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Treatment of critical-sized bone defects: clinical and tissue engineering perspectives

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Abstract   Critical-sized bone defects are defined as those that will not heal spontaneously within a patient's lifetime. Current treatment options include vascularized bone grafts, distraction osteogenesis, and the induced membrane technique. The induced membrane technique is an increasingly utilized method with favorable results including high rates of union. Tissue engineering holds promise in the treatment of large bone defects due to advancement of stem cell biology, novel biomaterials, and 3D bioprinting. In this review, we provide an overview of the current operative treatment strategies of critical-sized bone defects as well as the current state of tissue engineering for such defects.

Keywords   Critical bone defects · Bone tissue engineering · Bone healing · Fracture healing

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Introduction

The management of critical-sized bone defects remains a major clinical orthopedic challenge. Critical-sized bone defects are technically defined as those that will not heal spontaneously during the patient's lifetime [121]. Bone loss greater than 2 times the diameter of the long bone diaphysis is unlikely to result in union despite appropriate stabilization methods [56, 85]. Large bone defects can be secondary to bone loss from trauma, infection, tumor resection, or developmental deformities. The overall incidence of critical-sized bone defects is low. For instance, in one 10-year fracture registry, only 0.4% of all fractures at a level-1 trauma center were complicated by significant bone loss [63].

The condition of the soft tissues surrounding the bone defect is a major contributory factor to bone healing. Unlike other tissues, bone can regenerate and repair itself through the process of primary or secondary healing. However, this process is dependent on adequate vascularity, and avascularity is a major contributor to the pathogenesis of critical-sized defects [78, 129]. The majority of fractures heal by secondary healing. This process depends on osteogenesis, osteoinduction, and osteoconduction [30]. Specifically, mesenchymal stem cells (present in the bone marrow, granulation tissue, periosteum, surrounding soft tissues, and blood vessels) provide the osteoprogenitor cells that differentiate into osteoblasts and osteoclasts, while osteoinductive factors stimulate this differentiation. Osteoinductive factors are delivered by vasculature to the site of the fracture and include pro-inflammatory cytokines, growth factors such as those in the TGF-β superfamily (e.g., bone morphogenic proteins) and angiogenic factors such as VEGF. Finally, an osteoconductive scaffold, which permits bone growth onto its surface, is also necessary. In native fracture healing, this

scaffold is first provided by the hematoma and then by the cartilage callus [30].

Host factors can also adversely affect bone healing. Comorbid metabolic and systemic disorders such as diabetes, obesity, inflammatory arthritis, malnutrition, peripheral vascular disease, and hypothyroidism, as well as certain drugs such as NSAIDs, steroids, phenytoin, ciprofloxacin, and anticoagulants are known to increase healing time and risk of nonunion [2, 23, 39, 48, 51, 55]. Specifically, diabetes is associated with up to three times the risk of nonunion and a doubling of the time to healing [65, 75]. Pro-inflammatory cytokines associated with systemic inflammatory diseases including diabetes, obesity, and inflammatory arthritis are thought to activate osteoclasts and therefore create an imbalance between bone resorption and formation [23]. Inflammatory diseases are also associated with reduced expression of osteoinductive factors [114]. Finally, patient behaviors including smoking and alcohol abuse are also associated with significantly delayed union and increased risk of nonunion [39]. Alcohol inhibits cell proliferation [21]. Smoking is associated with atherosclerosis and reduced blood supply [104, 107]. Further, nicotine inhibits vascular ingrowth and early revascularization of bone and diminishes osteoblast function [26, 116]. Advanced age, venous stasis, burns, radiation, and obesity are additional predisposing factors [34, 48, 66].

There is a paucity of evidence to guide clinical treatment strategies for critical-sized bone defects, with no controlled studies comparing different techniques. Historically, large segmental defects have had a poor prognosis, often requiring amputation. Current therapeutic approaches include bone grafting, distraction osteogenesis, and the induced membrane ("Masquelet") technique. Although case series and retrospective reviews have reported relatively high rates of union following these techniques, each is associated with its own complications. Therefore, much recent research has been devoted to tissue engineering strategies that can provide all three of the factors deemed essential for bone healing: osteoconductive scaffold, growth factors for osteoinduction, and cells with osteogenic potential. The purpose of this review is to provide an overview of the current operative treatment strategies of critical-sized bone defects as well as the current state of tissue engineering for such defects.

Nonstructural bone grafts: cancellous autograft

Nonstructural bone grafts include autologous cancellous grafts, allografts in the form of mineralized allografts or demineralized bone matrices (DBMs), and bone graft substitutes including calcium sulfate, calcium phosphate, hydroxyapatite, and tricalcium phosphate. Cancellous autograft remains the gold standard void filler for

the treatment of bone loss during nonunion treatment. It provides osteoconductive (scaffold, allowing ingrowth of osteoblasts), osteoinductive (bone morphogenetic proteins and other growth factors which signal the differentiation of mesenchymal cells along the osteogenic lineage) and osteogenic (mesenchymal stem cells) factors [13]. Additionally, it is nonimmunogenic and does not carry the risk of transmissible infections [13]. However, for large segmental defects, the morbidity associated with harvesting the amount of graft required to fill the defect is substantial; additionally, the amount of graft that can be obtained is finite. Historically, autograft has been harvested from the anterior and/or posterior iliac crest. For iliac crest bone grafting, the rate of complications has been reported to be in the range of 20% [29, 43], with pain at the harvest site reported in 18% of patients at 2 years [29]. Recently, the Reamer/Irrigator/Aspirator (RIA) device has offered a new method of obtaining bone graft. The RIA addresses the reaming-related problems of thermal necrosis and embolism of marrow contents through simultaneous irrigation of the canal and suction of reaming debris [53, 103]. This same debris has recently been utilized as bone graft. In fact, RIA reamings contain multipotent stem cells as well as high levels of growth factors [120]. Additionally, the volume of bone graft obtained from RIA is significantly larger (25–90 cm^3) than that obtained from anterior iliac crest harvesting (5–72 cm^3), although comparable to that obtained from posterior crest harvesting (25–88 cm^3) [29]. Importantly, a review of 233 patients undergoing RIA showed only a 6% complication rate [29]. Complications reported after RIA include iatrogenic fracture, violation of the knee joint, and heterotopic ossification [24, 76, 110].

Over the past two decades, surgeons have begun to utilize bone morphogenic proteins (BMPs) as an alternative to, or in combination with, autologous bone grafts. BMPs are growth factors important in the formation of bone and cartilage as well as bone repair. BMP-2 and BMP-7 are the most clinically relevant of this group. In 2001, recombinant BMP-7 (OP-1) became FDA-approved for the treatment of tibial nonunions after a randomized control trial showed no difference in the rate of union in patients treated with BMP-7 plus intramedullary (IM) rod versus fresh autograft plus IM rod for tibial nonunion [36]. In 2002, BMP-2 was FDA-approved for the treatment of acute open tibial fractures after a randomized control trial comparing BMP-7 plus IM nail versus IM nail alone found a decreased rate of secondary intervention in the experimental group [46]. Since then, off-label use of these molecules has been adopted by many surgeons for treatment of nonunion sites at other locations [15, 35, 59]. Unfortunately, a Cochrane review recently found that the rate of union is not different for nonunions treated with BMPs versus autologous graft [RR 1.02 (95% CI 0.9 to 1.15)] [38].

Moreover, although BMPs have strong osteoinductive properties, surgeons need to be cognizant that they lack osteoconductive and osteogenic factors. This is particularly relevant for the use of BMPs in the treatment of critical-sized bone defects—if used for such applications, they must be combined with autologous bone graft or another scaffold (the current clinical delivery system is a collagen sponge) and supply of osteoprogenitor cells. More studies need to be performed evaluating the role of BMP plus autologous graft versus autologous graft alone.

Bone marrow aspirate represents another option for obtaining osteoprogenitor cells, as well as osteoinductive factors. However, it, like BMPs, has no osteoconductive properties. Some studies have reported union rates from 75 to 88% using bone marrow aspirate for the treatment of nonunions; however, they had no control groups [42, 52]. Moreover, it cannot be used alone for the treatment of critical-size defects.

Although autologous bone graft represents the gold standard for nonunions and has osteoinductive, osteoconductive, and osteogenic properties, its lack of structural support makes such grafts, with or without the addition of BMPs, less desirable for larger defects. The graft becomes partially resorbed and revascularization, and bridging occurs by creeping substitution with cells migrating from the well-perfused section into the graft, both of which lead to weakness of the reconstructed segment and increase the risk of iterative fractures [82]. Currently, cancellous autograft is recommended for voids less than 5 cm, with well-vascularized, healthy recipient sites, and that do not require structural integrity from the graft [43]. Therefore, for segmental bone defects larger than 5 cm, other therapeutic options are necessary. These options include allografts, vascularized fibular grafts, distraction osteogenesis and bone transport, and the induced membrane technique.

Nonstructural bone grafts: cancellous allograft

The benefits of cancellous allograft include availability of graft in the desired quantity, avoidance of donor site morbidity, the ability of the graft to provide structural support, and a relatively straightforward surgical technique [91, 92]. However, cancellous allografts are also not vascularized and therefore have limited ability to integrate with host bone. Additionally, they have no osteogenic potential as mesenchymal stem cells, osteoblasts, or osteoclasts are unable to survive under conditions of low oxygen tension [40]. Allografts are also associated with an increased risk of disease transmission as well as immunogenic response [93, 130]. Studies utilizing cadaveric allografts in critical-sized defects have demonstrated frequent complications including nonunion, fracture, and infection [5, 33, 37]. In one study of oncologic patients, the rate of nonunion was 75%, the rate of fracture was 13%, and the total rate of infection was 16% at an average of 5 years of follow-up [17]. However, the overall rate of allograft survival was 81%, consistent with most reports in the literature [5, 33, 37]. In general, cadaveric allograft is utilized mostly for massive oncologic-related defects in association with endoprosthetic repair and is not commonly utilized for defects arising in the setting of trauma or infection. Recently, orthopedic oncologists have begun to combine massive bone allograft and autologous vascularized fibular grafts, with promising results including improved rates of union (over 70%) [54, 68, 111].

Structural bone grafts: free vascularized fibular grafts

Vascularized autogenous cortical bone grafts, by virtue of maintaining their blood supply, allow the cells contained in these grafts including mesenchymal stem cells, osteoblasts, and osteoclasts to remain viable [128]. This preserves bone-remodeling ability even when the blood supply is inadequate at the recipient site. By contrast, conventional non-vascularized bone grafts are incapable of remodeling unless the recipient site has adequate blood supply. Together with the ability to provide structural support, vascularized grafts are ideal for the treatment of segmental long bone defects.

The size of the fibula matches that of both the radius and ulna and thus can anatomically match forearm deficits. For larger long bones such as the femur or proximal tibia, the fibular graft can be inserted into the medullary canal singularly, with another free fibular graft, or combined with allograft [14]. It may also be cut transversely and folded to match the width of the recipient bone site while preserving the vascular pedicle. This is particularly useful when considering the cross-sectional area of the femur compared to the fibula and can reduce the risk of stress fracture after weight bearing. Another advantage of the fibula is that it tends to hypertrophy in response to microscopic stress fractures, and studies have reported rapid hypertrophy when free fibular grafts are used for tibial or femoral defects [18, 27]. Rates of union after vascularized free fibular grafting range from 70 to 100% in retrospective studies of critical-sized defects secondary to a variety of causes including trauma, osteomyelitis, and tumor resection [28, 32, 67, 89, 96, 137, 139, 141]. Mean time to union is approximately 6 months [28, 89, 96, 139]. The rate of return to weight bearing and adequate functionality, when reported, is also generally high: over 96% in one study [71].

However, the rate of fractures after free fibular grafting in the lower extremities may range from 15 to over 40% [27, 28, 67, 89]. These fractures are thought to occur due to excessive mechanical stress or misalignment. For this

reasons, some authors recommend fibular grafts be used predominantly for the upper extremities alone [113, 128, 137]. Others have found lower rates of fracture by using conventional plates, external fixators, or locked plates in conjunction with the fibular graft, as well as implementing delayed weight bearing [70]. Still others hypothesize that stress fractures themselves are desirable because they induce bony hypertrophy through activation of bone remodeling pathways [108, 139].

Other reported complications include donor site morbidity, failed anastomosis, microvascular thrombosis, infection, and progressive deformities [6, 90, 133]. A systemic review of complications of free fibula grafts found an overall incidence of early donor site complications (including infection, dehiscence, delayed wound healing) of 9.9% for wounds closed primarily, and 19.0% for wounds requiring skin graft closure [72]. Late morbidities included chronic pain (6.5%), gait abnormality (3.9%), ankle instability (5.8%), limited range of motion (11.5%), and sensory deficit (7.0%) [72]. Overall, disadvantages of the fibular autograft include donor site morbidity, increased operative time, risk of fracture particularly in the lower extremities, and a challenging microsurgical technique. However, the fibula graft may still be useful in cases of segmental bone loss in the upper extremity, especially those with associated soft tissue loss [49, 94, 113, 128, 137].

Distraction osteogenesis and bone transport

Ilizarov first reported on the technique of distraction osteogenesis in the 1950s [9]. This technique utilizes the bone's natural capacity for regeneration under tension. A corticotomy is made in healthy bone, usually metaphyseal, at a distance from the defect site to create a free segment of living bone [9]. This is followed by distraction of the free segment toward the defect site, the future docking site, with bone production occurring de novo between the two corticotomy surfaces. The process of bone formation in the distraction gap is histologically similar to intramembranous ossification [10, 11]. Distraction-compression transport is achieved mechanically by attaching the segmental fragments to a circular external fixator with tensioned wires, which allows for distraction at the corticotomy site and eventually compression at the docking site.

The Ilizarov technique is divided into three periods. The first is the latency period, which is the time from corticotomy to the beginning of distraction during which callus is formed, usually 5–7 days. Next is the distraction period. A distraction rate of 1 mm per day has been shown to minimize the rate of premature consolidation, yet does not outpace the speed of vascular ingrowth [12]. Finally, once the bone fragment closes the defect, the consolidation phase begins. During this period, the new bone in the distraction gap bridges and corticalizes. The ring fixator is left in place during this time to allow the new bone to consolidate and become strong enough to withstand fracture, shortening, or bending. Historically, the length of this period has been described as two to three times the distraction period. Some authors have described an external fixator index (defined as the amount of time in months required per centimeter of distraction). Most report this index between 1.4 and 2.1 [97, 100, 101], although others have recently reported indices as low as 0.4 with the concomitant use of IM nails [32].

The Ilizarov technique has been reported with good results in all long bones in the body [102]. In a meta-analysis of lower limb segmental defects treated by bone transport, the overall union rate was 95% (range, 60–100%) [102]. Additionally, this technique can be used to correct alignments in any plane or direction including rotational deformities since it utilizes a circumferential ring fixator.

Although the Ilizarov technique is well established, disadvantages of the method include prolonged treatment times (up to years, depending on the length of the original defect), pin site infection (over 80% in some series [20, 47]), pin breakage, and the inconvenience and burden of prolonged circular external fixation [16, 97, 99, 102]. The soft tissues surrounding the defect are also manipulated and pulled, which can cause significant pain from muscle and nerve stretching, and can also cause neighboring joint contractures [98]. Finally, and critically, this technique requires significant cooperation from the patient, and patients must be informed of the time commitment involved in the procedure. Notably, although the amputation rate was only 2.6% in a meta-analysis of 37 studies of bone transport, half of these amputations were secondary to patient request [102]. This suggests that very careful explanation of the details of this technique and careful patient selection is important for this procedure.

Induced membrane technique

Recently, a new and promising technique has been increasingly utilized for the treatment of long bone segmental defects. In 2000, Masquelet first reported the results of this technique in a case series of 31 patients with segmental diaphyseal defects ranging from 5 to 25 cm [84]. This technique, which he termed "induced membrane," is a two-stage procedure that utilizes a temporary cement spacer initially, followed by subsequent bone grafting [83]. The first stage consists of radical soft tissue and bone debridement, and implantation of a polymethylmethacrylate cement spacer at the bone defect. Stabilization is provided by a plate or nail. The cement spacer induces a foreign body reaction, which causes a fibrous membrane to form around the spacer.

During the second stage, which is typically performed 6–8 weeks later, the membrane is incised and the spacer is removed, and autograft is packed into the pseudocapsule. If the amount of autograft is insufficient, allograft is added to the mixture [41].

The cement spacer provides several mechanical and biological advantages. First, it prevents hematoma formation and fibrous tissue ingrowth into the bone defect and maintains the potential space as well as bone length and soft tissue tension. Secondly, the spacer can be impregnated with high dose antibiotics, and lack of infection after 6 weeks is an indication of favorable conditions for bone grafting. Finally, the fibrous membrane which forms around the spacer is highly vascularized and contains various osteoinductive factors including bone morphogenetic protein-2, vascularized endothelial growth factor, transcription growth factor-beta-1, and IL-6, IL-8, type I collagen, and von Willebrand factor [3, 25, 106]. Additionally, the membrane contains osteogenic properties: mesenchymal stem cells have been shown to be present in the membrane in both animal and human studies [25, 45, 106]. This vascularized pseudomembrane therefore provides support to the implanted autograft, inhibits graft resorption, and facilitates graft vascularization and corticalization/osseous consolidation [25, 106]. By contrast, studies have shown that membranes induced in subcutaneous or intramuscular sites do not contain mesenchymal stem cells and are less well vascularized [50, 73].

Since Masquelet's first report in 2000, several case series and retrospective studies utilizing this method have been published (Table 1). However, there are relatively few such studies and no direct comparisons of the induced membrane technique to other methods. Therefore, a consensus on the efficacy of this method versus other methods has yet to be determined. The technique has been successfully utilized in a variety of causes of critical-sized bone defects, including osteomyelitis, tumor resection, infected nonunions, and post-traumatic bone loss. Results are favorable, with a union rate of 88–100% in trauma cases, although lower rates have been reported after tumor resection (Table 1) [4, 44, 62, 82, 105, 117, 119, 122, 135, 138, 140]. Unfortunately, the methodology of the relevant studies varies considerably with regard to type of fixation (screws, plates, nails, external fixators) as well as type of graft (autologous or allogeneic and with or without the addition of BMP). For bone grafting, fresh cancellous autograft is the gold standard. However, large defects require large amounts of bone graft, and harvesting autologous bone is limited in quantity and carries morbidity [43, 64]. The ideal graft composition, as well as type of fixation, is yet to be determined.

Another question to be addressed is the optimal time between the first and second stages. Masquelet initially recommended 6–8 weeks [82, 84]. However, studies examining the composition of the membrane have shown that the time of highest biological activity may be 4 weeks post-spacer placement [3, 50, 106]. For example, BMP-2 levels in the membrane peak at 4 weeks [106], and cells grown in culture from membranes between 2 and 4 weeks of age proliferated at a high rate while cells grown in culture from membranes taken at 6 weeks showed negligible proliferation [50]. The highest levels of vascularity and cell density were also found at 4 weeks [3, 45]. Based on these findings, some authors have advocated for earlier bone grafting. Several case series have reported successful union after grafting at 4 weeks [3, 136].

Reported complications of the induced membrane technique include infection, amputation, malunion, fracture, and nonunion necessitating reoperation and additional bone grafting. The rates of each of these complications is quite variable (see Table 1); however, infection is overall most common, ranging from 16.1% in Masquelet's original paper to 50% in a large study by Karger et al. of 84 patients [84]. Rates of reoperation range from 4 to 35% (Table 1). Karger et al. reported a mean number of 6.1 interventions from the first stage until union was obtained. Finally, cases of massive graft resorption have been reported in pediatric populations undergoing reconstruction after tumor resection [1, 22, 44]. In these patients, the second stage was not performed until after chemotherapy completion, 6 months after the initial stage, suggesting these failures could be due, at least in part, to delay and membrane devascularization. However, the considerable inhomogeneity present both within and between series in terms of defect cause, size, and location, as well as fracture complexity and associated comorbidities, precludes any definitive exploration of the rates of complications associated with the technique. Perhaps the most significant disadvantage of the Masquelet technique is the need for two surgeries. There has been a recent push toward a single-stage tissue engineered solution to surgically treat critical-sized bone loss.

Tissue engineering

The ideal tissue engineering construct for critical-sized bone defects would provide an environment that mimics the body's natural healing process. Such a graft would promote osteogenesis and angiogenesis, while having sufficient mechanical strength to promote integration with host tissues and facilitate load transfer under weight-bearing conditions. To date, no such construct has been developed. However, there are many promising techniques. This section will provide an up to date review of scaffold materials, growth factors, and methods of vascularization.

Scaffolds must be biocompatible, contain macro- and microporosity, have sufficient mechanical strength for load

Table 1 Comparison of studies utilizing the induced membranes technique including rate of union, time to union, average diaphyseal defect, and complications

Study	Study type	N	Indications	Defect size, mean (cm)	Union rate	Mean time to union, months	Infection	Other complications
Masquelet 2000 [79]	Prospective	31	Trauma	5–25	100%	4	16.1%	Refracture (12.9%), amputations (6.5%)
Schöttle 2005 [123]	Retrospective	6	Trauma, infected nonunion	6.5 (5–8)	83%	6.8	0%	Refracture (17%), thrombosis of free flap anastomosis (17%)
Ristiniemi 2007 [124]	Retrospective	23	Trauma	5.2 (3.5–10)	96%	10		Reoperations for osseous healing (35%), 17 reoperations for soft tissue complications (74%)
Zwetyenga 2009 [125]	Case series	4	Osteoradionecrosis	11.3 (9–14)	50%	Not reported	50%	
Stafford 2010 [126]	Retrospective	27	Trauma nonunion	5.8 (1–25)	88%	Not reported	4%	BKA (25%), repeat grafting (25%)
Apard 2010 [89]	Retrospective	12	Trauma infected nonunion, aseptic nonunion	8.5 (4–15)	92%	Not reported.	33%	
Donegan 2010 [127]	Retrospective	11	Trauma, infected nonunion, aseptic nonunion, tumor	8.5 (4–15)	91%	7.5	9%	Heterotopic ossification (18%)
Zappaterra 2011 [98]	Case series	9	Trauma, infected nonunion, aseptic nonunion, tumor. Upper extremity.	5.9 (2.5–8)	100%	14.5		
Villemagne 2011 [96]	Retrospective	12	Pediatric tumor resection		58%	4.1	0%	Fractures (42%), femoral varus deformities (17%)
Sales de Gauzy 2012 [94]	Retrospective	10	Pediatric trauma, infected nonunion	3.5	100%	11.5	60%	Functional limitations (40%)
Karger 2012 [91]	Retrospective	84	Trauma, infected nonunion	6.8 (6.4–7.1)	91%	14.9	50%	Malalignment (14%), 6.1 mean procedures to achieve union
Chotel 2012 [102]	Prospective	8	Pediatric tumor resection	15 (10–22)	88%	4.8	0%	Paradoxical graft resorption (13%)
Gouron 2013 [90]	Retrospective review	14	Pediatric tumor resection, trauma, pseudarthrosis	10.3 (3.8– 19.2)	100%	9.5	0%	Massive graft resorption (7%); reoperations 36%) (autogenous grafting, better fixation)
Accadbled 2013 [101]	Case series	3	Pediatric tumor resection	19 (15–22)	0	N/A	0%	Massive graft resorptions (100%)
Scholz 2015 [95]	Case series	13	Septic diaphyseal defects	8 (5.5–14.5)	100%	4.2	0%	Malalignment requiring reoperation (8%), functional deficits (15%) (ROM, leg length difference)
Olesen 2015 [128]	Retrospective	8	Trauma, infected nonunion	5 (3–9)	75%	7	0%	Malalignment (25%), grafting needed (12%)
El-Alfy 2015 [129]	Retrospective	17	Infected nonunion, osteomyelitis	7 (4–11)	82%	10	12% reinfections	6% refracture

transfer, and also be bioresorbable. Biocompatibility refers to the ability to support cellular activity without toxicity to the host. The interconnected porosity is necessary for ingrowth and diffusion of nutrients; unfortunately, porosity reduces mechanical properties such as compressive strength. Additionally, cortical bone and cancellous bone vary significantly in their mechanical properties, which makes it difficult to design a single optimum scaffold.

Ceramics are a common material for bone scaffolds and include bioactive glass, tricalcium phosphate, and hydroxyapatite. All are biocompatible, osteoconductive, and have pores to allow tissue ingrowth. However, ceramics are brittle [115]. To overcome this shortcoming, synthetic biodegradable polymers such as polylactic acid (PLA), polyglycolic acid (PGA), or poly lactic acid-co-glycolic acid (PLGA) have been used to coat bioglass, hydroxyapatite, and beta-tricalcium phosphate, improving their mechanical properties as well as increasing their osteogenic potential [7, 8, 61]. These materials are bioresorbable synthetic polymers, which serve as scaffolds with good osteoinductive qualities. Moreover, although alone these compounds have poor mechanical properties for load-bearing, as well as poor osteoconductive properties, when combined with ceramics they have significantly increased strength [87, 118]. Combining PLA, PGA, or PLGA with the strength of ceramics is therefore a synergistic partnership.

Natural substitutes used for scaffolds include collagen, alginate, and hyaluronic acid. These each have excellent porosity and viscosity to allow for cellular immobilization and release of cells and osteoinductive factors from the scaffold; however, they lack sufficient mechanical strength [57, 69]. Similar to polymers, they can be combined with other compounds such as hydroxyapatite or chitosan to increase strength. One study found that a collagen–hydroxyapatite scaffold containing osteoprogenitor cells resulted in near complete filling of a 3.5-mm calvarial bone defect at 5 weeks, with much higher rates of scaffold breakdown compared to pure hydroxyapatite scaffolds [134]. Similarly encouraging results have been reported in other collagen–hydroxyapatite scaffolds [77, 109]. This resorbability is unique compared to pure hydroxyapatite scaffolds—for example, in one study, pure hydroxyapatite scaffolds used in critical-sized defects did not show any signs of resorption even after 7 years post-implantation [80]. This lack of resorption may create inferior mechanical qualities in the new bone, as well as represent an infection risk. Additionally, the strength of collagen scaffolds has recently been improved by novel modifications of collagen cross-linking [132].

Next-generation synthetics improve on the osteogenic properties of polymer–ceramic scaffolds through a variety of mechanisms. The addition of bone marrow mesenchymal stem cells and the incorporation of growth factors into the scaffold have been shown to be associated with increased rate and quantity of bone formation compared to scaffolds without these additions [19, 74]. Additionally, the manner of release of growth factors is essential [60], and importantly, new technologies have allowed growth factors to be released from the scaffold continuously for up to 6 weeks, rather than all at once [58, 125].

Several studies using novel polymers to deliver growth factors have found increased bone formation at lower doses compared to the standard clinical delivery system for BMP-2 (collagen sponge). In one study, a polyethylene glycol synthetic polymer was used to encapsulate and deliver BMP-2 in a critical-sized defect in rat calvariums and showed significantly increased bone formation compared to collagen–sponge delivery systems [81]. In a sheep model of 3-cm tibial defects, implantation of synthetic scaffolds containing BMP-7 resulted in the generation of similar bone volume and torsional stiffness when compared to autologous bone graft [112]. Finally, the addition of integrin signaling has been shown to have exciting results: in scaffolds coated with GFOGER (which binds to cells via alpha2beta1 integrin) as a BMP-2 delivery carrier, the subsequent bone formed was equal in torsional strength to that of native bone [124]. Moreover, this delivery system outperformed that of collagen sponges. Specifically, the GFOGER coated polymer stimulated bone healing at levels of BMP-2 that produced no results when delivered from collagen sponges.

Ideally, a graft would encourage angiogenesis around or even within the graft in order to perfuse the forming tissue. Methods of increasing vascularity around the scaffold include seeding scaffolds with endothelial progenitor cells and angiogenic agents such as VEGF. Such seeding is associated with increased vascularization compared to non-seeded scaffolds and promotes increased bone healing when coupled with the addition of mesenchymal stem cells [123, 142].

A tissue engineered vascularized bone graft might someday achieve the ideal graft characteristics through the incorporation of cell-laden hydrogels and synthetic vascular grafts into a porous osteoconductive rigid frame. The soft hydrogel would be utilized to control the spatial and temporal distribution of cells and growth factors, while the incorporation of vessel graft beds would allow for immediate perfusion. A rigid channeled macroporous frame with structural and mechanical properties resembling those of bone would promote integration and facilitate load transfer [88].

Given the difficulty of engineering functional tissue that recapitulates the composition, biomechanical properties, and physiological performance of native tissues, in vivo bioreactors utilize the body to grow grafts for later transplantation to the defect site. A scaffold within a chamber can be implanted into the body, normally intramuscularly or adjacent to periosteum and the osteogenic and angiogenic

capacity of the body is exploited. With time, a vascularized bone graft is generated. The implanted chamber and scaffold can be modified to the desired geometry and harvested with vessels for the treatment of bone defects [86, 131].

Conclusions

Critical-sized bone defects remain one of the most challenging orthopedic conditions to treat. Several techniques have been described, each with their own unique advantages and disadvantages. Distraction osteogenesis and free fibular grafting have been the historical mainstays of treatment. Although good clinical results have been reported for both methods, distraction osteogenesis requires lengthy treatment times and is associated with a high incidence of pin site infections, while fibular grafts are highly technically demanding and may not be ideal for lower extremity defects. The more recent induced membrane technique is an increasingly utilized method with favorable results; however, it does require at least two stages and is not as predictable as would be hoped. Tissue engineering holds great promise in the treatment of large bone defects due to advancement of stem cell biology, novel biomaterials, and 3D bioprinting, but the clinical implementation will be dependent upon whether the outcome and efficacy of this approach can provide additional benefits compared to the current clinical treatments, as well as upon the collaborative movement on commercialization, quality and safety control, and regulatory issues.

Compliance with ethical standards

Conflict of interest   Drs. Roddy, DeBaun, Daoud, and Yang have no conflicts of interest to declare. Dr. Gardner reports personal fees from DePuy-Synthes, personal fees from KCI, personal fees from Miami Medical, personal fees from Biocomposites, personal fees from Pacira, outside the submitted work.

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