The potential impact of bone tissue engineering in the clinic

Bone tissue engineering (BTE) intends to restore structural support for movement and mineral homeostasis, and assist in hematopoiesis and the protective functions of bone in traumatic, degenerative, cancer, or congenital malformation. While much effort has been put into BTE, very little of this research has been translated to the clinic. In this review, we discuss current regenerative medicine and restorative strategies that utilize tissue engineering approaches to address bone defects within a clinical setting. These approaches involve the primary components of tissue engineering: cells, growth factors and biomaterials discussed briefly in light of their clinical relevance. This review also presents upcoming advanced approaches for BTE applications and suggests a probable workpath for translation from the laboratory to the clinic.

First draft submitted: April 20 2016; Accepted for publication: 11 July 2016; Published online: 23 August 2016

Keywords: 3D printing • additive manufacturing • biomaterials • bioreactor • bone tissue engineering • cell-based therapy

Bone tissue engineering (BTE)

Globally, bone is the second most transplanted tissue. More than two million bone graft procedures are performed annually worldwide, of which almost 1.6 million bone grafts are performed in the USA alone [1,2]. In 2010, revenue generated from the use of allogeneic and alloplastic bone graft substitutes was approximately US\$1.3 billion in the USA, and is expected to rise to US\$2.3 billion by the end of 2017 [3,4]. The standard of care treatment for critical size bone defects is vascularized autografts. However, drawbacks of this option include lack of supply, donor site morbidity and infection. Additionally, vascularized autografts often require complex alterations to reconstruct the external shape or cortical distribution of the defect site [1,5]. As alternatives, allogeneic and xenogeneic bone grafts face their own challenges such as lack of vascularization/blood supply, immunogenicity, disease transmission and, in some cases, donor shortage [6].

The goal of BTE is to regenerate viable skeletal elements through the use of biomaterials, cellular therapies and/or growth factors. Tissue engineering, in general, is depicted in Figure 1 as a three-legged stool. BTE is particularly important when treating critical size defects, which are defined as those that are too large to be healed through natural processes alone [7]. More specifically, the objective is formation of remodeled bone that can sustain normal stress–strain trajectories and/or properly protect underlying structures. For this reason, the majority of BTE research has focused on the development of biomaterials that simultaneously provide a scaffold for tissue growth and facilitate the production of cortical bone function where needed.

Current challenges in translating BTE therapies to the clinic

Despite the amount of money and time already invested, clinical progress in BTE has, unfortunately, been minimal [8,9]. The

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Figure 1. Tissue engineering represented as a stool resting on three legs made of growth factors (and other bioactive molecules), cells and scaffolds.

most commercially successful BTE products in the clinical market are Infuse® (Medtronic Inc., MN, USA) and ACTIFUSE (Baxter International Inc., IL, USA) [10]. These and several other products, described in Table 1, have limited US FDA-approved indications and represent a miniscule fraction of what has been tried experimentally.

We propose that insufficient consideration of the requirements of the regulatory pathway and the cost of bringing new products to the clinic are the major reasons for the disparity between the research investment and clinical payoff in BTE. To date, most BTE research has focused on the generation of entire, new bone segments from a single material with uniform material properties; composite implants combining different biomaterials, including inert biomaterials, such as metals, polymers and ceramics, are rarely proposed. Furthermore, little attention has been paid to patient-specific implant architecture and mechanical function or to designing viable strategies that would restore site-specific function [11]. Patient-specific implants could be achieved through the use of computer-aided design (CAD) [12,13]. However, procedure-planning and implant-CAD software rarely include modeling of implant mechanical properties or the anticipated *in vivo* biomechanical load. The exclusion of biomaterials from the discussion of standard medical practice (i.e., regenerative medicine) is exemplified by the lack of experiments using standard immobilization hardware during the initial phases of bone healing in animal model work. Full therapeutic pathways, including fixation and the need to transition from immobilization to load bearing, are rarely discussed in reports of BTE therapy studies. A notable exception is a very recent compilation by Farré-Guasch *et al.*, which discusses additive manufacturing strategies for oral and maxillofacial surgery [14].

Although researchers are often interested in gene expression and the different stages of healing, translation of this information into therapies that present a staged healing process that includes site-specific

remodeling has not yet become a reality. A staged healing strategy could, for example, involve multiple, minimally invasive surgical procedures during the regenerative process (e.g., the addition of new bone constructs to extend healed/formed bone or removal of fixation hardware to prevent stress shielding/concentration). Laboratory and clinical procedures could produce useful data for the development of staged regenerative bone interventions, which may be a more successful restoration strategy than one-size-fits-all regenerative models. In this regard, useful data may include the maximum amount of bone regeneration that can be achieved per surgery, postoperative rates of bone formation, types of infection, and stress-shielding that can be expected if fixation hardware is not removed.

BTE comprises three elements: cells, growth factors (and other bioactive molecules) and scaffolds, depicted in Figure 1 as a three-legged stool. Currently, no clinical therapies simultaneously utilize all three of these elements. However, these elements, in conjunction with bioreactors, may reveal a comprehensive restoration strategy. Recent research into bioreactor pre-culturing of bone implants has emphasized the study of co-culturing multiple cell types, staged growth factor dosing and the use of shear and compressive forces to drive fate [15]. However, as yet, there are no widespread bioreactor approaches used in the clinic to prepare bone repair implants (see section titled “Pre-culturing strategies: static versus perfusion bioreactor culture”).

Recent successes in BTE

Recent laboratory research into cell-based BTE therapies has explored strategies utilized in other tissue types. Specifically, hydrogel-based cell-printing technologies or lyophilized, thicker decellular extracellular matrix with secondary cell seeding approaches have proven successful for thin tissue regeneration (e.g., skin) [16,17]. Although these methods have worked in mostly sheet-like tissue types, there is notable concern that fixation-free technologies may not be applicable to non-sheet-like organs such as bone, especially given its critical structural and protective functions. Furthermore, bone as a 3D tissue, has complex maintenance requirements, especially in regards to vascularization [18].

Cell printing, also known as ‘bioprinting’, is an established method by which cells suspended in a hydrogel medium can be directly printed into 3D structures. This technology allows one to control the placement of cells in precise 3D arrangements. However, clinical concerns over the maintenance of cell viability while undergoing the shear stress of printing and prior to implantation in increasingly complex 3D structures are valid. In addition, a single cell type is

unlikely to be sufficient for tissue formation since tissues are complex structures formed from combinations of multiple cell types; thus, printing of more than one cell type in the same environment is another important consideration for future bioprinting research. Furthermore, large 3D constructs often require a prohibitive number of cells and also pose the problem of nutrient delivery and cellular waste-product removal. Therefore, we expect that a great deal of further research will be necessary to apply bioprinting to large-scale tissue engineering applications, especially the sizeable 3D tissues that will be necessary for clinically relevant therapies [19,20]. Conversely, notable early success has been observed by culturing 3D printed scaffolds inside

of body compartments (e.g., intraperitoneal, intramuscular, subcutaneous and dermal junctional locations). These so-called *in vivo* bioreactors may control which types of cells may inhabit each space and respond to site-specific needs (e.g., muscle attachment, marrow production, immune function, vascularity, mechanical loading) [21]. These systems provide physiological and vascular support to the scaffolds, promoting cellular in-growth and/or maturation. Such scaffolds can be placed *in situ* for restoration of a specific defect and can then, secondarily, be translocated to the defect site once the tissue construct and its vasculature have sufficiently matured. Scale-dependent geometry and mechanical function are potential starting points for the design of

Table 1. List of some of the most successful commercially available bone tissue-engineered products along with their description.

No.	Company	Product	Product description
1	Medtronic Inc., Minneapolis, MN, USA	Infuse® bone graft	Solution containing rhBMP-2 and an ACS used to fill the LT-CAGE® lumbar tapered fusion device
2	Baxter International Inc., Deerfield, IL, USA	Actifuse	Silicate substituted calcium phosphate
3	Orthovita Inc. Malvern, PA, USA	Vitoss	Bioactive glass and calcium phosphate
4	Olympus Biotech, Hopkinton, MA, USA	OP-1® Putty	rhBMP-7, Type I bovine collagen matrix and putty additive carboxymethylcellulose sodium
		OP-1 Implant	Osteoinductive and osteoconductive bone graft material consisting of collagen matrix, 1 g of Type I bovine collagen, 3.3 mg of rhBMP-7 and 2–3 cc of saline
5	DePuy Orthopaedics, Inc., Warsaw, IN, USA	Vertigraft® Allograft	Variety of structural allografts and an array of osteobiological properties, including, but not limited to, VG1 ALIF, VG2 PLIF, VG2 RAMP and VG2 TLIF
		Symphony® I/C Graft Chambers	Osteoinductive and osteoconductive product comprised of a mixture of allograft cancellous chips and DBM. Also contains naturally occurring cascade of BMP's and growth factors.
		Restore® Orthobiologic Soft Tissue Implant	Resorbable scaffold to reinforce weakened or damaged soft tissue repair including rotator cuff, patellar, Achilles, biceps, quadriceps and other tendons
		Optium DBM® System	Includes both Optium DBM® Gel and Putty. Both contain osteoinductive and osteoconductive properties
		Healos® Fx Injectable Bone Graft Replacement	An osteoconductive and easy to use bone graft replacement which provides a scaffold. It has potentially osteogenic properties when autologous bone marrow is added.
		Healos Bone Graft Replacement	Osteoconductive matrix comprised of cross-linked collagen fibers that are fully coated with hydroxyapatite. When combined with BMA, product provides an environment for osteoprogenitor cell attachment, proliferation, and differentiation
		Conduit® TCP Granules	Osteoconductive synthetic porous ceramic graft material made of tricalcium phosphate which provides a scaffold for bone cell attachment

ACS: Absorbable collagen sponge; BMA: Bone marrow aspirate; DBM: Demineralized bone matrix; allograft bone particle.

Figure 2. Path for clinical translation of bone tissue-engineered biomaterials, cell-based therapies and growth factors (and other bioactive molecules) that prove effective, first preclinically and then in clinical trials.

scaffolds that can succeed in bioreactor preculturing and subsequent healing of an implantation site.

In this review, we will present BTE research, emphasizing a strategic view of cells, growth factors (and other bioactive molecules), scaffold materials and fabrication strategies, and bioreactors that might soon be

part of staged bone therapies. Such methods may facilitate the rapid translation from the laboratory setting ('benchtop') to the patient within the clinical setting ('bedside').

Commercially available BTE therapies for bone deficit

The pathway for translation of BTE research to the clinic is presented in Figure 2. In order to meet clinical demand, commercially developed therapies have emphasized each of the three legs of the tissue engineering stool: cell-based therapies, growth factor-based (and other bioactive molecules) therapies, and/or biomaterial-based therapies. Therefore, we will review the commercially available therapies for BTE based on their alignment with these three elements.

Cell-based therapies

Five general bone progenitor cell types are currently been used for bone regeneration: embryonic stem cells; fetal stem cells (e.g., fetal mesenchymal stem cells [MSCs]); human umbilical vein endothelial cells; induced pluripotent stem cells (iPSCs) [22,23]; and adult stem cells (e.g., bone marrow stromal cells, bone marrow-derived MSCs, adipose-derived MSCs [24,25], muscle-derived MSCs) [26,27], dental pluripotent stem cells [28], and iPSCs. Thus far, bone marrow-derived MSCs have shown the greatest bone repair potential. A significant advantage of bone marrow-derived MSCs as compared with other sources is the lack of MHC class II cell surface markers and co-stimulators prior to end-stage differentiation [29]. They are, therefore, not visible to the immune system [30]. This characteristic is expected to enable the use of allogeneic bone marrow-derived MSCs for the clinical treatment of bone defects [31]. In summary, advantages associated with MSCs derived from bone marrow are ready commercial availability, ease of culture, maintenance of differentiation potential, increased osteogenic potential and, finally, upon culturing at high passage number their osteogenic commitment phenotype is lost only in terminal lineages [32].

Bone marrow- and adipose-derived stem cells have been used in investigational trials in the USA and in clinical trials for bone repair in countries other than the USA [33]. However, there are a negligible number of cell-based, regenerative bone therapies approved by the FDA in the USA. In part, this is due to the high cost of demonstrating clinical safety, efficacy, and compliance with regulatory oversight [34]. Other than that, we speculate 'the lack of understanding by researchers' and generally the 'high expense' of clinical translation of strategies that have shown promise in the laboratory as the major causes.

Figure 3. Various signaling pathways involved in cell signaling. Many of these pathways such as the Wnt, hedgehog and MAPK signaling pathways are involved in bone formation. The MAPK pathway is activated by growth factors such as EGF, FGF and PDGF. These growth factors may also be useful in bone tissue engineering (Source: [48]).

Figure 4. Diagrammatic representation of osteoblastic differentiation of progenitor bone cells in response to BMP signaling via the Smad pathway. BMP attaches to extracellular matrix (A) through the binding of integrins (B). The cascade of events after the binding of BMP (C) to its receptors (D) include the activation of R-SMAD (SMAD 1, 5 and 8) through phosphorylation (E), thereafter, binding with SMAD 4 to form a complex that enters the nucleus and binds to DNA causing the transcription of osteoblastic genes.

The use of stem cells is strictly regulated by the FDA. In particular, the FDA requires that cells be “minimally manipulated, labeled, or advertised for homologous use only, and not combined with another drug or device” [35,36]. Without prior FDA approval, cells can only be used clinically if they are autologous, used on the same day of extraction, and are not manipulated by additional factors, drugs, or devices. Without prior FDA approval, cells can only be used clinically if they are autologous, used on the same day of extraction, and are not manipulated by additional factors, drugs, or devices. The use of bone progenitor cells after more than ‘minimal manipulation’, such as *in vitro* expansion, is not currently approved by the FDA for use in a bone repair therapy [37,38]. Furthermore, embryonic stem cell- and induced pluripotent stem cell-based therapies are not expected to appear in the clinic in the near term given the

current FDA stance on the use of these cells. However, despite these challenges, the FDA has approved therapies that utilize MSCs and many are in clinical trials. Same-day isolation of bone marrow-derived cells has been employed in a number of clinical applications [39,40]. Regenexx™ (Regenerative Sciences, Inc., Broomfield, CO, Canada) provides bone marrow-derived stem cells for nonunion fractures. Autologous bone marrow-derived osteoprogenitor cells along with allograft material (demineralized bone matrix and cancellous bone) have been used clinically as Osteocele plus® (NuVasive, Inc., CA, USA) [41,42]. Some other examples of commercially available, cell-based allograft products include ‘Ovation OS’ (Osiris Therapeutics, Inc., MD, USA), which contains allogeneic osteoblasts and MSCs, growth factors and extracellular matrix within cancellous bone matrix. Another such product is ‘Trinity evolution’ which comprises allogeneic cancellous bone along with osteoprogenitor cells and osteogenic cells inside a matrix with demineralized cortical bone [43].

Table 2. Complication arising due to the use of growth factors (i.e., BMP-2 and BMP-7) for bone tissue engineering.		
Growth factor	Complications	Ref.
BMP-2	Swelling/inflammation in the neck, painful seroma, ectopic bone formation, retrograde ejaculation	[61–72]
BMP-7	Bone resorption	[73,74]

Growth factors in scaffold-based or cell-based therapies
Growth factors are cell-signaling molecules that can modulate cellular behavior. Various signal transduction pathways involved in cell-signaling are presented in Figure 3. The main factors (e.g., growth factors and other bioactive molecules) involved in bone healing include osteogenic (bone morphogenetic proteins, activins, among

others), angiogenic (PDGF, VEGF, among others), inflammation-control (TNF α , interleukins, interferon- γ , among others) and systemic factors (Vitamin D, growth hormone, calcitonin, parathyroid hormone, among others) [44–47]. These cell signaling molecules have been studied extensively in the laboratory but few have been used in therapies that have gone through clinical trials.

RUNX2 acts as a key transcriptional factor for osteoblastic differentiation. As MSCs proceed down osteogenic lineages, *RUNX2* plays a vital role in the differentiation of osteogenic precursors to preosteoblasts. Moreover, *RUNX2* also controls the expression of osteogenic markers such as alkaline phosphatase and osteocalcin [49]. For BTE applications, researchers have tried to incorporate molecules such as N-acetyl cysteine within scaffolds to promote *RUNX2* expression. N-acetyl cysteine incorporated scaffolds have significantly increased *RUNX2* production, and thus, enhance osteogenic differentiation of MSCs. The expression of *RUNX2* in osteoblastic lineage cells has been well studied. At the molecular level, the expression of *RUNX2* is directed by heterodimeric Smad proteins. Smad proteins are recruited and activated via BMP signaling pathways [50–52]. As shown in Figure 4, when BMP binds to its receptors, receptor-regulated Smads (R-Smads, which are a complex of Smads 1, 5 and 8) become phosphorylated. These phosphorylated R-Smads then bind to Smad 4 and enter the nucleus. This Smad complex binds to the DNA, leading to the transcription of genes involved in osteoblastic differentiation (e.g., *RUNX2*).

Two BMPs, which are cytokines belonging to the TGF- β family, are FDA-approved for clinical use: BMP-2 (Infuse[®], MN, USA) [53] and BMP-7, which is coupled with bovine collagen type I as its carrier (OP-1[®], Olympus, MA, USA) [54]. The clinical performance of each of these products has been comparable to autografts in certain BTE applications [55,56], and both products have received FDA approval for limited indications [55,57].

rhPDGF (Augment[®] Bone Graft, BioMimetic Therapeutics, TN, USA) is also commercially available in some countries. This product uses β -tricalcium phosphate as the carrier vehicle [54,58]. It has been used to treat foot, ankle, and distal radius fractures clinically in Canada, Australia and New Zealand, and it is currently under review by the FDA and commercially available in the USA.

Despite the success of various bone-promoting growth factor-based products, it is worth mentioning that many of these molecules have very powerful local or paracrine and, in some cases, pleiotropic effects. Reported complications associated with the use of BMP-2 and BMP-7 include ectopic bone formation, bone resorption and/or remodeling at the implanta-

Table 3. US FDA 510(k) medical devices approved for bone applications between 2011 and 2015.

No.	Medical devices	Devices (number)
Replacement-based medical devices		
1	Bone implant systems	47
2	Bone plates/fasteners	44
	Total	91
Regeneration-based medical devices		
1	Allograft	6
2	Bone tissue engineered biomaterials	30
	Total	36
Medical devices for indirect bone applications		
1	Hemostatic bone putty	10
2	Hearing systems	6
3	Imaging processes	5
4	Bone aspiration needles	7
	Total	28

tion site, severe swelling/inflammation around the site of use, pain, and seroma (Table 2). Additional concerns include the potential for induction of malignancies, oncologic recurrences, and teratogenic effects [59]. Given the limitations and concerns regarding pleiotropic effects from whole cytokine molecules (e.g., BMP-2), confidence in their localized use will likely be tempered. Locally metabolized, scaffold-bound ligands [60], with or without circulating bioactive molecule support, may prove to be a more viable therapeutic. That is, the use of functionalized scaffolds to control cell attachment, proliferation and/or differentiation. Irrespective of their use in circulating or bound molecules, it appears that BMP-2 and PDGF will continue to play a role in clinical bone therapies as will more recently researched cytokines (e.g., BMP-7). The adoption of these molecules is likely to be enhanced by the use of carriers with reliable release kinetics.

Biomaterial-based therapies

Bone substitute materials now in clinical use include nonresorbable metallic, ceramic and polymeric biomaterials or resorbable ceramic and/or polymeric biomaterials [75]. For the purpose of this review, we define biomaterials-based products as products without any cells or growth factors. Commercially available BTE biomaterials are present mostly as alloplastic bone void fillers [76–78]. These materials do not claim to encourage bone formation, neither do they pro-

Table 4. US FDA 510(k) cleared bone tissue-engineered (resorbable) medical devices approved between 2011 and 2015.

Product Name	Company	Composition	Form	Property
2015				
NanoBone	ARTOSS GmbH, Rostock, Germany	Phase-pure nonsintered nanocrystalline hydroxyapatite + highly porous silica gel matrix	Irregular granulate	Osteoconductive and resorbable
CS bone graft	BioStructures, LLC, CA, USA	Biphasic mineral granulate + porous type I collagen matrix + bone marrow aspirate	Strip	Resorbable
2014				
Bioactive bone graft putty	BioStructures, LLC	Calcium phosphate granules + bioactive glass granules + resorbable polymer carrier	Putty	Osteoconductive and resorbable
nanOss Bioactive 3D	Pioneer Surgical Technology, Inc., MI, USA	Porous hydroxyapatite granules + porous porcine gelatin-based foam matrix	3D construct	Osteoconductive and resorbable
PRO-DENSE bone graft substitute	Wright Medical Technology, Inc., TN, USA	Calcium sulfate-calcium phosphate composite	Paste	Osteoconductive and resorbable
Solum IVBone void filler	SpineSmith Partners, LLP, TX, USA	Porous hydroxyapatite granules + Type A porcine gelatin based carrier	Granular	Osteoconductive and resorbable
BioSphere putty bioactive bone graft	Synergy Biomedical, LLC, PA, USA	45S5 bioactive glass particles + inert moldable carrier	Putty	Osteoconductive and resorbable
SKaffold NMX	Skeletal Kinetics® LLC, CA, USA	Calcium phosphate powder + bioinert polyethylene glycol-based polymer	Putty	Resorbable
NovaBone MacroFORM	NovaBone Products, LLC, FL, USA	Bioactive calcium-phospho-silicate granules + collagen binder	Loose granules, composite plug and composite block	Osteoconductive and resorbable
NovaBone BIOACTIVE strip	NovaBone Products, LLC	Bioactive calcium-phospho-silicate granules + a collagen binder	Strip	Osteoconductive and resorbable
2013				
TRS cranial bone void filler(TRS C-BVF)	Tissue Regeneration Systems, Inc., MI, USA	Polycaprolactone, hydroxyapatite + octacalcium phosphate	Porous implant via 3D printing	Osteoconductive and resorbable
MeResorbable bone void filler	AG Digital Technology Corp., Taiwan, Republic of China	Surgical grade calcium salt mixture + mixing solution	Putty	Osteoconductive and resorbable
Pro-Dense bone graft substitute	Wright Medical Technology, Inc.	Calcium sulfate + calcium phosphate	Injectable paste	Osteoconductive and resorbable
Biosphere putty	Synergy Biomedical, LLC	45S5 bioactive glass particles + inert moldable carrier	Putty	Osteoconductive, bioactive and resorbable
2012				
SKaffold bone void filler and autograft extender	Skeletal Kinetics, LLC	Calcium phosphate/calcium sulfate powder	Putty/paste	Resorbable
Polyfuse – neurobone	Atpac Medical LLC, NV, USA	β-tricalcium phosphate + polyphosphate	Granule and porous strips	Resorbable

Table 4. US FDA 510(k) cleared bone tissue-engineered (resorbable) medical devices approved between 2011 and 2015 (cont.).				
Product Name	Company	Composition	Form	Property
Apaceram bone graft substitute	HOYA Corporation, MD, USA	Synthetic hydroxyapatite	Interconnected porous scaffold	Osteoconductive and resorbable
OsseoFit porous tissue matrix	DSM, PA, USA	β -tricalcium phosphate + polylactic acid	Different preformed cylinders, wedges, among others, of size max. 25 mm diameter	Resorbable
Bone Plus™ BCP Eagle Eye	MegaGen Implant Co., Ltd., South Korea	Hydroxyapatite (60%) + β -tricalcium phosphate (40%)	Interconnected porous sticks or granules	Osteoconductive and resorbable
Bicera resorbable bone substitute	Wiltron Corporation Ltd., Taiwan, Republic of China	Hydroxyapatite (60%) + β -tricalcium phosphate (40%)	Interconnected porous sticks or granules	Osteoconductive and resorbable
2011				
Vitoss bone graft substitute	Stryker, MI, USA	Calcium phosphate + Type I bovine collagen	Porous implant	Osteoconductive and resorbable
NovaBon putty – bioactive synthetic bone graft	NovaBon Products, LLC, FL, USA	Calcium-phosphorus-sodium-silicate (Bioglass) + a synthetic binder	Putty	Osteoconductive, bioactive and resorbable
NanOss bioactive	Pioneer Surgical Technology, MI, USA	Calcium phosphate + a porcine gelatin carrier	Granules	Resorbable
NovaBone MacroPor-Si ⁺ – bioactive bone graft	NovaBone Products, LLC	Calcium-phosphorus-sodium-silicate (Bioglass) + a synthetic binder	Putty	Osteoconductive, bioactive and resorbable
ReproBone	Ceramisis Ltd, Sheffield UK	Hydroxyapatite (60%) and β -tricalcium phosphate (40%)	Interconnected porous sticks or granules	Osteoconductive and resorbable
NanOss Bioactive 3D	Pioneer Surgical Technology, MI, USA	Calcium phosphate	Interconnected porous implant	Osteoconductive and resorbable
Interface bioactive bone graft	BioStructures, LLC, CA, USA	Bioactive glass	Granules	Osteoconductive, bioactive and resorbable
NovaBone dental morsels – bioactive synthetic bone graft	NovaBone Products, LLC	Calcium phospho-silicate (Bioglass)	Interconnected bulk porous form	Osteoconductive, bioactive and resorbable
IngeniOs HA synthetic bone particles	Zimmer Dental, CA, USA	Pure phase hydroxyapatite	Open-cellular highly porous morsels	Biocompatible, bioactive, osteoconductive, resorbable
Cohesion bone cement	Vexim, France	Liquid component of methyl methacrylate + powder component of polymethylmethacrylate + benzoyl peroxide	Putty, paste	Resorbable

Table 5. US FDA 510(k) cleared allograft-based medical devices approved between 2011 and 2015.

Product name	Company	Composition	Form	Property
2013				
OsteoSelect demineralized bone matrix putty	Bacterin International, Inc., MT, USA	Demineralized processed human bone + carboxymethylcellulose + phosphate buffered saline	Putty	Biocompatible, biodegradable, osteoconductive, osteoinductive, and resorbable
MTF bone void filler	Musculoskeletal Transplant Foundation, NJ, USA	Demineralized processed human bone + gelatin + sodium hyaluronate	Putty	Osteoconductive, osteoinductive, resorbable
Magnifuse bone graft	Shilog Medical Supply, OK, USA	Human allograft cortical bone tissue (both demineralized and nondemineralized fibers) in sealed poly(glycolic acid) mesh pouch	Graft	Osteoconductive, osteoinductive and resorbable
2012				
MTF bone void filler	Musculoskeletal Transplant Foundation,	Demineralized processed human bone + gelatin + sodium hyaluronate	Putty	Osteoconductive, osteoinductive, resorbable
Optecure	Exactech Inc., FL, USA	Polymer powder, demineralized bone matrix + corticocancellous bone chips + mixing solution	Putty	Osteoinductive and resorbable
2011				
DBX™ demineralized bone matrix putty	Musculoskeletal Transplant Foundation	Demineralized bone matrix + sodium hyaluronate	Putty	Osteoinductive and resorbable

MTF: Musculoskeletal Transplant Foundation.

pose any hematopoiesis at the defect area. Synthetic biomaterials are often made up of ceramic (calcium phosphate, calcium sulphate, β -tricalcium phosphate, synthetic hydroxyapatite, among others) or polymer (polycaprolactone, gelatine, sodium hyaluronate, collagen, among others) components, either individually or in combination. Various bone graft substitutes used through the year 2010 have been presented in recent literature [5]. The most current progress within this area was surveyed by searching on the keyword 'bone' on the FDA's 510(k) cleared medical devices website between the years 2011 and 2015.

The resorbable and nonresorbable bone medical devices approved between the years 2011 and 2015 are presented in Table 3. Additional complete information about FDA 510(k) approved BTE and allograft biomaterials is contained within Tables 4 & 5. As seen in Tables 3 & 4, the most common form of bone biomaterials used clinically are mixes of polymer and ceramic ingredients to form either moldable putties or injectable pastes. For example, NovaBone Putty® (FL, USA) is a Bioactive Synthetic Bone Graft; Acumed's Callos® (OR, USA) and, ProModel® Bone Void Fillers (OR, USA) are types of putties or pastes that can be

employed to fill small, subcritical size bone defects or voids [79].

Other less frequent forms of BTE biomaterials approved for clinical use include interconnected porous structures such as granules, strips, or 3D implants (e.g., Apaceram Bone Graft Substitute [Hoya Corporation (Pentax [Ricoh]), Tokyo, Japan]-hydroxyapatite, Bicera Resorbable Bone Substitute [Hannox International Corp., Taipei, Taiwan]-hydroxyapatite- β tricalcium phosphate, among others, as summarized in Table 3). One important observation is that almost all of these biomaterials are intended for defects which will not immediately incur significant loads (e.g., dental extraction fillers). While polymer and ceramic pastes and putties will continue to have wide use, it is likely that these materials will be refined by adding a local surface functionalization. Functionalization with ligands or the controlled release of whole growth factors are likely to become part of many regenerative bone therapies and/or as substrates for cell-based therapies once current preclinical and translational studies now near completion become supporting material for clinical trials (see section titled "Cell-based therapies").

Future BTE practices in the clinic

There are three broad categories of BTE biomaterials: amorphous – these materials do not have any defined 3D shape and are used to fill defect sites, for example, putty/paste, granules/particles, among others; mold-based – these materials have a 3D shape, usually with nonuniform porous structures, for example, solvent casting particulate leaching, gas foaming, thermally induced phase separation, cryogelation, among others; 3D printed (i.e., additive manufacturing) – these materials are fabricated via computer aided manufacturing technologies and are 3D structures that may have well-defined porous architecture. 3D printed devices, for example, may be fabricated by selective laser sintering among other methodologies [80–84]. In spite of the distinct advantages of additive manufacturing approaches, these techniques have to overcome major obstacles in order to yield successful patient-specific defect therapies. Considerations of external geometry and soft tissue shape coupled with the internal pore architectures needed to promote tissue infusion and vascularization are formidable obstacles. As such, optimization of these criteria in additive manufacturing approaches must occur for the translation of 3D constructs of a clinically useful size.

3D printing (additive manufacture)

3D printing is a method by which implants may be manufactured based on specific geometries and shapes as manipulated via CAD files. Complex pore geometries can be fabricated and rendered in ways that no other technique can easily or affordably replicate. Furthermore, a workpath from patient radiological images to implant CAD software to a 3D printer is extremely efficient. These methods can be used for the preparation of patient-specific implants for use in research and/or clinical applications [85–89]. The primary restrictions are the requisite expertise, access to implant CAD software, access to an appropriate 3D printer, and access to FDA-approved, 3D printable, implant material. The available biocompatible metals, ceramics and polymers that can be 3D printed have a relatively limited range of material properties. If resorbable materials are used, it is important to note that highly accurate 3D printing allows one to reduce the thickness of walls separating pores. That reduces the surface area/mass ratio of the wall and may speed resorption and aid in tissue ingrowth and the subsequent remodeling of bone.

3D printing's entry into the clinic

3D printing of implants for cranial repair started well over a decade ago with the use of inert implant

materials, such as polymethylmethacrylate [90]. Most 3D printing of BTE scaffolds has been studied via *in vitro* cell-based [13,91–93] or preclinical animal model studies [94]. However, recently, some groups outside the USA have started clinical trials to analyze the efficacy of 3D printed BTE materials. For example, Probst *et al.* have studied the application of a 3D printed polycaprolactone-tricalcium phosphate biodegradable scaffolds fabricated via fused deposition modeling [95]. Their application was the treatment of osseous defects created iatrogenically during reconstructive surgery for congenital craniosynostosis. They found that these 3D printed implants integrated with the surrounding tissue after a period of 6 months [96,97].

A 3D printed porous medical device has recently received FDA 510(k) approval as a cranial bone void filler [95]. This product is the Tissue Regeneration Systems (MI, USA) Cranial Bone Void Filler (TRS C-BVF) which is fabricated by laser sintering. It is also composed of polycaprolactone. The introduction of 3D printed, resorbable scaffolds for subcritical size defects is an important landmark for BTE's path to the clinic.

Preculturing strategies: static versus perfusion bioreactor culture

Many researchers have developed preculturing strategies for resorbable solid and hydrogel scaffolds seeded with bone progenitor cells and, in some cases, vascular progenitor cells or native vasculature. The critical steps in this process are the attachment, proliferation and maturation of bone and vascular progenitor or end-stage cells. Two types of preculturing devices, static and dynamic, have been studied. Static preculture involves the expansion of cells on a biomaterial matrix under static conditions of cell culture, whereas, dynamic preculturing occurs inside a bioreactor [98]. Dynamic preculture in bioreactors can mimic some aspects of an *in vivo* environment, including the incorporation of shear stress due to media circulation or direct mechanical stimulation [15,99–101]. Unlike entirely *in vivo* therapies, the use of bioreactors makes it easy to prevent the risk of pleiotropic effects of high doses of bioactive molecules and to avoid attachment of competing, undesirable cell types (e.g., fibroblasts). The goal of some bioreactor approaches is to create scaffolds coated with precultured bone extracellular matrix that may be perceived by the host as a bone graft [102–104]. Three types of bioreactors may be used for BTE. They are: rotating wall, spinner flask and flow perfusion, among which, flow perfusion bioreactors have been found most suitable due to uniform cell seeding and the media flow coming in direct contact with the cell-seeded biomaterials causing predictable shear stress to the cells [15,100–101,105].

Figure 5. A hypothetical model for a work path that could be used in the future to prepare a patient-specific synthetic bone graft.
CAD: Computer-aided design.

Co-culture of MSCs along with endothelial cells in a hydrogel such as fibrin can also lead to the formation of vessel-like structures in what has been termed a 'pre-vascularized' tissue-engineered bone graft [105,106]. One difficulty for cell-seeded solid-cured polymer, ceramic or composite scaffolds or hydrogel scaffolds to gain entry in the clinic is that there is little experience with them in the clinic. These products will require centralized production, at least initially, and careful oversight to insure safety and efficacy. A hypothetical pathway for preparing a patient-specific 3D, precultured BTE graft is represented in Figure 5.

Conclusion

A very small array of BTE strategies incorporating scaffolds, cells and growth factors (or other bioactive molecules) have made their way to the clinic. Most alloplastic bone repair products continue to be inert, however some are considered 'bioactive' if bone ongrowth is possible. Additive manufacturing strategies (3D printing) offer opportunities for implants with patient-specific external geometries and complex pore design that may help insure tissue ingrowth and resorption. Cell printing can be used to suspend bone progenitor and vascular progenitor cells together in a hydrogel. Bioreactor preculturing of these or cell-seeded, solid-cured scaffolds may lead to prevascu-

larized bone grafts and/or bone extracellular matrix-coated bone implants. Bioreactor strategies offer the benefit of avoiding pleiotropic effects by sequestering high doses of growth factors outside the body and limiting the types of cells which attach to scaffolds to solely bone and/or blood vessel progenitor cells.

Future perspective

In this review, regenerative medicine and restorative strategies that utilize tissue-engineering approaches to address bone defects within a clinical setting were discussed. All the described strategies have advantages and disadvantages. However, the most promising strategy may be the combination of growth factors and MSCs attached to 3D scaffolds using bioreactor perfusion. As described previously, new 3D printing (additive manufacturing) techniques are very promising for tissue-engineering applications. Their use for patient-specific, 3D printed scaffolds are expected to be used in the near future in order to accomplish more efficient bone regeneration and translation of new BTE therapies to the clinic.

Acknowledgements

The authors thank Ms Megan Posukonis and Ms Brianna Swan for assistance with the preparation of references. The authors also thank Dr. Jason Walker, Ms Alison Claybon, and Ms Arghavan Farzadi for assistance in editing the manuscript.

Disclaimer

Opinions, interpretations, conclusions and recommendations are those of the authors and are not necessarily endorsed by the US Department of Defence.

Financial & competing interests disclosure

The authors acknowledge partial support from NIH grant R01-DE013740, NIH grant R01-AR061460, the Army, Navy, NIH, Air Force, VA, and Health Affairs to support the AFIRM II effort under award No. W81XWH-14-2-0004. The US Army Medical Research Acquisition Activity is the awarding and administering acquisition office for award No. W81XWH-14-2-0004. Some of the implant design and fabrication technology discussed in

this review have been patented and assigned or licensed to Osteoplastics, LLC (Shaker Heights, OH, USA). Other technology discussed in this manuscript is the subject of sponsored research agreements with 3DBioResins (Pepper Pike, OH, USA) and 3DServePros, LLC (Pepper Pike, OH, USA). D Dean is a co-founder and has an ownership stake in all three companies. D Dean has received compensation from Osteoplastics, LLC. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Executive summary**Cell-based therapies**

- Bone marrow-derived mesenchymal stem cells (MSCs), lack MHC class II cell surface markers and co-stimulators prior to end-stage differentiation and therefore are not visible to the immune system. Bone marrow-derived MSCs have shown good potential for use in cell-based bone repair therapies.

Growth factors (and other bioactive molecules) in scaffold-based or cell-based therapies

- Locally metabolized ligands, with or without support of freely circulating bioactive molecules, may be a safe and effective for the production of functionalized bone tissue engineering scaffolds or other medical devices.

Biomaterial-based therapies

- Polymer- and ceramic-based biomaterials can be refined by local surface functionalization using ligands and/or attached growth factors (or other bioactive molecules) as part of regenerative bone tissue engineering therapies.

3D printing

- Bone scaffolds with complex pore geometries can be fabricated and rendered by using computer-aided design to prepare shapes that are then 3D printed in biocompatible, resorbable or inert materials.

Preculturing strategies: static versus perfusion bioreactor culture

- Flow perfusion bioreactors are most suitable following cell seeding and ensure media flow that comes in direct contact with the cell-seeded biomaterials and causes predictable, non-deleterious shear stress to the seeded cells.

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