in vivo* bone formation in calvarial defects in rabbits [52]. The study with MG-63 cells seeded on TCP scaffolds showed higher cell seeding efficiency *in vitro* while alginate gel assisted cell seeding with BMP-2 showed osteocalcin and osteoid deposition *in vivo* [53].PLGA scaffold coated with BMP-2 and PDGF polyelectrolyte on transplantation in calvarial bone defect rat model induced mechanically competent local bone formation [54].

The osteogenic growth factor bFGF has a potential to accelerate bone regeneration when used with MSCs [44].

Also use of bFGF with gelatin hydrogels have resulted in improved bone regeneration in skull defects of rabbits [55] and monkeys [56]. The mesoporous bioactive glass nanospheres used for the delivery of FGF2 and FGF18. Rat MSCs culture with these growth factors showed cell proliferation, cellular mineralization *in vitro* and their transplantation into rat calvarial defects revealed bone formation with higher bone volume and bone density [57]. The PDGF stimulate VEGF secretion and contributes to the osteogenic lineage and helps to formation of new bone by differentiation of MSCs in presence of BMP *via* Wnt signaling [45] and chitosan-TCP [58]. The combination of PDGF and IGF-1 with aqueous gel transplanted to periodontitis affected teeth in beagle dogs' revealed cementum and new bone formation [59]. The PDGF with deproteinized bovine bone mineral showed higher bone regeneration as compared to β-TCP in calvarial defect rabbits models [60]. The patients with alveolar defects transplanted with PDGF, hMSCs seeded on biphasic scaffolds, three month post-surgery revealed more than 50% bone repair [61]. In a clinical trial patients with one localized periodontal osseous defect treated with PDGF and β-TCP, 36 month follow up revealed filling of potential bone defect [62].

When BMSCs cultured on VEGF-silk-fibroin-chitosan scaffolds showed significant cell attachment, cell proliferation compared to BMSCs cultured on silk-fibroin-chitosan scaffolds [63]. VEGFs incorporated PLGA scaffolds showed proliferation of endothelial cells and apatite formation revealing osteogenic and angiogenic potential [64]. Osteoblasts cultured on AD-VEGF activated chitosan-HA showed attachment, proliferation, differentiation *in vitro* and *in vivo* with neo-vessel formation in newly formed ectopic bone [65]. VEGFs, when used synergistically with BMP-4 [47]and BMP-2 [66] enhanced bone formation than VEGFs alone (Table 1).

IGF-1 is secreted by mature osteoblasts and stimulates *in vitro* and *in vivo* proliferation and differentiation of osteoblasts [46]. Human periodontal ligament stem cells treated with exogenous IGF-1 showed the *in vitro* osteogenic differentiation and *in vivo* there was mineralization in the tissues [67]. The IGF transplanted with MSCs in the mice models improved the bone fractures through the callus mineralization and autocrine osteogenic effects via IRS-1 signaling [68]. IL-3,induces BMP2 and activate Smad1/5/8, enhancing the differentiation of MSCs in to the osteoblasts and bone regeneration, both *in vitro* and *in vivo* [69].

4 Bone tissue engineering with scaffolds

Scaffolds are porous 3D matrices that act as temporary templates for cell adhesion and proliferation, while providing mechanical support until formation of new tissue at the diseased area [70]. Scaffolds can also mimic the natural extra cellular matrix (ECM) [70] without activating host immune response or secretion of toxic metabolites [71]. A variety of materials such as metals [72], ceramics [73], natural [74] and synthetic polymers and their combinations (Table 2) have been explored for replacement and repair of damaged or traumatized bone tissues.

The metallic materials such as Stainless steel, Co-Cr alloys and Ti alloys etc. [72] are in use over 100 years for bone replacements due to their mechanical properties [75]. However, these materials are corrosive and release cytotoxic ions [75] and often suffer from the wear and stress-shielding effect on transplantation into the human body [76]. Stainless steel is the most common bone implant material because of its combination of properties like mechanical properties, biocompatibility, corrosion resistance and cost effectiveness [77]. Nickel free stainless steel implants are recent focus of metallic bone implants [77].

Biocompatibility and osteogenesis were observed with corrosive resistant implants made from Tantalum (Ta), Hafnium (Hf) Niobium (Nb), Titanium (Ti), Rhenium (Re) [78]. The properties of pure metals can be enhanced by alloying the different types of metals. Co-Cr alloys are wear resistant but possess corrosion properties [79]. The coiled wire and particle form of Co-Cr alloy and Ti implants are found to be devoid of inflammatory response upon transplantation [80].

Ti and Ti alloys like Titanium-Aluminum (6%)-Vanadium (4%) alloy (Ti6Al4 V) have excellent tensile strength, resistance to corrosion [81], lower modulus and superior biocompatibility as compared to stainless steel, Co based alloys [82]. Nickel-titanium alloy called Nitinol (NiTi) possesses shape memory effect, biocompatibility, super-plasticity, damping properties [81, 83].

Ceramics such as HA [76], bioactive glasses [84], calcium phosphate [73] are widely used for bone repair. These are similar to the inorganic component of bone and possess chemical and structural similarity to the native bone [74]. Being natural component of bone HA is biocompatible, biodegradable, biomimetic and bioactive in nature has been widely used in different types of scaffolds as major or partial component. For example, HA and its derivatives like nano-HA, bovine derived porous HA (BDHA) [22, 85].

Calcium Phosphate ceramics are biocompatible, safe, cost effective, easily available and show lower morbidity hence widely used as bone substitutes, coatings, cements, drug delivery systems and tissue engineering scaffolds [73]. The mechanically stable 3D printed calcium silicate scaffolds showed *in vitro* mineralizationand *in vivo* osteogenesis [86]. Bio-mimetic composites of calcium phosphate and mixtures of chitosan, hyaluronic acid found to have biodegradability and good biocompatibility with

Table 1 Cells for bone tissue engineering

Cells for bone tissue engineering	Tissue repair	References
ESCs	Osteoblast differentiation but Teratoma formation in SCID mice	[24, 25]
BMSCs	Osteoblast differentiation; osteoinduction; osteogenesis; mineralization; *in vitro* & *in vivo* bone regeneration	[22, 29, 63, 109, 138, 147, 149, 153]
ADSCs	Osteoblast differentiation	[29, 35, 137]
DPSCs	Mineralization; *in vivo* bone regeneration	[40–43]
AFSCs	Osteoblast differentiation; *in vivo* bone regeneration	[39–41]
UCMSCs	Osteoblast differentiation; mineralization	[35, 36, 38]
iPSCs	Osteoblast differentiation; mineralization; *in vitro* & *in vivo* bone regeneration	[30–33]
MG63 cells	Osteoblast differentiation	[53]
MC3T3-E1	Osteoblast differentiation	[88]
Osteoblast cells	Biocompatibility; mineralization; *in vivo* bone regeneration	[87, 94, 123, 124, 136]

Table 2 Types of scaffolds used for bone tissue engineering

Type of scaffolds	Type of study	References
Metals		
Lotus type porous nickel free stainless steel	*In vivo*	[76, 77]
Cobalt-Chromium (Co-Cr) & Ti alloys	*In vivo*	[72, 79, 80]
Ti6Al4 V alloy	*In vitro*	[81, 82]
Nitinol (NiTi) alloy	*In vitro* & *In vivo*	[81, 83]
Ceramic composites		
BDHA scaffolds	*In vitro*	[22, 85]
calcium silicate scaffolds	*In vitro* & *In vivo*	[86]
calcium phosphate composite	*In vitro*	[87]
Bioglass 45S5	*In vitro* & *In vivo*	[84, 89–91]
BCP scaffolds	*In vitro*	[94]
Bioactive glass-Strontium	*In vitro*	[88]
Polymers		
Collagen composites	*In vitro* & *In vivo*	[74, 97–99]
Chitosan-gelatin-nano silica nanocomposite	*In vitro*	[102]
Chitosan-forsterite composite	*In vitro*	[95]
nHA-chitosan-CMC	*In vitro*	[105]
EDC treated Gelatin scaffolds	*In vivo*	[106]
PGA-PLA scaffolds	*In vitro*	[109]
PLLA-HA nanocomposites	*In vivo*	[115]
PLGA-nHA composite	*In vitro*	[119]
PDLLA-nHA-PPy-Alg scaffolds	*In vitro*	[117]
PCL, PCL-PLGA-HA, PCL-TCP-nHA	*In vitro*	[26, 99, 120, 121]
PCL-HA-CNTs; PCL-MNPs	*In vitro* & *In vivo*	[122, 123]
PLA, PLA-HA, PLA-HA-GO	*In vitro*	[124]
PHB, PHB-gelatin, PHB-gelatin-nHA	*In vitro*	[117]
Carbon materials		
nHA + SWCNT scaffold	*In vitro*	[128, 129]
SWCNT networks, rGO	*In vitro*	[130]
HA-GN composites	*In vitro*	[127]

osteoblasts cells [87]. The 3D printed bioactive glass-Strontium mesoporous scaffolds showed apatite formation and proliferation and differentiation of MC3T3-E1 cells *in vitro* [88]. Bioglass 45S5 showed good osteogenic cellular activities, osteocalcin synthesis, and calcified extracellular matrix production along with formation of calcified bone nodule [84, 89], hence proposed for bone tissue engineering [90] alone or in combination [91].

Biphasic calcium phosphate (BCP), which is made up of varying concentration of HA and β-TCP, possesses controllable biological and chemical properties and has become preferred choice for promoting bone ingrowth over other calcium phosphate ceramics [74, 92, 93]. For example, 3D printed BCP scaffolds dynamically cultured with rat osteoblasts and BMSCs showed increased osteoinduction, ALP activity and mineralization [94]. Like metals, ceramics too lacks degradability in a biological environment, and their limited processability [95, 96] can become a hurdle in tissue engineering.

Polymers are widely used in biomaterial applications worldwide. For bone tissue engineering natural polymers such as collagens, glycosaminoglycans(GAG), starch, chitin, and chitosan are used [74] which possess good biocompatibility but have poor mechanical strength [74]. Natural polymers are biocompatible which advantageous for cellular adhesion. In some cases, these polymers may contain pathogenic impurities which can exhibit immunogenicity. Other disadvantages include less control over their mechanical properties, biodegradability, batch-to-batch variability and limited supply can affect the cost efficacy [74].

Collagen is most accepted scaffold among all due to its biocompatibility and availability. Type I collagen which constitutes >90% of the organic mass of the bone [97] promotes proliferation and differentiation of human MSCs in to the osteoblasts *in vitro* and osteogenesis *in vivo* [97, 98]. The composite scaffolds of collagen-apatite [13], BSP-collagen composite scaffolds [99] are known to support bone repair. Collagen in combination with ceramics like HA, silk fibroin-HA, GAG exhibits good biocompatibility and bone regeneration properties [74]. A natural polymer chitosan is biocompatible, biodegradable, hydrophilic [100] and stimulate the differentiation of osteoprogenitor cells [101]. It is observed that chitosan-gelatin scaffold, chitosan-gelatin-nano silica nanocomposite scaffolds showed improved bioactivity and cellular behavior [102] as compared to control chitosan. Interconnected porosity and mechanical strength of chitosan scaffolds can be improved by reinforcement with additives like forsterite (FS) nanopowder without altering its biocompatibility [95].

Combinations of natural and synthetic polymers like corn starch with functionalized polycaprolactone are widely used in preparation of composite scaffolds for bone tissue engineering [17]. These biodegradable scaffolds not only promote osteogenic differentiation [103] but also shows adequate mechanical properties with highly interconnected pores and porosity [17]. Natural polymers like Bacterial cellulose derived from *Acetobacter xylinum* (ATCC 53582) [104], Carboxymethyl cellulose (CMC) incorporated nHA-chitosan (nHA-chitosan-CMC) [105] composite, 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) treated gelatin scaffolds [106] and Modified cellulose-poly (vinyl alcohol) (PVA) [107] are some of the promising scaffold for bone tissue regeneration.

Unlike natural polymers, synthetic polymers have advantage of reproducibility, large scale production with controlled properties of strength, degradation rate and microstructure. Poly (α-hydroxy acids), including poly(-galactic acid) (PGA), poly(lactic acid) (PLA), and their copolymer PLGA, are the most popular and widely used synthetic polymeric materials in bone tissue engineering. When degraded, PGA, PLA [108] and PLGA [99] secretions are nontoxic, natural metabolites, and are eventually eliminated from the body in the form of carbon dioxide and water. The 3D printed PGA-PLA scaffolds found to be biocompatible with BMSCs [109]. Also composites *viz.* PCL-$CaCO_3$ [110], HA-gelatin [111], silk-HA [112], PLA-HA [113] and triphasic HA-collagen-PCL [114] have been used for bone regeneration applications.

A wide range of PLLA based composites like PLLA-HA, PLLA-gel, PLLA-gel-HA, PLLA-apatite have been studied by various groups worldwide. Composite polymers prepared using combination of PLLA with various other materials increased its suitability for bone regeneration compared to the plain PLLA scaffolds [100]. Formation of new bone trabeculae with complete repair of bone was seen in nano-composites scaffold like PLLA-HA [115] or PLLA-Gel-HA with negligible complement activation [116]. The poly-D, L-lactic acid (PDLLA) materials in combination with additives like nHA, polypyrrole-alginate (PPy-Alg), chitosan have demonstrated good cytocompatibility, hydrophilicity, bioavailability and compressive strength [117], along with mineralization and osteogenesity [118]. PLGA-HA composite foams demonstrated comparatively higher density, compressive modulus and compressive yield strength [119]. PCL alone [26] or in combination with other polymers like PLGA-HAcomposite [99], TCP, nHA [120] have been observed to increase porosity, tensile strength and cellular activities than rest of the scaffolds [121]. The porous PCL-HA-CNTs (Carbon Nano Tubes) composites prepared by 3D printing with comparable compressive strength of trabecular bone revealed HA bioactivity, cell adhesion and spreading properties seemly to regenerate bone [122]. Magnetic nanofibrous PCL scaffolds prepared by incorporating

magnetic nanoparticles (MNPs) (PCL-MNPs). These PCL-MNPs showed apatite formation with simulated body fluid *in vitro*. Osteoblasts were adhered and penetrated in to PCL-MNPs and expressed osteogenic genes as compared to pure PCL. Also *in vivo* there were bone regeneration in segmental bone defects and neo-vessel formation [123].

PLA, PLA-HA and PLA-HA-GO scaffolds have showed osteoblast growth and proliferation on their surface [124]. Another poly (3-hydroxybutyrate) (PHB) based nanofibrous scaffolds namely PHB, PHB-gelatin, PHB-gelatin-nHA and PHB-gelatin have demonstrated similar results along with higher level of ALP activity and matrix biomineralization in presence of MSCs [117]. The biomorphic scaffolds like demineralized bone matrixes, calcined animal bone and decellularized ECMs derived from various tissues are known to promote differentiation of ASCs, MSCs, ESCs, iPSCs in to the osteoblasts and supported bone regeneration [125, 126].

5 Carbon materials and their use in bone tissue engineering

Due to the similar dimensions, carbon nano-materials are considered to be physical analogue of ECM components like collagen fibers [127]. Various forms of carbon materials or their composites like single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), and grapheme oxide (GO) have been investigated for their efficacy in tissue engineering in last couple of years. The nHA-SWCNT scaffold in chitosan enhanced the mechanical properties suitable for bone tissue engineering. These scaffolds are found to have osteoblast adhesion and proliferation [128], biocompatible and non-toxic cellular compatibility properties [129].

The SWCNT networks and rGO are chemically similar in nature, but differ by topographical features, with rGO exhibiting higher biocompatibility than the SWCNT [130]. In other hand rough, porous HA-graphene nanosheet (GN) composites contributes to increased fracture properties of HA based scaffolds with post mineralization apatite formation *in vitro* [127].

6 Surface modification of scaffolds

Altering the physicochemical surface properties can change biocompatibility, influence cell adhesion and growth; can improve wear resistance and corrosion resistance properties of material to be used as biomaterial. The surface modification can be achieved by various methods (Table 3) such as coating by self-assembled film/electrolyte multilayers, surface gradient, surface activation, and surface chemical reaction. Stainless steel screws when coated with bisphosphonate increased new bone formation around implants [131]. Similarly Co-Cr alloy coated with HA showed superior osteogenesis and integration than uncoated alloy [132].

Osteoblasts were able to adhere and proliferate on composites of β-TCP-HA scaffolds coated with alginate [71]. The uniform Ca-P-polydopamine composite nanolayer on β-TCP bio-ceramics results in improved surface roughness and hydrophilicity of β-TCP bio-ceramics. These composites when seeded with hBMSCs showed cell attachment, proliferation and alkaline phosphatase activity and expression of bone related genes (ALP, OCN, COL1 and Runx2) [133]. The interconnected porous β-TCP scaffolds improved by ZnO showed good mechanical properties like compressive strength, stiffness, fracture toughness and micro hardness. These scaffolds showed bioactivity, biodegradability *in vitro* and cell attachment, proliferation [134]. Porous 45S5 Bioglass® based scaffolds fabricated and coated with poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) revealed higher porosity with increased interconnected pore structure and high mechanical properties [70] hence ideal candidate for bone tissue engineering (Tables 4, 5).

The corn starch-ethylene-vinyl alcohol (50/50 wt %) based scaffolds when coated with Ca-P showed compressive modulus of 224.6 and compressive strength of 24 without affecting normal cellular activity, expression of osteopontin, collagen type I and alkaline phosphatase activity (ALP) [135]. PDLLA foams and PDLLA foams coated with Bioglass® particles showed complete covering with HA in 28 days of incubation in SBF. Osteoblasts were attached and spread on both PDLLA uncoated and coated foams [136]. While in another *in vitro* study with SBF, the HA formation was slower in uncoated composites than coated composites of PDLLA [108]. The 3D printed polydopamine coated PLA composite showed cell adhesion, cell cycle progression, increased ALP activity, osteocalcin on culturing with hADSCs [137]. Dextran coated polyvinyl formal (PVF) sponges with water holding capacity showed more adhesion, proliferation, and differentiation of BMSCs *in vitro* along with increased DNA content, ALP activity, osteocalcin content, and calcium deposition [138].

7 Bioreactors for bone tissue engineering

A bioreactor is a culture system to proliferate the cells through dynamic culture and restrained environment [139]. The limitation of nutrient transfer in the 3D tissue engineering scaffolds can be overcome by continuously mixing media and by convectively transporting nutrients to cells

Table 3 Surface modification of scaffolds for bone tissue engineering

Surface modified material	Coated by material	Study outcomes	References
Stainless steel screws	Bisphosphonate	New bone formation	[131]
Co-Cr alloy	HA	Osteogenesis; implant integration	[132]
β-TCP-HA scaffolds	Alginate	Osteoblasts adhesion, proliferation	[71]
β-TCP scaffolds	Ca-P-polydopamine	Cell attachment, proliferation and mineralization	[133]
β-TCP scaffolds	ZnO	Cell attachment, proliferation	[134]
45S5 Bioglass® based scaffolds	PHBV	Improved porosity, mechanical properties	[70]
corn starch-ethylene-vinyl alcohol based scaffolds	Ca-P	Normal cellular activity, osteogenic expression	[135]
PDLLA foams	Bioglass® particles	Osteoblasts adhesion, proliferation	[136]
PLA composite	Polydopamine	Normal cellular activity, osteogenic expression	[137]
PVF sponges	Dextran	Cell attachment, proliferation, osteogenic expression calcium deposition	[138]

Table 4 Growth factors for bone tissue engineering

Growth factors	Tissue repair	References
BMPs	Osteoblastic differentiation; *in vivo* bone formation	[18, 50–54]
FGFs	Mineralization; *in vivo* bone regeneration	[44, 55–57]
PDGFs	Stimulate VEGF secretion; osteogenic lineage differentiation; *in vivo* bone regeneration	[45, 58–62]
VEGFs	Osteogenic and angiogenic potential; bone formation	[47, 63–66]
IGFs	Osteogenic differentiation; mineralization	[46, 59, 67, 68]

Table 5 Bioreactor systems for bone tissue engineering

Sl. no.	Bioreactor systems	Culturing of under bioreactor	Aftermaths	References
1	Biaxial bioreactor	Umbilical cord blood endothelial progenitor cells & hBMSCs + PCL-TCP	Mineralization, ectopic bone formation	[145]
2	Perfusion bioreactors	hBMSCs + collagen/silk	*In vitro* bone formation	[147]
3	Flow Perfusion bioreactors	Goat bone marrow stromal cells seeded with biphasic calcium phosphate	*In vivo* bone formation	[148]
4	Multiplate Xpansion bioreactor	Human periosteum derived stem cells	*In vivo* bone formation	[152]
5	Hollow fibre bioreactors	hBMSCs + semipermeable polyethersulphone	Osteoblastic differentiation of hBMSCs	[153]

through bioreactor [140]. Various studies revealed potential role of bioreactors in the cell seeding [141], cell proliferation [142] and differentiation of MSCs in to osteoblasts [143] with mineralization and calcium deposition [144]. The umbilical cord blood endothelial progenitor cells and hBMSCs seeded with PCL-TCP scaffolds dynamically cultured into biaxial bioreactor showed mineralization as well as calcium deposition and subcutaneous implantation in to NOD/SCID mice showed ectopic bone formation as compared to static culture [145].

Among different bioreactors, for example, spinner flasks, rotating wall systems, and a perfusion system (Fig. 3), the latter has potential applications in bone tissue engineering [139]. Perfusion bioreactors increase mass transfer, removes waste and seed scaffolds dynamically by controlled distribution of cells compared to static culture

[146].For example, the study with hBMSCs cultured on collagen/silk scaffolds in three different environments *viz.* static dish, spinner flask and perfusion system showed highest *in vitro* bone formation in perfusion system [147]. The goat bone marrow stromal cells seeded with biphasic calcium phosphate cultured in perfusion system proliferated homogeneously on scaffolds and after implantation in to nude mice showed bone formation [148]. In comparison with static culture, hBMSCs cultured on the PLGA-PCL scaffolds in perfusion systems, when implanted into femoral condyle defects in rat, showed rapid bone regeneration [149].

Rat MSCs seeded on PCL scaffolds cultured under engineered flow perfusion bioreactor demonstrated cell adhesive, remodeling, structural proteins as well as HA [150]. The hMSCs seeded with Poly (L-lactide-co-caprolactone) cultured in the dynamic conditions showed calcification, expression of osteogenic genes and induction of osteogenic lineage [151]. Human periosteum derived stem cells cultured in the multiplate Xpansion bioreactor showed proliferation of cells and *in vivo* bone formation [152]. The hBMSCs separated by semipermeable polyethersulphone cultured in hollow fibre bioreactors maintained their immunophenotype and osteoblastic differentiation capacity [153]. Flow perfusion culture of rat MSCs seeded on PLA scaffolds increased the growth and proliferation of MSCs with higher ALP Activity [154].

8 Bone tissue engineering and future perspectives

From the first attempt of bone regeneration by Urist [155], the field of bone tissue engineering has grown rapidly to develop bone substitute which is more close to natural bone or to regenerate bone using different approaches. Advanced studies in bone tissue engineering in recent past both *in vitro* and *in vivo* have explained the potential of variety of cells to differentiate into osteoblasts and the supporting role of growth factors and/or biomaterials. Most of these studies have revealed the biocompatibility, biodegradability, osteoinductivity, osteoconductivity, osteogenicity and/or physico-mechanical properties. Some *in vivo* studies showed repair of bone defects or bone regeneration. However, complete replacement of defective bone using biomaterials is still not achieved. Creation of functional bone in laboratory condition using cell therapy is still a challenge, although different types of stem cells have shown osteogenic lineage differentiation. Because of many functional problems like mechanical strength, host immune integration, vascularization, etc. in development of bone or bone substitute that can mimic natural bone, clinical trials in human are still at bay. So far, researchers have shown successful use of biomaterials or scaffolds growth factors, and cells for bone tissue engineering, alone or in combination. However, when it comes to clinical application of these materials as bone substitute, it is difficult to obtain approval from regulatory bodies for clinical trials. The future direction should focus on establishing an ethical threshold that is effective and obtainable for future researchers to partake in more high-level studies within the clinical setting. Another reason for only few approved bone substitute for clinical trials, is the difficulties in performing pre-clinical large animal trials. High research and development costs, in combination with the current regulatory environment, present a challenge to high-quality evidence-based study.

Biomaterials for orthopedic implants have great financial impact all over the world. In U.S. alone it was

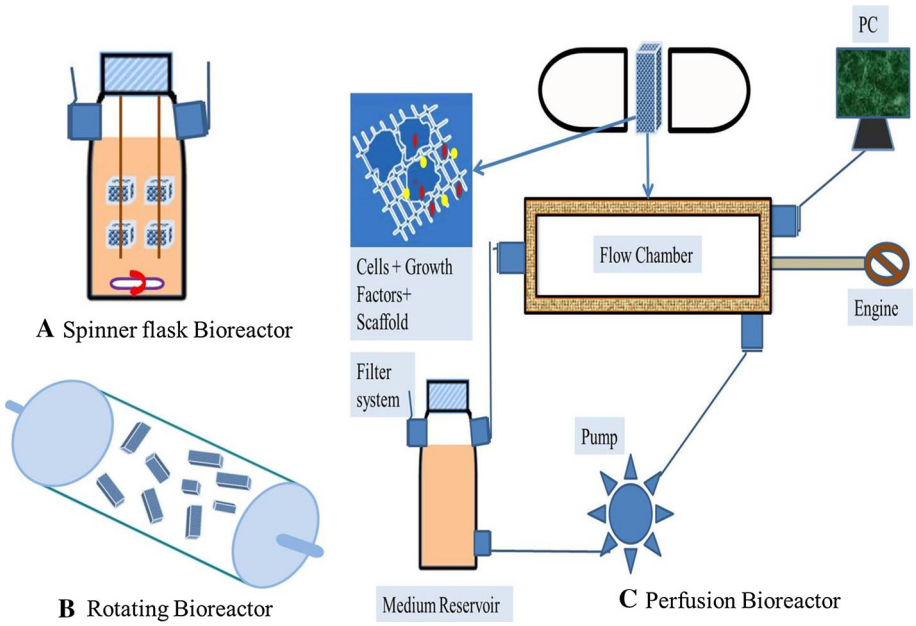

Fig. 3 Schematic diagram of bioreactors: **A** spinner flask bioreactor, **B** rotating bioreactor, **C** perfusion bioreactor. Cells, growth factors filled 3D constructs cultured in bioreactors can be used to regenerate bone tissues

predicted that the biomaterials for orthopedic implants will costs as much as $3.5 billion by the end of 2017 [156]. Patient specific manufacturing of bone substitute also adds in to the cost of therapy. Hence, further efforts are required to develop cost effective, bio-mimicking constructs which can replace defective bone in reality. Such bone tissue engineering constructs will surely bring fruitful treatments in curing bone defects *via* bone replacement or by regeneration. As research at the cellular level continues to expand, the opportunity for growth is limitless, with stem cell-based applications and tissue engineering potentially setting the stage for how more effective and cheap bone substitute/regeneration treatments are carried out both today and in the future.

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Compliance with ethical standards

Conflicts of interest Authors have no potential conflicts of interest.

Ethical Statement There are no animal experiments carried out for this article.

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