# Current Concepts in Scaffolding for Bone Tissue Engineering


## Abstract

Bone disorders are of significant worry due to their increased prevalence in the median age. Scaffold-based bone tissue engineering holds great promise for the future of osseous defects therapies. Porous composite materials and functional coatings for metallic implants have been introduced in next generation of orthopedic medicine for tissue engineering. While osteoconductive materials such as hydroxyapatite and tricalcium phosphate ceramics as well as some biodegradable polymers are suggested, much interest has recently focused on the use of osteoinductive materials like demineralized bone matrix or bone derivatives. However, physiochemical modifications in terms of porosity, mechanical strength, cell adhesion, biocompatibility, cell proliferation, mineralization and osteogenic differentiation are required. This paper reviews studies on bone tissue engineering from the biomaterial point of view in scaffolding.



## Keywords

Bone tissue engineering; Scaffolds; Regeneration;


## Abbreviations

BCP: Biphasic calcium phosphate

BMP-2: Bone morphogenetic protein 2

CP: Calcium phosphate

ECM: Extracellular matrix

HA: Hydroxyapatite

NiTi: Nitinol, a metal alloy of titanium with nickel

PAA: Poly(acrylic acid)

PBT: Poly(butylene terephthalate)

PCL: Poly(ε-caprolactone)

PEG: Poly(ethylene glycol)

PGA: Polyglycolide

PH: potential of hydrogen

PLA: Polylactide

PLGA: Poly(l-lactide-co-glycolide acid)

PLLA: Poly(L-lactic acid)

PPF: Poly(propylene fumarate)

PU: Polyurethane

PVA: Polyvinyl alcohol

RE: rare earth elements

SBF: simulated body fluid

TCP: Tricalcium phosphate

TGF-β: Transforming growth factor beta

VEGF: Vascular endothelial growth factor

## Introduction

The incidence for all fractures among United States white population in 2010 was 4017/100,000 (1). High rates of bone vulnerability to trauma and fractures have attracted extensive researches in the bone tissue regeneration field. Bone has a hierarchical and complex structure that supports its diverse mechanical, biological and chemical functions. The heterogeneous and anisotropic structure of bone is composed of optimized irregular arrangement and orientation of macrostructures (such as cancellous and cortical bone), microstructures (like osteons, and single trabeculae), sub-microstructures (such as lamellae), nano-structures (like fibrillar collagen), and sub-nanostructures (such as minerals, and collagen molecules) (2). These components are architecturally designed to fulfill the functional needs of each particular bone. The mechanical properties of bone are made by its component phases and hierarchical structural organization (3). These properties are defined as compressive and bending strengths as well as the fracture toughness (4). Collagen and hydroxyl-carbonate apatite are the main components of bone with a porosity of 10-30% in the outer layer of the cortical bone and 30-90% in the inner layer of the cancellous bone. Some bones like ribs are more involved in tensile stress, while others, like talus, are under heavy compressive strength.

Any missing piece of bone due to traumas, tumors, avascular necrosis, and/or infections must be replaced with a proper functional alternative. Normally, the healing process starts with an inflammation phase, starting immediately after fracture and lasting up to several days, during which, the blood clot at the fracture cite initiates a stable framework for new bone formation. The clot is later replaced with fibrous and collagenous tissue, the soft callus, which will be hardened weeks after fracture. Bone remodeling will happen during

several months after the fracture. Autografts from different bones (fibula, iliac crest, ribs, etc.) are harvested and used for substitution of small missing bones; however, large bone voids are challenging. Tissue engineering has introduced new hopes as combination of cells, scaffolds, and biofactors for bone regeneration. Scaffolds are the masterpiece of bone tissue engineering. A bone scaffold is the 3D matrix that allows and stimulates the attachment and proliferation of osteoinducible cells on its surfaces. The following concerns must be considered in designing bone scaffolds: 1) biocompatibility in terms of cell attachment and proliferation as well as lack of toxicity and inflammatory reactions; 2) biodegradability for programmed safe substitution of the scaffold material with osteoid deposition (5); 3) mechanical properties to bear weight during the amelioration period (6); 4) proper architecture in terms of porosity and pore sizes for cell penetration, nutrients and waste transfer, and angiogenesis; 5) sterilibility without loss of bioactivity; and 6) controlled deliverability of bioactive molecules or drugs (7).

Probably, seeding cartilage cells onto bone spicules by Green in early 1970 was the first attempt for tissue scaffolding. Since then, seeding cells on properly engineered scaffolds from biocompatible biomaterials was suggested for new tissue formation (8). Bone scaffolds are optimally expected to have both osteoconductive and osteoinductive properties. Osteoconduction is the process whereby the scaffolds provide inward migration of osteoinducible cellular elements such as mesenchymal cells, osteoblasts, and osteoclasts, as well as the supplementary vasculature (9); whereas, osteoinductivity refers to inducing the differentiation of cells from different lineages into osteogenic cells (10). Various synthetic and natural, biodegradable and non-biodegradable materials have been used in the fabrication of bone scaffolds through different methods (11). Among polymers,

ceramics, metals, and composites, each has their specific resorption, surface reactivity, and biocompatibility properties that affect osteoconduction and osteoinduction (12).

Incorporation of growth factors into the scaffold biomaterial can improve osteogenesis and angiogenesis. Fibroblast growth factor (FGFs), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EPG), beta transforming growth factor (TGF-β), and bone morphogenic protein (BMP) are among the known growth factors used in scaffold for promoting bone plasticity (9, 13).

The current study has aimed to review the different materials commonly used in fabrication of scaffolds for bone tissue engineering applications. Generally, from the materials point of view, scaffolds for bone tissue engineering can be categorized into four classes: polymeric, ceramic, composite, and metallic scaffolds.

## Polymeric scaffolds for bone regeneration

Generally, polymeric materials provide more controllability on physiochemical characteristics of scaffolds such as pore size, porosity, solubility, biocompatibility, enzymatic reactions, and allergic response (14, 15).

Synthetic polymers were introduced for their excellent mechanical properties. They consist of aliphatic polyesters such as poly(lactic-acid)(PLA), poly(glycolic-acid)(PGA), and poly(caprolactone)(PCL), and their copolymers which are the most commonly utilized polymers in bone tissue engineering (16, 17). They are biocompatible, biodegradable, and can be easily fabricated into different shapes (18). They also can mechanically support demands for a wide range of applications in orthopedics (19). Other synthetic polymers in

bone tissue engineering includes poly(methyl methacrylate), poly(e-caprolactone), poly hydroxyl butyrate, polyethylene, polypropylene, polyurethane, poly(-ethylene terephthalate), poly ether ketone, and poly sulfone (20). Although, some synthetic polymers like Poly(propylene fumarate) (PPF) show high compressive strength and a controlled degradation time (21); however, they lose their strength due to rapid degradation in vivo and created local acidic environment which can make adverse tissue responses (22, 23). List of common polymeric scaffolds are presented in Table 1.

Natural polymeric scaffolds are composed of extracellular biomaterials in 3 classes (24): 1) proteins (collagen, gelatin, fibrinogen, elastin, keratin, silk, . . .); 2) polysaccharides (glycosaminoglycans, cellulose, amylose, dextran, chitin, . . .); and 3) polynucleotides (DNA, RNA) (25, 26). Extracellular matrix (ECM)-based scaffolds have been suggested as most similar ones to the original tissue. They have also shown osteoinductive properties. This group of natural scaffolds could be cell-derived (cells are used to generate new bone tissue or seeded onto a supporting matrix) or tissue-derived (bone tissue is directly used) (27-29). In contrast with autogenous ECM-based scaffolds, allogenous and xenogenous constructs should be devitalized or decellularized to avoid host immune response. Although, autogenous scaffolds have minimum immunological rejections; high histocompatibility; high osteoconductive, osteoinductive, and osteogenic properties; however, their application has been limited due to the need for additional surgery, donor site morbidity, and lack of availability. Allogeneic and xenogenic scaffolds have osteoconductive and osteoinductive effects with no need for additional surgery and donor site morbidity; however, they are limited due to the risk of disease transmission and immunogenicity. Availability is the main problem with the allogenous ECM-based scaffolds.

Although xenogenous scaffolds are abundant, they are limited due to DNA or mutation transfer (9, 30, 31). Strong human immune response to the residual cellular components of xenogeneic grafts is the main cause of transplant rejection. Transplantation of xenografts triggers inflammatory, immune, and coagulatory responses. Osteoblastic differentiation of human mesenchymal stem cells has been reported with porous bovine cartilage matrix derived scaffolds (32). Although, natural polymers have shown a great biocompatibility and controlled biodegradation; poor mechanical properties is the major concern with them as bone scaffolds (33, 34). The mechanical properties, biodegradability, and consistency from batch to batch are hardly controllable in naturally derived biomaterials. These biopolymers fail to provide sufficient architectural support and protection for the osteogenic cells. Also, immunogenic reactions and pathogen transmission due to the impure content in natural biopolymers may also happen (35).

## Ceramic scaffolds

Bone tissue consists of about 70% of hydroxyapatite (HA) and 30% of collagen by weight (36). Bioceramics almost mimic bone tissue and provide a higher osteoblasts adherence and proliferation compared to other materials (12, 37). Calcium phosphate ceramics (CPCs) have been greatly studied for bone tissue repair as tunable bioactive materials (38). Their physiochemical properties result in osteoconduction and osteoinduction. Hydroxyapatite, tricalcium phosphate (TCP), and their combination as biphasic and amorphous calcium phosphates (BCPs and ACPs) are common types of CPCs used in bone tissue engineering (23, 39). Recent studies have shown that modification of the mechanical strength, dissolution rates, and biocompatibility of the scaffold can be done through addition of

calcium phosphate (40). Doping β-TCP scaffolds with SiO2 (0.5%) and ZnO (0.25%) has been shown to upgrade the compressive strength to 2.5-fold and increase cell viability up to 92% (41). Solubility and surface topography are the most significant factors that influence cell behavior. Therefore, designing CPCs with suitable physical and chemical properties, and osteoinductive potential may improve their bioactivity in vivo (39).

Although the mechanical strength of ceramics is superior compared to polymers, it is still inferior to natural bones especially in terms of tensile and torsion strength. Also, HA has a great compressive (500-1000 MPa) and bending strength (115-200 MPa) in comparison with cortical human bone (100-230 and 50-150 MPa respectively); however, its fracture toughness (1 MPa $m^{0.5}$) is much less (2-12 MPa $m^{0.5}$) (4).

## Composed structures as optimized scaffolds

Recently, bioactive composite materials have been suggested to combine the advantages of two or more different materials (metallic, ceramic, and polymeric materials) (20). Composite materials improve the scaffold properties and allow controlled degradation for tissue engineering applications (42, 43). Excellent mechanical properties and osteoconductivity have made polymer/ceramic composites as promising materials for bone tissue engineering (44, 45). Composites of main natural bone bioceramics including CP, HA, and TCP with PLLA, collagen, gelatin, and chitosan have been greatly used as scaffolding materials for bone repair studies (11, 46-49). Reinforcement of high density polyethylene (HDPE) and PLGA with HA has introduced the structures that mimic and match bone properties as well as matrices for bone mineralization and cell differentiation (20). Calcium phosphate (CP)-polymer composites combine mechanical integrity and

bioactivity together (23). Collagen/bioglass nanocomposites have shown early mineralization and upgraded ALP expression (50). Simple calcium phosphate coating method on metals, glasses, inorganic ceramics and organic polymers (such as PLGA, PS, PP, and silicone), collagens, and silk fibers can improve biocompatibility or enhance the bioreactivity for orthopedic applications (11, 51). Mechanical reinforcement of these composite scaffolds has not yet matched the bone tissue demands in vivo.

The proliferation and differentiation rate of human mesenchymal stem cells on Fe foam coated with calcium-phosphate have shown to be higher than on uncoated samples (52). However, although, the coating enhances bioactivity, it inhibits the degradation of Fe foams (53). Addition of phosphorus increases the compressive yield which is comparable to the typical bone. Fe alloys have shown faster in vitro degradation compared to the pure form (54). Making porous structure from all biodegradable metals affects mechanical and degradation properties of the construct, the cell regeneration, and degradation product transport in the structure (55). Metallic scaffolds gridded with carbon and Ta deposits have shown high biocompatibility in animal experiments. Trabecular networks have shown appropriate bone growth and high stability; therefore, they can be used in orthopedic implants and instruments (56-60). Incorporation of Cobalt (Co) in meso-porous bioglass scaffolds have been shown to induce hypoxia that increased bone marrow-derived stem cell proliferation, differentiation, and bone-related gene expression (61).

## Metallic scaffolds in bone tissue engineering

Iron (Fe) and magnesium (Mg) based metals such as Mg-RE (rare earth) alloys, Mg-Ca, pure Fe, Fe-Mn alloys, and Fe foam have been used for bone scaffold (52, 62-65).

196 Fe has a 211GPa elastic modulus, higher than Mg (41GPa) and its alloys (44GPa) and 316L
197 stainless steel (190GPa) (66). However, inflammatory response and systemic toxicity have
198 been observed with in vivo implantation of Fe stents in descending aorta of rabbits (65).

199 Magnesium (Mg) and its alloys are other metals that are used in bone tissue engineering.
200 Bio-resorbabililty, high biodegradability, suitable mechanical properties, non-inflammatory
201 responses, and bone cells activation support have been counted as its characteristics (67-
202 69). Mg-based implants have shown superior increase in bone area in comparison with
203 PLA. Their corrosion layer also has been observed to contain calcium phosphates (67).
204 Porous Mg has better degradation behavior (slower hydrogen evolution) and slower
205 decrement of compressive yield strength in simulated body fluid (SBF) immersion tests
206 (70). Mg and its alloys have a wide range of elongation (from 3% to 21.8%) and tensile
207 strength (from 86.8 to 280MPa). Its elastic modulus (41–45GPa) is closer to that of the
208 bone compared to other metals (71). Very quick pure Mg corrosion produces hydrogen gas
209 at a high rate that is too to be handled with by the host tissue (67). Addition of 0.4–4wt%
210 REs, and other trace elements such as Cd and Al, has shown to decelerate the corrosion rate
211 of alloyed Mg (72). Also porosity and pore size modifications can adjust its stiffness and
212 strength range to that of bone; however, higher porosity decreases the corrosion resistance
213 of Mg. Cerium, neodymium, calcium, and praseodymium are used in orthopedic
214 applications with Mg alloys (64, 73). High corrosion and toxins of Mg has limited the
215 application of this metal in medicine (74). Early stages of in vivo biocompatibility
216 studies of Mg scaffolds have recently been started (75).

Titanium (Ti) porous scaffolds have also been studied as bone replacement materials (76). These elements are not biodegradable and do not integrate with biomolecules. Surface modifications has been suggested to improve Ti bioactivity (77). Ti and its alloy particles have shown inhibition of bone-cell proliferation and reduction in bone formation markers (78). Oxidization ($TiO_2$), surface of modification, and combination of chrome-cobalt (Cr-Co) alloys and stainless steel with titanium alloys can improve its biocompatibility. Titanium-aluminum-vanadium alloys (ASTM F1472, ASTM f136, ASTM F110) possess better mechanical properties compared to pure titanium and can be used in joint implants. Non-toxic alloys of beta titanium like Nb, Ta, and Zr are also offered (79). Biocompatibility has been increased in the 2nd generation of titanium alloys like Ti-15 Mo-5Zr-3Al, Ti-15Zr-4Nb-2Ta-0.2Pd, Ti-12Mo-6Zr-2Fe, and Ti-29Nb-13Ta-4.6Zr. Titanium and titanium alloy trabecular networks are used in spine surgery (80). Incorporation of TGF-β and BMP has shown to improve the osteoinductivity of titanium and its alloys (13, 81). Porous titanium and its alloys can be used in permanent implants due to their good mechanical properties (82-84). Nitinol (NiTi), a metal alloy of titanium with nickel, has shown high biocompatibility and significant plasticity for bone scaffolding; it has been also used for nail manufacturing and spine separator in scoliosis treatment (85-87). Nitinol-coating of stainless steel surfaces results in higher biocompatibility. The use of NiTi alloys has been banned in America and Europe due to allergic response and toxicity problems of Ni ions (88).

Tantalum (Ta) is widely used in bone tissue engineering and knee replacement surgeries. The similar elasticity of Ta to bone can decrease the imposed stress levels (56).

Metal implants are light-weight, strong, biocompatible, and osteoconductive; but they may inhibit of bone formation markers, stimulation of bone loss or resorption, show poor osseointegration with the surrounding bone due to the stiffness difference, and release toxic ions by corrosion which may cause inflammatory responses (9, 12, 31). Metal scaffolds are usually unrecognizable by biological factors too. They act more as permanent implants than scaffolds.

Although, an optimal scaffold for bone tissue engineering is still a question, none of the studied materials alone has fulfilled the bone scaffold requirements. Polymers are great for designing controllable biodegradability beside osteoconductivity; however, they are weak in mechanical resistance. Ceramics have better mechanical strength and are osteoinductive; however, they are vulnerable to fracture. Hence, recent researches have been shifted towards composite materials with incorporation of biomolecules. Proper integration between ceramic particles and polymeric matrix is necessary for the improvement of mechanical performance (87). Modification of scaffold chemistry, cells seeding, and growth factors like TGF-β, BMP, and VEGF can improve osteoinductivity and angiogenesis (23, 53, 88, 89). Beside, scaffold pore size and porosity can control the rate and efficiency of delivery.

According to the conventional definition, scaffolds are meant to be biodegradable. Metals have introduced as mechanically strong materials, but, they are non-biodegradable. Therefore, none of suggested materials could be perfect to be used for bone scaffolds unless the definition boarders are trespassed. Binary combinations of polymer/ceramic,

polymer/metal, or metal/ceramic composite materials have been reported as mechanically strong scaffolds; however, they have not matched the original bone tissue yet.

## Summary and Conclusion

Considering the low mechanical properties of polymeric, ceramic, and composite biomaterials as well as lack of biocompatibility of metals, an optimal scaffold for bone tissue engineering applications can only be a well-orchestrated multiphasic construct composed of all biocompatible materials. The core of such an optimal structure can be composed of a ceramic-coated biocompatible metal in order to compensate the mechanical properties. The next phase might be an osteoinductive composite loaded with proper growth factors. Surface modifications can be done by biomolecules like collage and/or gelatin. Aligned porosity with adjusted pore sizes that allow angiogenesis must also be considered. As these scaffolds with metallic cores trespass the regular definition of degradability, they will be the next generation of scaffold/prosthesis complexes, the "ScaTheses". The scaffold part will play its role and degrade in a time manner, while, the metallic portion will stay much longer in the body, without interrupting the bone physical integrity and hence function.

## Disclosure Statement

The authors declare no conflict of interest regarding this manuscript.

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