Review

Recent advances in bone tissue engineering scaffolds

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Bone disorders are of significant concern due to increase in the median age of our population. Traditionally, bone grafts have been used to restore damaged bone. Synthetic biomaterials are now being used as bone graft substitutes. These biomaterials were initially selected for structural restoration based on their biomechanical properties. Later scaffolds were engineered to be bioactive or bioresorbable to enhance tissue growth. Now scaffolds are designed to induce bone formation and vascularization. These scaffolds are often porous, made of biodegradable materials that harbor different growth factors, drugs, genes, or stem cells. In this review, we highlight recent advances in bone scaffolds and discuss aspects that still need to be improved.

Bone scaffolds

Bone tissue engineering is a complex and dynamic process that initiates with migration and recruitment of osteoprogenitor cells followed by their proliferation, differentiation, matrix formation along with remodeling of the bone. Major advances in bone tissue engineering with scaffolds are achieved through growth factors, drugs and gene deliveries. Bone scaffolds are typically made of porous degradable materials that provide the mechanical support during repair and regeneration of damaged or diseased bone [1]. Requirements for an ideal scaffold are highlighted in Box 1.

Biomolecule delivery

Scaffolds are also used to deliver biomolecules that can facilitate bone tissue engineering. Biomolecules integrated into scaffolds are proteins/growth factors such as TGF-β, BMP (see Glossary), IGF, FGF, and VEGF. These growth factors control osteogenesis, bone tissue regeneration, and ECM formation via recruiting and differentiating osteoprogenitor cells to specific lineages [2]. Therefore, incorporating different growth factors and other biomolecules are of special interest for bone tissue engineering. For example, IGF helps in migration of different bone cells required for bone healing whereas BMPs induces early stage proliferation and differentiation of osteoprogenitor cells [3,4]. In animal models, it has been shown that introducing specific biomolecules can enhance the union of nonunion type (a fracture that does not heal by itself after several months) bone fractures [5]. The effective

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Glossary

45S5 Bioglass®: High silica containing glassy bioactive/bioresorbable material that was first proposed in 1969 as an alternative to conventional bioinert materials for bone tissue repair [66].
Alkaline phosphatase (ALP): an osteoblast differentiation marker [67].
Bone marrow-derived stem cells (BMSC): bone marrow stromal cells. These are adult stem cells isolated from samples of bone marrow [68].
Bone morphogenetic protein (BMP): critical in embryonic skeletal development, bone formation, maturation, and repair. Also known as 'growth and differentiation factors (GDFs)'. Activated BMPs induce the transcription of specific genes intracellularly through Smad proteins [3].
Cancellous bone: the inner part of a bone that hosts the bone marrow and responsible for blood cell generation; often called 'spongy bone' due to its resemblance to sponge or foam. Cancellous bone is weak in mechanical properties: Young's modulus is between 0.1 and 2 GPa and the compressive strength is between 2 and 20 MPa [14].
Cortical bone: the outer part of a bone that is dense with high strength (100 and 200 MPa) and high modulus (15–25 GPa). The primary role of cortical bone is to provide structural support to the body and to protect vital organs [14].
Embryonic stem cells (ESC): These cells are derived from embryos and are pluripotent and can differentiate into all somatic cell types [69].
Extracellular matrix (ECM): self-assembled macromolecules generally consisting of collagens, noncollagenous glycoproteins, hyaluronan, and proteoglycans. It works as a reservoir for different cytokines and growth factors [70,71].
Fibroblast growth factor (FGF): secreted glycoproteins that are sequenced in ECM and cell surface by heparan sulfate proteoglycans. FGFs are released from the ECM by heparinases and regulate cellular proliferation, survival, migration, and differentiation [72].
Human umbilical cord mesenchymal stem cells (hUCMSC): multipotent cell line capable of differentiating in to osteoblasts, chondrocytes, and adipocytic cells [69].
Human umbilical vein endothelial cells (HUVEC): derived from umbilical vein and used to study angiogenesis [69].
Insulin-like growth factor (IGF): Regulates several key cellular processes, including proliferation, movement, and inhibition of apoptosis. Expression of IGF regulates anchorage independent growth and eventual activation of P13K (phosphatidylinositol 3-kinase) [4,56].
Laser engineered net shaping (LENS^{TM}): a layer-by-layer SFF process that uses a high power laser (between 500 W and 2 kW) to melt metal powders to form 3D structures based on CAD data. A laser is focused onto a metal substrate to create a molten metal pool where metal powder is externally fed into the metal pool in a controlled environment. Moving the substrate in the X–Y direction creates a pattern and fill material in the desired area forming a layer. The next layer is built on top of the previous layer. This procedure is then repeated until the entire body is produced. Apart from the macrostructure, the pore structure can also be controlled in LENS processed parts [20,21].
Mesenchymal stem cell (MSC): a multipotent cell line capable of differentiating into osteoblasts, chondrocytes, and adipocytic cells [73].
Osteoconductivity: a property of materials that allows bone cells to adhere, proliferate, and form extracellular matrix on its surface and pores [14].
Osteoinductivity: a property of materials that induces new bone formation through biomolecular signaling and recruiting progenitor cells [14].
Parthenogenetic ESCs (PESC): ESCs derived from human oocytes that is an alternative stem cell source for tissue repair and regeneration [74].
Solid freeform fabrication (SFF): a generic term used to describe three dimensional layer-by-layer printing of any object without any part-specific tooling from its computer-aided-design (CAD) file. The process has been successfully used to fabricate polymer, ceramic, metal, and composite scaffolds for bone tissue engineering [15–17].
Transforming growth factor-β (TGF-β): protein superfamily related to bone that stimulates recruitment and proliferation of mesenchymal cells, their differentiation into osteoblasts and/or chondrocytes, and ECM production [75].

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Vascular endothelial growth factor (VEGF): an angiogenic signal that increases the permeability of endothelial cell to extravasate and lay down a provisional ECM. VEGF is responsible to generating new blood vessels in the tissue [76,77].

incorporation of biomolecules and growth factors in scaffolds could reduce wound healing time and thus help in patient recovery.

Angiogenesis in bone scaffolds

Bone is highly vascularized; therefore, the performance of a bone scaffold is dictated by its ability to induce new blood vessel formation [6–8]. *In vivo* conditions, supply of oxygen and nutrients are essential for the survival of growing cells and tissues within scaffolds [8]. The inflammatory wound healing response induces spontaneous vascularization after scaffold implantation [6], although it takes weeks to form a complex network of blood vessels. Osteoconductive or osteoinductive bone scaffolds do not induce vascularization. Moreover, improper and insufficient vascularization leads to oxygen and nutrient deficiency, which may result in non-uniform cell differentiation and cell death [9]. VEGF can be used to induce a complex network of blood vessels throughout a scaffold [6,10–13].

This review focuses on recent advances in biomolecule-incorporated scaffolds and their osteogenic and angiogenic properties. We first discuss physical and mechanical properties of bone scaffolds (Table 1) and their fabrication techniques. We then discuss *in vitro* responses and *in vivo* osteoconductive properties of these scaffolds. Finally, we discuss the role of various biomolecules delivery on osseointegration and angiogenesis in the bone tissue engineered scaffolds. We conclude with critical issues and future developments of scaffolds for next generation bone tissue engineering.

Design and fabrication of scaffolds

Bone is a natural composite of collagen and hydroxycarbonate apatite with 10–30% porous hard outer layer, i.e., cortical bone; and 30–90% porous interior, i.e., cancellous bone. Mechanical properties of bone vary widely from cancellous to cortical bone which along with complex geometry makes it difficult to design an "ideal bone scaffold" (Box 1). The key factors for an ideal scaffold for bone tissue engineering are: (i) macro- (pore size >100 μm) and microporosity (pore size < 20 μm); (ii) interconnected open porosity for *in vivo* tissue in-growth; (iii) sufficient mechanical strength and controlled degradation kinetics for proper load transfer to the adjacent host tissue, (iv) initial strength for safe handling during sterilizing, packaging, transportation to surgery, as well as survival through physical forces *in vivo*; and (v) sterile environment for cell seeding [6,19].

Fabrication techniques

Among various fabrication techniques, SFF based techniques are probably the most widely studied for fabricating 3D interconnected porous scaffolds [15–17]. SFF is a general approach in which 3D parts are printed layer-by-layer based on a computer-aided-design (CAD) file. There are many commercial SFF techniques available for different materials. Figure 1a,b shows schematics of the 3D printing process. First a CAD file is created according to the geometry and porosity of the scaffold (Figure 1a). The 3D printing system has a deposition bed, a feed bed, a powder spreader, a print head, and a drying unit (Figure 1b). Figure 1c shows a 3D ceramic scaffold printer (R-1 R&D printer by ProMetal, Ex One Company, Irwin, PA). Initially, the printer head sprays the binder on the loose powder according to the specific CAD file, followed by lowering the deposition bed and raising the feeder bed. A metallic roller then evenly

Box 1. Requirements for an ideal scaffold

The biomechanical system of bone is complex so that the following requirements for an ideal scaffold are diverse.

- *Biocompatibility* One of the primary requirements of any bone scaffolds is biocompatibility; a term which has been described in many ways. Biocompatibility of a scaffold is described as its ability to support normal cellular activity including molecular signaling systems without any local and systematic toxic effects to the host tissue [79]. An ideal bone scaffold must be osteoconductive where the scaffold allows the bone cells to adhere, proliferate, and form extracellular matrix on its surface and pores. The scaffold should also be able to induce new bone formation through biomolecular signaling and recruiting progenitor cells, a property known as osteoinduction. Furthermore, an ideal scaffold needs to form blood vessels in or around the implant within few weeks of implantation to actively support nutrient, oxygen, and waste transport [14].
- *Mechanical properties* The mechanical properties of an ideal bone scaffold should match host bone properties and proper load transfer is important as well. Mechanical properties of bone vary widely from cancellous to cortical bone. Young's modulus of cortical bone is between 15 and 20 GPa and that of cancellous bone is between 0.1 and 2 GPa. Compressive strength varies between 100 and 200 MPa for cortical bone, and between 2 and 20 MPa for cancellous bone. The large variation in mechanical property and geometry makes it difficult to design an 'ideal bone scaffold' [14].
- *Pore size* A must have property for scaffolds is interconnected porosity where the pore size should be at least 100 μm in diameter for successful diffusion of essential nutrients and oxygen for cell survivability [6]. However, pore sizes in the range of 200–350 μm are found to be optimum for bone tissue in-growth [80]. Furthermore, recent studies have indicated that multi-scale porous scaffolds involving both micro and macro porosities can perform better than only macro porous scaffolds [30]. Unfortunately, porosity reduces mechanical properties such as compressive strength, and increases the complexity for reproducible scaffold manufacturing. Researchers have explored porous scaffolds using polymers, ceramics, composites and metals. Strength of dense bioceramic materials matches close to the cortical bone, and different polymers to that of cancellous bone, however ceramic–polymer composite scaffolds are typically weaker than bone. Porous metallic scaffolds meet the mechanical requirements of bone, but fail to provide the necessary implant-tissue integration and add the concern related to metal ion leaching [22].
- *Bioresorbability* Bioresorbability is another crucial factor for scaffolds in bone tissue regeneration [79]. An ideal scaffold should not only have similar mechanical properties that of the host tissue, but also be able to degrade with time *in vivo*, preferably at a controlled resorption rate and eventually creating space for the new bone tissue to grow. The degradation behavior of the scaffolds should vary based on applications such as 9 months or more for scaffolds in spinal fusion or 3–6 months for scaffolds in cranio-maxillofacial applications. Naturally, design and manufacturing of multi-scale porous scaffolds having ideal composition including targeted biomolecules, mechanical properties and related bioresorbability are some of the key challenges today towards their successful implementation in bone tissue engineering [14,37].