

Review article

Skeletal regeneration for segmental bone loss: Vascularised grafts, analogues and surrogates



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ABSTRACT

Massive segmental bone defects (SBD) are mostly treated by removing the fibula and transplanting it complete with blood supply. While revolutionary 50 years ago, this remains the standard treatment. This review considers different strategies to repair SBD and emerging potential replacements for this highly invasive procedure. Prior to the technical breakthrough of microsurgery, researchers in the 1960s and 1970s had begun to make considerable progress in developing non autologous routes to repairing SBD. While the breakthrough of vascularised bone transplantation solved the immediate problem of a lack of reliable repair strategies, much of their prior work is still relevant today. We challenge the assumption that mimicry is necessary or likely to be successful and instead point to the utility of quite crude (from a materials technology perspective), approaches. Together there are quite compelling indications that the body can regenerate entire bone segments with few or no exogenous factors. This is important, as there is a limit to how expensive a bone repair can be and still be widely available to all patients since cost restraints within healthcare systems are not likely to diminish in the near future.

Statement of Significance

This review is significant because it is a multidisciplinary view of several surgeons and scientists as to what is driving improvement in segmental bone defect repair, why many approaches to date have not succeeded and why some quite basic approaches can be as effective as they are. While there are many reviews of the literature of grafting and bone repair the relative lack of substantial improvement and slow rate of progress in clinical translation is often overlooked and we seek to challenge the reader to consider the issue more broadly.

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1. Introduction to bone defects

1.1. Definition of the problem

Patients are poorly served by current solutions for segmental bone defects (SBD). This review will summarize current and emerging technologies to regenerate bone that are likely to impact SBD treatments as well as provide a new examination of underlying principles and earlier works that predate vascularized free bone grafts. Bone defects can be subclassified in the following three categories: 1) Small bone defects causing little or no structural instability, 2) critical sized bone defects < 5 cm or 3)

segmental bone defects >5 cm. (Fig. 1) [1]. Although each bone defect has its own challenges, small bone defects can generally be treated with shortening of a bone, using small non-structural autograft/allograft, or usage of one of the many bone graft substitutes available. When defects affect structural stability or clinical outcome/function, then shortening is no longer optimal and more complex reconstruction is required mainly because spontaneous healing will not occur. In general, this is the case for defects of 2–5 cm. When defects become greater than 5 cm, the so-called massive defects, then reconstruction of this type of defect is even more challenging. Massive defects are seen after trauma, cancer surgery, and osteomyelitis. It is to be noted that a wide variety of preclinical models have been used to study defects [2,3]. These models have varied solutions that differ in size depending on the bones and animal species and can range from as little as 2 × 2 mm cylinder for rat femur [4] to 3 cm for sheep tibia [5]. The reader is

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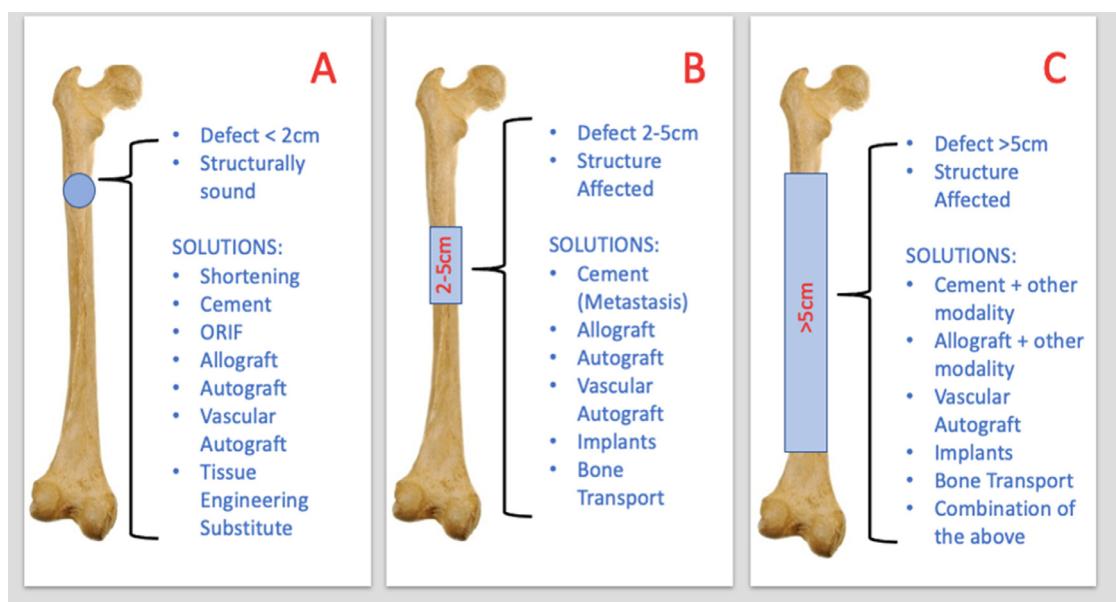


Fig. 1. Bone defects and current clinical solutions; A: Small bone defect <2 cm, B: critical sized and C, segmental bone defects. (ORIF- Open reduction internal fixation).

directed to other texts specifically dealing with this topic [6–8]. It is hard to generalize about the numbers of patients suffering from massive bone loss as injury rates and causes vary. For example in Kampala, Uganda the prevalence of traumatic bone loss following open long bone fractures was 26.5% and 61.1% of these involved ≥ 2.5 cm loss with gunshot injuries being the leading cause of traumatic bone loss. At the Edinburgh Orthopaedic Trauma Unit, Edinburgh, Scotland, significant bone loss is seen in only a small minority of all fractures. Between 1988 and 1998, fractures with bone loss accounted for 0.4% of all fractures, but was 11.4% of open fractures. The majority of these fractures were high energy wounds of the tibia, >10 cm, with extensive soft tissue injury and contamination with a smaller proportion involving additional vascular injury, (Gustilo grade 1–3 IIIB and C). The mean age of patients admitted with fractures with bone loss was 37 years and 71% of these patients were male [9], more than 50% of high energy fractures are caused by traffic accidents and high falls [10].

In massive bone defects, bone grafts (autografts or allografts) are at risk of resorption and usually require more complex reconstruction. Therefore, many different solutions have been proposed with their own set of pros and cons to address these segmental bone defects [11]. Some solutions include bone transport, combinations of implants/allograft/cement, or custom implants. Hence, bone deficits are approached differently depending on the anatomical site, the size, and the structural integrity of the bone in question, as well as the etiology that caused the defect [12,13]. In other words, segmental bone defects related to infection will differ from those of a large tumor resection. Patients with large segmental bone loss incur significant hospital costs (e.g. originating from fracture: \$86,453 (95%CI: 62,027–120,498), osteomyelitis \$156,818 (95%CI: 112,970–217,685) or non-union \$93,910 (95%CI: 52,851–166,865)). Despite state of the art treatment, patients with large segmental bone loss from osteomyelitis have a 14.5% risk of amputation [14].

Over several discrete eras in biological, surgical, technological, and materials science advances, different bottlenecks to improvements in bone repair have been overcome. For example, prior to widespread application of microsurgical bone transplantation developed in the mid-1970s, scientists tried to improve outcomes

of mainly large allografts - the state of the art alternate to autograft at that time [15]. While not immediately successful at that time due to several roadblocks such as resorption, infection and lack of integration, these experiments provide fascinating insights that are relevant today. Many of these reports have been dismissed as old concepts yet the data can be extremely valuable in helping guide current strategies. For example, the stated goal of many contemporary bone regeneration 'scaffolds' is to maintain viability of tissue by incorporating or promoting [16–18] nutrient ingress through pores and channels. However, in apparent contradiction to current goals, it has been thought as early as 1940 that necrosis of transplanted marrow was a prerequisite for bone formation [15,19]. Burwell proposed that the purpose of a cancellous allograft scaffold was in fact to reduce marrow cell survival by reducing the available surface for neovascularization, thereby resulting in the release of an agent, still unidentified, that induced bone formation. One can speculate through today's lens, that this was a beginning of the identification of the role of immunology of the whole organism in bone health and healing- since damage associated molecular patterns (DAMPs) [20,21] are known to initiate from dying tissue. It was observed that marrow transplants without a supporting scaffold were squashed flat and rarely yielded new bone formation but when loaded in a scaffold more bone formation did occur. That was attributed to the greater necrosis of marrow constrained in a volume, at least several mm thick in each axis [15]. The reader is directed to the first six chapters of the classic text by Urist, O'Connor and Burwell [22], for further historical insights on attempts to make allografts better through the use of marrow. These are also the authors that first postulated Bone Morphogenetic Proteins (BMP).

The breakthrough of vascularized bone free flaps in 1975 [23] made improvement of allograft osteogenicity less of a priority. At around the same time, two more important pieces of the bone biology puzzle were uncovered, BMP and mesenchymal stromal cells (MSC) [24,25]. Today after decades of research and thousands of publications on these two subjects, we are still not completely clear as to their clinical benefit and limitations outside of the research laboratory. BMP delivery has provided an effective product in some indications and patient groups. Other bioactive

molecules have also shown potential to generate bone but are not clinically available and this topic is reviewed in detail elsewhere [26–28].

MSC, originally conceived as an off the shelf non-immunogenic source of new mesenchymal tissue, has perhaps created more questions than answers, as it is becoming clear that MSC role in bone formation is far from fully clear [29].

We find ourselves today at a hiatus. Recombinant BMP products are not cheap and therefore cannot be used extensively given limited hospital operating budgets. At times their benefit is not clear cut, possibly a factor in them not being reimbursed in some regions since their commercial launch two decades ago. More importantly, the use of BMP cannot be ubiquitous given that they are contraindicated in patients with a cancer history, thoracic spine surgery, or in growing pediatric patients, which account for many reconstructive cases. There is a growth in studies looking at technologies to improve efficacy and hence reduce the dose, cost and risk of exogenous bioactive protein delivery, and ultimately this may overcome some limitations of this approach, e.g. [30–34].

Despite clinical grade MSC being available in many major hospitals, there is no clear indication for the technology in musculoskeletal regeneration. Current research orientation on better understanding osteoimmunology seeks to try to bridge gaps in our knowledge and hence the ability to manipulate skeletal regeneration [35]. While a fairly recent term, 'osteoimmunology' has been studied for some time by dental researchers wishing to better understand periodontal bone loss that can occur near to sites of gingival infection and inflammation [36].

The term 'bone defect' is used loosely by researchers. Bone has an incredible ability to regenerate and repairs itself without scarring in ideal circumstances. While empty critical sized defects are the staple preclinical negative control in the literature, it is clinically of little meaning. In environments with access to modern hospital treatments, patients are not left with untreated bone defects. Often any one of autografts, allografts, or any of the essentially similar bone graft substitutes will adequately support healing in most small defects. This review is focused on massive segmental bone defects caused by the loss of an entire bone segment that cannot reliably be healed by allografts or substitutes, or for which preparing an autograft usually requires harvesting a large, vascularized bone flap.

As well as reviewing major areas of advancement and activity, it is valuable to consider whether the extent of the need for improved segmental skeletal regeneration can support further technological breakthroughs. The numbers of people injured through armed conflict, to the extent it is controllable or even measurable, is allegedly reducing or relatively stable [37,38]. The general trend is for a reduction in motor vehicle accidents, certainly as a result of public policy enforced by law and various innovations such as seat belts, airbags, advanced safety systems and in Europe as of 2022 initiation of speed limiters. However, medium- and low-income countries still have a huge mortality from motor vehicle accidents which is the 7th most prevalent cause of disability adjusted life years for all ages globally and the leading cause for population aged 10–24 and 24–49 and the cost in trauma that includes bone defects is significant compared with GDP. Looking ahead then we posit that cancer patients in the first world- a growing population in whom bone metastasis is relatively common- are likely to experience longer post treatment life expectancy thanks to successes in the 'War on Cancer' as we approach the half century anniversary of the National Cancer Act [39]. What were intended as short-term end of life reconstructions are becoming less and less adequate solutions for these patients. Rebuilding cancer survivors has sadly been less of a national priority for many countries than the War itself, something that needs to change rapidly if we are to be able to serve this growing need.

1.2. Bone vasculature: Effects of its loss

One of the main issues surrounding the use of a vascularised bone graft is the morbidity associated with its harvest. Attempts at substitution aim to replace a blood supply and cortical (possibly also cancellous) bone. Appendicular bones generally consist of cortical bone tubes surrounding variable quantities of cancellous bone and marrow. Cortical bone is structurally the most important as it transmits the most load and represents about 80% of the bone mass. However, it is not a simple impervious layer surrounding the underlying cancellous bone and other structures. Up to 30% of its cortical bone volume is occupied by vascular channels [40]. In general, vascular channels are supplied by separate systems: nutrient arteries, periosteal, and epiphyseal vessels. Cortical bone vascularization is precisely organized and consists of Haversian canals containing blood vessels and nerve fibers, surrounded by osteons and interconnected by Volkmann's canals perpendicular to their axes. In addition to this network, a recently discovered system of blood supply in cortical bone has emerged as being the major effector of blood circulation: transcortical capillaries (arterioles or venules) account for over 80% of arterial and 59% of venous blood flow. They are a direct connection between the marrow, endosteal and periosteal circulation [41]. In cancellous bone, an extensive mesh of vessels and vascular sinuses surrounds the central artery and vein [42] aiding bone marrow metabolism as it generates white and red blood cells, that migrate from the bone marrow and reach the systemic circulation. In this network, different types of vessels and their corresponding endothelial cells subtypes have been identified [43] and the complex arrangement of cells around those different vessels has been described [44,45]. The topic of skeletal vascularization is intensely studied in the fields of bone development and vascular calcification where the concept of the bone-vascular axis is under a process of intense discovery [46] the reader is directed to recent reviews of bone vascularization for more detail [47–52].

Different cells and tissues have specific physiological oxygen requirements and are reliant on a sufficient blood flow to maintain their viability and function. If the blood flow is sufficiently reduced or interrupted, tissues ultimately die as they exceed their ischemic limit. This is why vascularized grafts have been used, since bone viability can be maintained throughout transplantation and engraftment. Oxygen can be a major limiting factor of tissue survival during ischemia as it is essential for oxidative phosphorylation. It is not uncommon to find clinical situations in which oxygen concentrations fail to meet metabolic requirements or even are low enough to cause mild ischemia (e.g. microvascular deficiency due to diabetes can impair healing). Loss of blood supply to a bone is either acute or chronic and both inform us about requirements for healing and maintenance of bone. Acute loss of blood supply most often occurs due to injury, typically after a fracture. A well-orchestrated healing that is very successful in small defects occurs through inflammation and other steps. This process will create mineralized tissue that then remodels to form bone [53,54]. However, some fractures heal slowly or not at all resulting in a delayed union or nonunion. This particularly happens in high energy injuries or large defects. Non-unions occur when the bone lacks adequate stability, blood flow, or both. They also are more likely if the fracture results from a high-energy injury because severe injuries often impair blood supply to the broken bone due to greater stripping of vascular elements from the bone.

Chronic loss of blood supply can take place over months and may result from genetic, pathological or pharmacological endothelial abnormalities and can cause avascular necrosis (AVN). AVN is defined as cellular death of bone components due to interruption of the blood supply. Sequelae include bone structure collapse, pain, loss of joint function, and long-term joint damage. AVN is most commonly encountered in the hip, femoral and humeral heads as

well as the femoral condyles, but small bones can also be affected [55–58]. It is noteworthy that loss of blood does not result in instant loss of functionality but that this takes time, maybe as long as years and is dependent on the severity of ischemia and the healing response.

The effect of ischemia during bone transplant surgeries have been studied and it has been shown that an ischemic time of >5 h for total joint transplant in dogs resulted in more complications and flap loss that increased as the ischemic time increased [59]. Mice studies of an ischemic period followed by reperfusion, have shown that 6 h of ischemia resulted in more than a third of empty lacunae and partially necrotic bone marrow [60]. This correlates with a study by Yuan *et al.* where in a photo-thrombosis induced AVN model they found that osteocyte death starts within 3 h of ischemia [61]. Conditions and type of bone element affect survival. Fresh bone marrow has been shown to remain fully viable as a cell suspension for up to 2 days at 4 °C, and hematopoietic stem and progenitor cells survive circulation arrest and may reconstitute hematopoiesis after 12 h of ischemia [62]. Bone marrow mesenchymal stem cells have been shown to survive more than 24 h without reduced or total absence of oxygen or glucose [63–65]. Comparison with other organs shows the liver can only withstand less than 15 min of warm ischemia without drastically affecting tissue survival [66], and after 60 min kidneys transplant function is altered [67]. Skeletal bones receive only 7% of the total blood content and 5% of the blood flow rate despite having a much larger volume and weight than other organs [68].

When bone is damaged or necrosis occurs due to a trauma or a disease, the first step of healing consists mainly of dead tissue resorption before reconstruction/remodeling becomes the dominant process. Vascularization plays an important role because it will deliver immune cells to the dead bone to resorb it, but will also nourish the cells and allow repopulation. However, avascular necrotic bone has been shown in some cases to present no specific evidence of inflammation [69]. Core decompression treatment for early femoral head AVN is thought to allow the invasion of blood vessels and the subsequent removal and remodeling of the dead tissues [70,71]. Similarly, apposition of a vascularized bone graft onto non-viable bone has been shown to allow remodeling and revascularization of the dead bone in a non-union [72]. In all, the life span of bony elements before, during, and after surgical treatment are not clearly defined. Approaches in surgery and research have been devised to repair or recreate bone and its vascularization. We review the current different clinical and experimental techniques used, the interplay between new bone formation and the vascularization and their relative successes in achieving their goal.

2. Current surgical strategies for improved segmental bone defect regeneration

Current popular approaches to the clinical reconstruction of large bone defects center around four techniques: i) Bone grafting, ii) distraction osteogenesis, iii) the Masquelet (induced membrane) technique and iv) the use of arteriovenous loop and vascular bundles. They involve the use of different surgical techniques to repair bone or induce its regeneration [73–75].

2.1. Bone grafting

Avascular autologous bone grafts are generally harvested from the iliac crest or metaphyseal bone [76] (as morsels, granules, strips, etc.) and directly transferred to the repair site. Living bone transplants (vascularized autograft) were initially performed as pedicle grafts [77], resulting from the development of vascular anastomosis in the 1960s, and nowadays the most common sites

for free flap transplant involve resection of either the patient's fibula (including the peroneal or anterior tibia vessels) or iliac crest (including the deep circumflex iliac artery) which are reshaped, transplanted to the defect site and the arterial and venous vessels microsurgically anastomosed [78,79]. In the period between the development of vascularized bone pedicles, (vascularized bone that is moved without being disconnected from its blood supply) and free flaps, (vascularized bone that is detached from the host vasculature and then reconnected at location distant to the bone harvest site), there was a period of discovery on how bone heals, the role of various elements such as osteocytes, marrow and periosteum [15], and how allografts and synthetic substitutes could be improved.

An important question to consider is, given that any bone tissue based graft will ultimately remodel, what is the benefit of repairing segmental bone defects with vascularised bone compared with avascular bone? Within the same defect size range, the process of healing is different between these two types of graft. Firstly, one must recall that the fate of cells in avascular grafts is limited. Osteocytes and osteogenic cells within transplanted avascular cancellous chips mostly die, except those near the surface and close to an adjacent blood supply [15,80]. Avascular grafts remodel through the removal of dead bone by new cell invasion (mostly from the ends abutting viable bone) but vascular grafts resorb initially more homogeneously as a result of pre-existing viable cells and the vascular network. Vascularized autografts undergo 50% less resorption than avascular bone grafts and result in double the amount of bone formation [81]. Despite practical advantage in use of allograft (ease, no donor site morbidity, and larger volume of supply) this deficiency of avascular bone grafts for segmental bone defects is not yet resolved. Recently a fairly low tech but seemingly effective technique was reported by Gupta *et al.* [82]. By filling the intramedullary canal of avascular allograft with PMMA cement, early allograft resorption and hence loss of mechanical strength was averted [82,83]. It is notable that physical compartmentalization of the healing volume using one of the oldest and biologically inert biomaterials is an effective technique and allows manipulation of healing by physically controlling routes of graft-host interaction.

Vascular grafts have a higher bone turnover [81,84], and so have a resultant lower compressive strength than avascular graft over the first 4 weeks after surgery, but this then increases significantly. Late bone torsion strength has also been shown to be superior for vascularized grafts when compared to avascular grafts; for example even when using ribs to replace tibial defects [85,86]. This has been reviewed previously [85] and the main points are summarized in (Fig. 2) [87]. The lack of initial load bearing capacity has led to the development of a vascular fibular graft reinforced within a metal cage for multi-vertebral body defect [88]. This construct creates biomechanical stability in the first weeks after surgery until the graft regained its mechanical strength allowing extensive load bearing skeletal replacement, Fig. 3. The pros and cons of vascularised bone grafts have been recently thoroughly reviewed [89].

Avascular bone grafts are relatively effective for some bone reconstructions (e.g.: scaphoid non-union treatment [90], small mandibular segments [91]) and for segmental defect repairs up to 6–9 cm [76,90–94] with acceptable success rates (80% or more). However success drastically drops (<50%) for massive segmental defects >6–9 cm [76]. These graft repairs are prone to infections or nonunions.

A higher union rate for the reconstruction of large segmental defects (>6–9 cm) is obtained by autogenous vascularized bone free flaps [92,95]. Improvements of vascularized over non-vascularized autografts include higher union rate, improved bone graft survival, improved mechanical strength to failure [84,96], and lower infection rates [76,84,90,96–98]. This may be in part due to

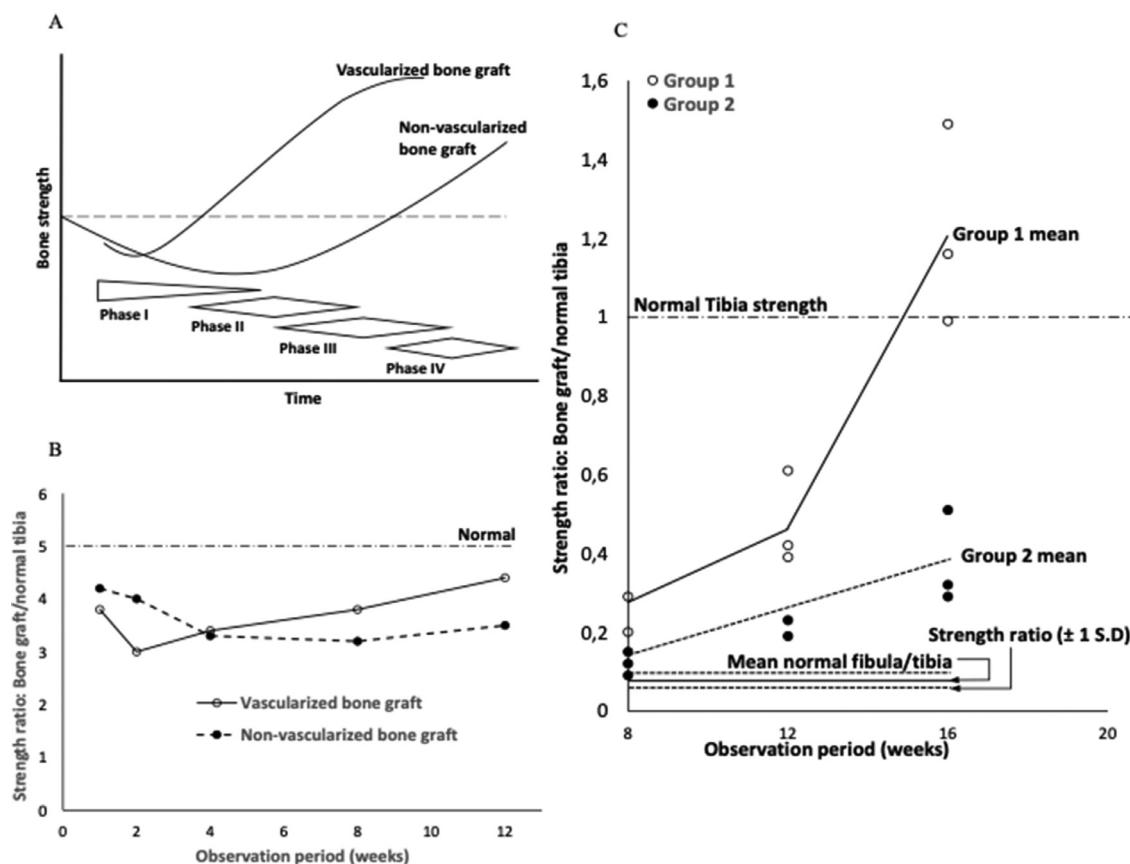


Fig. 2. (A) Graphical representation of the changes in bone graft strength over time. Phase I – Revascularization; Phase II – bone replacement; Phase III – bone consolidation; Phase IV – Hypertrophy. (B) Graph exhibiting relative c= strength of vascularized and non vascularized bone grafts compared with normal bone in rat. (C) Graph comparing strength of dogs' fibulae used as either vascularized bone grafts (group 1) or non-vascularized bone grafts (group 2) to replace a 4 cm defect in the tibia, the transferred vascularised fibula was stronger than normal tibia at 16 weeks. Re-drawn from [87].

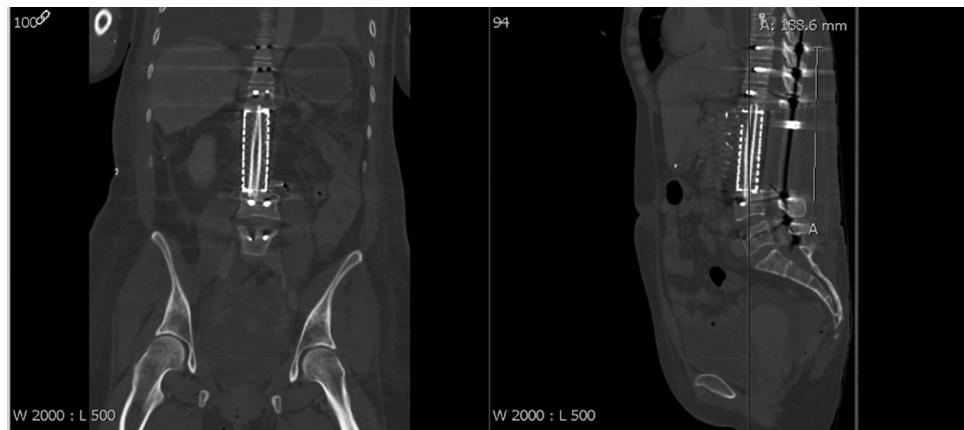


Fig. 3. Post operative CT scan of Synmesh cage and vascular fibula construct placed in the lumbar spine after en bloc resection. Left Coronal CT scan Cut and Right Sagittal CT scan cut. Reproduced with permission from Hatchell et al. [88].

a result of a direct vascular supply of leukocytes. Hemotherapy, while an old technique, has successfully used white blood cell fractions to treat a variety of ischemic infections. Treating chronic skin wounds with total blood has been shown to re-establish healing [99,100] and treatment with peripheral blood mononuclear cells directly isolated from blood has been shown to improve diabetic gangrene [101]. Peripheral blood mononuclear cells have been shown to be effective in fracture healing [102] and cartilage repair [103]. However, there is no direct evidence to support this the idea that blood's main function is to supply haemoatopoietic cells and it

is possible that neutrophils and monocytes migrate through tissue extravascularly in the absence of a direct blood vessel route. However, adequate vascularization of the graft host bed is often sufficient to reduce infection. If the host bed is not well vascularized, implantation of vascularized tissue, (e.g.: myocutaneous flaps) can be used to support the graft and may prevent infections [104,105]. While vascularized grafts present advantages for large reconstructions when compared to non-vascular grafts, they require microsurgical anastomosis, a specialized and demanding technique not always available. In addition, patients undergoing vascularized

bone graft harvest as part of reconstruction are hospitalized for a mean of 14.3 days longer (range, 8 to 36 days) than patients undergoing equivalent procedures with non-vascularized bone grafts, at least in cases of mandibular reconstruction [76]. Nonetheless, virtual surgical planning using tools such as computer-aided design/manufacturing combined with additive manufacturing technologies may improve significantly the operative efficacy and care (e.g.: minimally invasive approach, shape and positioning of the transplant, decrease of surgery time, better accuracy and consistency) [106–111].

Bone allograft without vascularization can be used fresh, frozen, freeze-dried or demineralized formats [77]. Allografts do not participate in osteogenesis, but when inserted adjacent to viable bone, allografts are repopulated to form a new bone. Large defects have been repaired with allografts [112–114] without any special technique for vascularizing the graft, and failure resulted mainly from infections (12%), fracture (16%) and non-union (17%). Failure was most prevalent in patients that had undergone chemotherapy [112–114]. Furthermore, these complications have been shown to be reduced when the allografts were covered with vascularized tissue flaps [115,116]. Unfortunately, the processes of freezing, impacting and rinsing, irradiating, freeze-drying, demineralizing, and deproteinizing allograft aim to reduce its immunogenic potential but may also decrease graft mechanical properties and repopulation potential [117–123].

Bone marrow is seen as a ready supply of viable bone elements. The osteogenic potential of bone marrow appears to have first been reported in experiments in rabbits more than 150 years ago [124]. However, autologous marrow grafts alone do not always form bone, yet in combination with different kind of scaffolding materials (such as cancellous bone allograft or porous bone substitute) ectopic bone formation was observed consistently [15,125–127]. Burwell put forth the view that necrosis of marrow was a requisite for bone formation since when marrow alone was implanted into soft tissue it became flattened and so diffusional limitations were minimized [128,129]. Bone marrow is formed of various cell types amongst which some are osteogenic under the right conditions. Bone marrow mesenchymal stem cells (BMSC) became identified as a stromal subpopulation of marrow that could form mineralized nodules in vitro and could be differentiated into adipocytes and cartilage. Scaffold supported BMSC regenerative strategies have dominated the literature for the past 20–30 years. Clear success in repairing segmental defects that would avoid the need for harvesting bone flaps has been elusive. It was recently identified that marrow osteogenic potential was also dependent on immune cells inside the marrow [130]. This work indicated that monocyte derived cells and not MSCs form bone upon transplantation. This finding is likely to herald progress in our ability to manipulate skeletal tissue therapeutically. It is well known since Burwell [131] that the use of total bone marrow with fresh allograft has a similar osteogenic potential to fresh marrow-containing autograft. In other words, marrow, a tissue that can be harvested in ample volumes under local anesthesia and that is one of only a few human tissues that has the capacity to self-regenerate can impart the same ‘gold standard’ bone defect healing as autograft to acellular and non-viable bone grafts. It also indicates that cells found in the mineralized matrix of bone, (periosteum, endosteum, osteoblasts and osteoclasts, and osteocytes) may not be required to induce bone formation. While this work predates the identification of the MSC and BMP it is important to consider because the mechanism by which it works, and the role of necrosis remains undiscovered. There has been a recent renaissance of this work and clinically it was very recently confirmed that there were no significant differences in treatment success rate between autograft and marrow loaded allograft [132]. It has been found to result in successful bone repair in diaphyseal critical sized defects in humans [133]. Similarly, addition of bone

marrow to xenografts have been shown to significantly enhance bone formation when compare to empty xenograft [134,135], resulting in healing not significantly different from autograft in rabbit models [136]. While somewhat less studied due to concerns over prion diseases there are approximately one sheep, pig or cow for every two humans [137]. They may represent a plentiful supply of high strength acellular bone matrix given their lifespan is typically 12 months.

Replicating the outcome of bone autograft without harvesting bone from a living patient is then possible. Recently there has been a case report of successful repair of a large (hemi-mandibular) defect utilizing marrow, allograft, and BMP [138]. Another case report details a near hemi mandibular defect repaired with a frozen humeral allograft consisting of a bone diaphysis cut longitudinally and perforated. A vascular free flap was placed inside what was formerly the medullary cavity that was also marrow loaded. In the words of the authors, the technique ‘can be considered as a real bone tissue engineering demonstrating the possibility to rebuild large bony defects without vascularized bone’ [139]. However they did use a vascular free flap as part of the construct.

2.2. Distraction osteogenesis bone transport

This well-established technique is widely reviewed elsewhere [140] and is included briefly here because it perhaps the most prevalent route to repairing massive segmental bone defects without large autograft harvest. In addition, we consider studies confirming a role of angiogenesis in successful distraction osteogenesis.

The bone transport strategy, pioneered by Ilizarov (lengthening distraction osteotomy) and later refined by Cattaneo, is based on the principles of osteogenesis by distraction of an incompletely mineralized, evolving, and therefore extendable callus (Fig. 4) [140–144]. A low energy osteotomy of the cortex performed proximal to a defect site and maintaining the peripheral and central blood supply of the periosteum and medullary canal respectively is first performed followed by fixation [142,145,146]. Then follows a latency period during which a callus is formed between the bone ends. After new bone has formed in the gap, the bone segment is gradually distracted by approximately one millimeter per day until the desired bone length is reached [74,147–150]. A too low or too high elongation rate leads to a premature consolidation of the callus or slowed osteogenesis, respectively [151]. When the newly formed bone reaches the required length, a period of immobilization is necessary for its consolidation [152].

It is a technique that is often used in limb reconstruction surgery [145,146,153,154] and in the craniofacial region [155–159] for its ability to manage complex deformities and defects by simultaneously treating the bone defect and soft tissue loss. Its success has been attributed to the “tension-stress” effect, a phenomenon where tissues that are subjected to gradual, consistent traction forces become metabolically activated, leading to significant tissue regeneration [145,146].

Adequate blood supply is essential for this technique. It has been shown that angiogenesis is required for successful bone induction during distraction osteogenesis [161–164]. Blocking angiogenesis in a rat mandibular defect model resulted in no histologic or radiographic evidence of bone formation between the two osteotomy fronts [162]. Given its ability to mobilize the patient immediately following the initial osteotomy, bone transport has certain advantages. However, it is technically difficult with potential for complications relating to the quality of regenerate, axial deviations, infection [165] (5 to 30% [166]), and nerve overstretching (peripheral nerve complications between 2 and 15% [167]). As the process extends over a period of time during which the bones and surrounding tissues are being manipulated also leads to spe-

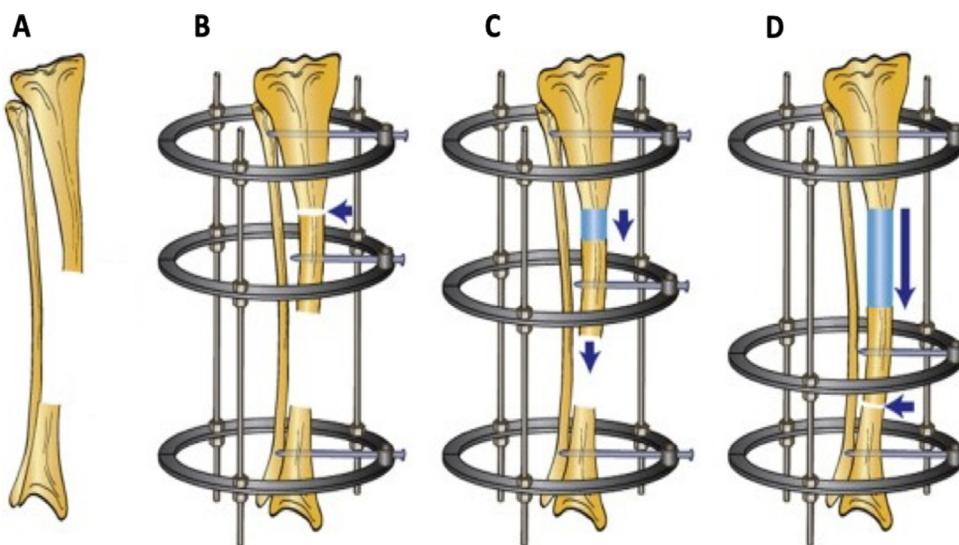


Fig. 4. Description of bone transport technique. (A) Showing the segmental bone defect. (B) Application of the external fixator and performing a proximal tibial osteotomy. (C) Start of distraction and transport of the healthy segment to close the bone defect distally. (D) End of distraction and completion of bone transport. Reproduced and modified with permission from Makhdom et al. [160].

specific complications during the surgery, the distraction, and post-distraction [165]. Adjuvant bone grafting is frequently needed to obtain complete consolidation and prevent repeat fractures and non-union [168–170]. The success of the procedure is contingent on adequate soft tissue coverage over the defect, which on occasion requires a free tissue flap transfer [171], compounding the risk of significant donor site morbidity [75]. Finally the discomfort to the patient is considerable with use of the distraction device for months. A number of recent reviews summarize historical and new developments in this field in relation to segmental bone defect repair [172–176].

2.3. Masquelet technique

More recently the Masquelet technique also known the induced membrane technique was initially described by Klaue *et al.* (1995) [177]. It is a two-stage technique for the repair of large (up to 25 cm) and contaminated segmental bones defects [150] (Figure). The first step consists of a debridement of the damaged tissues, the insertion of a polymethyl methacrylate (PMMA) spacer in the bone defect (often loaded with antibiotics to control open wound contamination), and stabilization with fixators. This spacer (i) prevents the invasion of the defect by a fibrous tissue that would hinder bone regeneration, (ii) induces a foreign body reaction and (iii) induces the formation of a vascularized membrane around the spacer. The spacer is removed (with minimal disruption of the membrane) after 4 to 8 weeks and the membranous capsule is packed with a morselized cancellous allograft, and then closed before definitive fixation.

The spacer features (e.g., composition, size, porosity, roughness, active substances) appear to have a significant influence on the membrane formation and final outcome. For example, PMMA induced membranes provide an environment more conducive to bone regeneration than titanium induced membranes, and polyvinyl alcohol spacers do not result in membrane formation [179,180], which makes the assumption that PMMA is totally inert as noted in section 2.1 seem improbable.

Despite a rate of bone union reaching up to 87%, this technique has been associated with a high risk of complications (54% reported in children [181]) when compared with donor site complications for vascularized allografts (6 to 19% [182]). The need for additional surgery, failure of one or both the surgical steps, with

fractures of the graft site, joint stiffness and deformity are all observed drawbacks [181,182].

Many studies have been conducted to characterize and determine the benefits of this induced membrane [150,183–188]. The membrane organization act as a synovial like epithelium on the inside, and the outer part is constituted of fibroblasts, myofibroblasts, and collagen [189] also including inflammatory cells and osteoprogenitor cells [190,191]. Although the cell types and cytokines present in this highly vascularized tissue changes throughout its maturation [188,192], the re-vascularization and regeneration of the defect once the bone graft is placed are promoted through the generation of angiogenic and osteogenic factors [186,188,192].

A recent study by Durand *et al.* [193] highlights that induced membrane failure is independent of the type of bone or fixation, IM maturation time, or other patient characteristics. Failure was associated with defective membrane properties which included decreased cellularity, decreased osteoprogenitor content, and impaired ECM remodeling thus pointing to the importance of the induced membrane. In addition, another pilot study by Tarchala *et al.* advanced the concept that the primary source of osteogenic factors for bone regeneration was the native bone ends and not, as often believed, the induced membrane [194]. To date the mechanism by which the technique is active is not fully understood but certainly the inflammatory responses would be different in the healing zone in the first and second surgeries. To our knowledge the role of the immune response in the Masquelet technique's action has not been systematically studied. The Masquelet technique originated from a serendipitous discovery following interpretation of unexpectedly good healing following staged surgeries to manage soft tissue infection prior to addressing skeletal repair. While different methods have been developed in order to improve the technique, they are largely incremental and until it is understood how a 'bioinert' material like PMMA can result in massive segmental bone regeneration it seems unlikely that we can fully harness the regenerative capacity of the adult skeleton. This technique is in continuous development and evaluation and the reader is directed to recent reviews focusing solely on this topic [195–203].

2.4. Arteriovenous loop and vascular bundles

In the late 1950s, development of microsurgical anastomosis of small blood vessels ($1 \text{ mm} \leq$) allowed transplantation of various

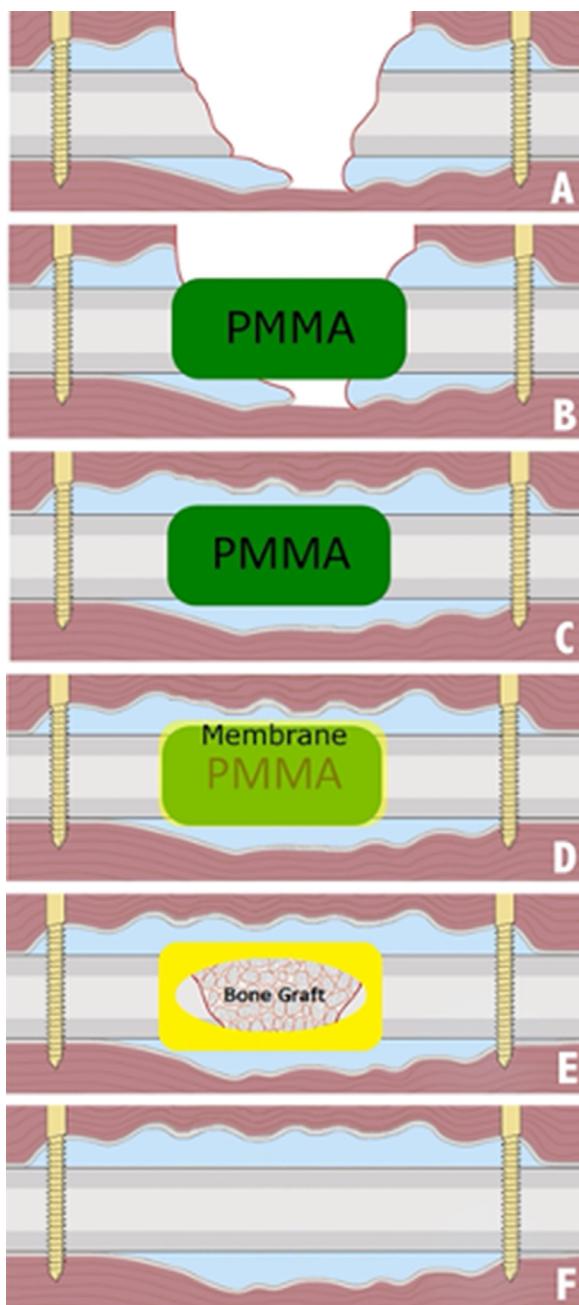


Fig. 5. The Masquelet technique. Images on the left and right depict step-by-step of the two-staged Masquelet technique. On the left from top to bottom A) First Surgery, Critical-sized bone defect irrigated and debrided at both bone ends. B) PMMA cement fills the defect, surrounding both proximal and distal bone ends. This step can involve further fixation with an external fixator, a locking plate, or intramedullary nail (patient/surgeon dependent). C) Surrounding soft tissue healing with PMMA in place. D) A membrane forms around the PMMA. E) Second Surgery: Induced membrane exposed, cut open, and PMMA removed. F) Defect site filled with bone graft (various combinations of auto/allograft used), and membrane sutured closed. Adapted with permission from Tarchala et al. [178].

tissue flaps and replantation of digits as reviewed by Fang et al. (2014) [77]. The first free vascularized bone flap was reported in 1975 [23] and this led to several variants and anatomical specific flaps. This technique also led to attempts to salvage ischemic limbs by reconstructing the arterial and venous vascular system [204,205]. Hori et al. were one of the first to compare regeneration of non-vital and necrotic bones by promoting revascularization with a diverted flow-through arteriovenous bundle (AVB), an

artery and an arteriovenous loop (AVL i.e. an anastomosis between an artery and a vein effected with a vein graft) [206]. The AVB, and to a lesser extent the AVL (since the vein lost patency ~70% cases,), induced the proliferation of capillaries around the necrotic area. This promoted the concomitant resorption of the non-vital bone and formation of new bone tissue. This discovery was applied to the clinical treatment of sclerotic and necrotic bones in the hand (Kienboeck's disease) and also partial bone unions after 3 to 6 months. Tanaka et al. investigated the underlying mechanisms leading to the formation of a new vascular network from AVL and both flow and non-flow AVB [207]. They reported that: (i) AVL had a stronger revascularization potential than AVB, (ii) neovascularization arose directly from the arterialized vein from which vascular sprouts directly arborized, (iii) endothelial sprouting of the artery was not observed. They proposed that endothelial sprouting was induced by the inflammation, hemorrhage and coagulation due to the surgical act itself and the modulation of the physiological flow within the vein walls (AVL and non-flow AVB) [207].

Although this strategy is already used for salvaging ischemic soft tissues [208–211] direct clinical applications of AVL and AVB for the regeneration of large bone defects is to date limited. Several reasons may explain this, such as (i) the complexity of the bone environment and the very slow healing process compared to other tissues, (ii) the lack of adapted biomaterials to host this intrinsic vascularization and to promote vascular and bone formation, (iii) the complexity of the surgical procedure that relies on microsurgery, which may not be a common skill in the orthopedic field. A few clinical cases have been reported mostly in Germany [127,206,212–217], to treat osteomyelitis, local osteosclerosis or necrotic bone without any bone harvesting. However, recently Horch et al. [212] combined an AVL and a bone filler (autograft or biphasic calcium phosphate granules loaded with autologous bone marrow) to treat large (8 cm tibia and 4 cm distal radius) bone defects generated after debridement of osteomyelitis. After the final follow-ups (2 and 3 years, respectively), the patients were healed with no residual osteomyelitis and resumed normal function. Currently a clinical trial is underway to use synthetic biomaterials and AVL to treat mandibular defects [218] (Estimated Primary Completion Date June 30th 2029 - ClinicalTrials.gov Identifier: NCT04001842). This could initiate a positive dynamic for the transfer of the extensive in vivo research performed on small and large animal models which have already shown exciting prospects: e.g., vascularized bone tissue engineered constructs, ectopically prefabricated transplantable synthetic vascularized bone.

The main advantages and disadvantages of reconstructive techniques for SBD are summarised in Table 1. Clearly the reasons for bone loss affect outcomes. Recently outcomes for segmental bone defect repair have been compared in a high quality study comparing reports only for fracture related infections with a mean bone defect of 6.6 cm (range 1.0–26.0), of which 82% were localized at the tibia [229]. Some of the key findings are reproduced in Fig. 6. The overall efficacy of VBG is evident as fastest time to union and lowest complication and revision rate.

3. Experimental and preclinical techniques

Having considered the state of the art and the most promising approaches in development we look ahead to concepts that are at the preclinical stage that also offer the potential to regenerate the segmental defects of the skeleton.

3.1. In vivo pre-vascularization strategies

The concept of an in vivo bioreactor reported in 2000 by Tanaka et al. [207,230,231], Stevens et al. [232] and Holt et al. [233] represents an interesting approach. It has been described as “taking

Table 1

Summary of the main pros and cons of each SBD reconstructive technique.

Technique	Union Rate	Compatible with tumor resection	Advantages	Disadvantages
VBG	95% Mean length 9.1 cm (4.5–16 cm) [76,90–94]	Yes [6]	High success rate esp. longer defects	Morbidity of harvest, (11% of complications) [89] poor anatomical fit
Allograft bone	76% Mean 8.3 cm, (3 to 14 cm) [52]	Yes [219]	No Harvest Readily available No microsurgery Long history of use	Lower union at lengths >6 cm Prone to resorption
Masquelet	82% Mean 5.9 cm (0.5 to 26) [220]	Yes [221]	Allows infection control and coverage No microsurgery Shorter history of use	Slow 2 stage procedure. Union 6 months (1.4 to 58.7) after first surgery. Potentially lower success in pediatric patients [222] Mechanism not fully understood
Bone Transport	59% Mean 5 cm, (range, 1.5–14.5) without use of supplementary graft at docking site [223]	Yes [224]	No limit to length Can correct deformity	Pain Increased infection risk Slow, e.g. 6.8 cm defects required 17.21 months (range, 11–24) fixation compared with 10.15 months (range, 8–14) for Masquelet More fair/poor functional outcome 52.65 vs 15% for Masquelet [225]
Arteriovenous Loop/ Arteriovenous Bundle	Insufficient population size Case reports and follow ups positive [214–217,226]	AVL for reconstruction of soft tissues already in use[227]	Creates tissue without harvest Closed technique	Can require 2nd surgery, but in situ cases reported Requires microsurgery AVL can occlude, failure rate 4 and 24% for single and two stage procedures and complication rates of 16% and 24% [228]

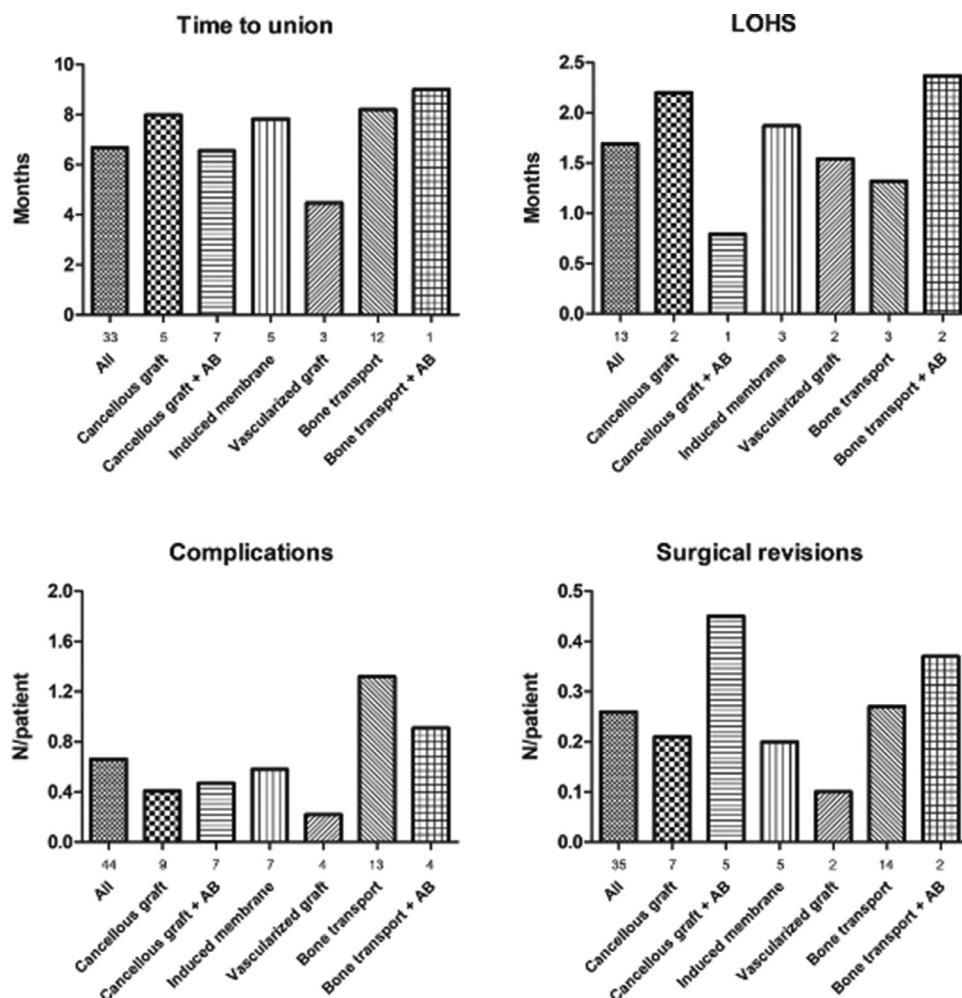


Fig. 6. Time to union, length of hospital stay (LOHS) (expressed as months), complications and surgical revisions (expressed as average number per patient) for mainly tibial fracture infection related segmental bone defect repair, (AB-Antibiotic, number in X axis is number of studies pooled). (Reproduced from [229] H. Bezstarost, W. J. Metsemakers, E. M. M. van Lieshout, L. W. Voskamp, K. Kortram, M. A. McNally, L. C. Marais & M. H. J. Verhofstad, Management of critical-sized bone defects in the treatment of fracture-related infection: a systematic review and pooled analysis Archives of Orthopaedic and Trauma Surgery volume 141, pages 1215–1230 (2021), reproduced under Creative Commons Attribution 4.0 International License, <http://creativecommons.org/licenses/by/4.0/>).

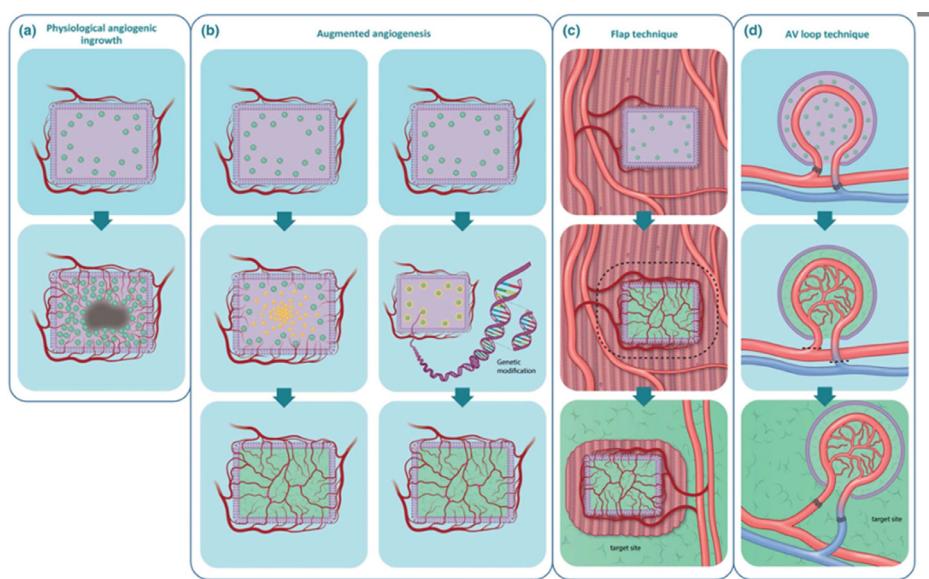


Fig. 7. Scheme showing the different *in vivo* pre-vascularization approaches in tissue engineering. (a) Physiological angiogenic ingrowth into a tissue graft. The lack of adequate and timely vascularization may lead to cell necrosis in the core of the graft. (b) Enhancement of the naturally occurring angiogenic response within the target site by either loading the graft/scaffold with proangiogenic factors (left panel) or by genetic modification of the graft cells to induce enhanced secretion of angiogenic factors (right panel). (c) The flap technique consisting in preimplantation in muscle tissue to pre-vascularize the graft, followed by a transplantation to the target site. (d) Arteriovenous (AV) loop technique, similar to the flap technique consisting in an encapsulated graft incorporating an AV loop to facilitate initial perfusion until further vascularization can be established. The vascularized graft then can be transplanted to the target site. Reproduced from open access article by Rademakers *et al.* [235].

advantage of the body's intrinsic self-regenerative capability, the traditional triad elements or a combination thereof are cultivated using the body as a bioreactor at the damage site or within ectopic sites capable of supporting neotissue formation" [234]. Different approaches to *in vivo* pre-vascularization have been developed as summarized in Fig. 7 and discussed fully in a recent comprehensive review [176]. Generally, the strategies are i) augmenting angiogenesis by capillary ingrowth either by loading the graft/scaffold with proangiogenic factors or by genetic modification to induce enhanced secretion of angiogenic factors. Ectopic implantation prior to usage at the target site using either a flap technique involving preimplantation usually in muscle tissue to pre-vascularize the graft [235] or an AVL technique, similar to the flap technique, but established at any location, and consisting of an encapsulated graft incorporating an AVL that facilitates initial perfusion as further vascularization is established [235–237].

3.1.1. Enhancement of angiogenesis

Utilization of pro-angiogenic factor (e.g., VEGF, FGF) loaded scaffolds for bone repair have shown contradictory results in vascularization - from no improvement [238] to an increased vascularization [239–243]. Similarly contradictory results were obtained for bone formation from no improvement [239,242,244] to increased bone formation [238,241]. This may suggest that vascularization during bone healing may not be the major factor at play when aiming to enhance bone healing. Furthermore, VEGF has been shown potential to promote osteoclast survival [245] and bone resorption both *in vitro* and *in vivo* [246–248]. This may in turn impair bone formation [249].

3.1.2. Pre-implantation and transplant

The flap technique is comparable to the vascularized free flap technique. This technique allowed clinical surgical bone reconstruction using bone mineral, bone carrier, β -TCP, or hydroxyapatite that is pre-implanted in titanium cages. Stem cells (bone marrow or adipose derived) or bone morphogenic proteins are added to the cages. This technique has been successful however the number of studies is limited. Furthermore, the clinical nature of these

studies limits the analysis that can be performed to appreciate the bone quality/quantity and its vasculature.

3.1.3. AVL-AVB

Spalthoff *et al.* demonstrated in a large animal model (sheep) that formation of clinically relevant volumes of bone could be ectopically generated using β -TCP scaffold seeded with autologous bone marrow and perfused by an AVL [250]. Tatara *et al.* [251] succeeded in the reconstruction of a large mandibular defect (sheep) by pre-implanting BCP granules in a chamber in contact with the animal rib, followed by transplantation of the intercostal pedicle into the defect. Despite compelling results, the need to sacrifice a main artery to transplant the vascularized bone construct may be a practical limitation. A recent study by our group proposed an alternative to solve this issue using exclusively dispensable tissues. Vascularized and transplantable bone volumes were subcutaneously generated using a microporous 3D-printed monetite scaffolds, loaded with autologous bone marrow, and perfused by a single vein (rat model) [126,252] (Fig. 8).

Generating personalized and transplantable large, vascularized bone volumes without recourse to processed cells, bioactive substances, sacrifice of healthy tissue or complex microsurgery techniques (e.g., AVL) would represent a significant progress in reconstructive surgery.

3.2. *In vitro* pre-vascularization strategies

Many approaches for engineering vascularized bone use the formation of organoids or a biomaterial scaffold seeded with different types of endothelial cells combined with cells intended to proliferate and repair the defect. Typically, the endothelial cell source may be endothelial progenitor cells (EPC) or human umbilical vein endothelial cells (HUVEC), osteoblasts or stem cells (mesenchymal derived from marrow, adipose tissue or other sources or induced pluripotent stem cells. Human iPSC-derived iMSCs improve bone regeneration in mini-pigs [253,254].

Stem-like cells capable of differentiating into more than one mesenchymal lineage have been identified in several readily ac-

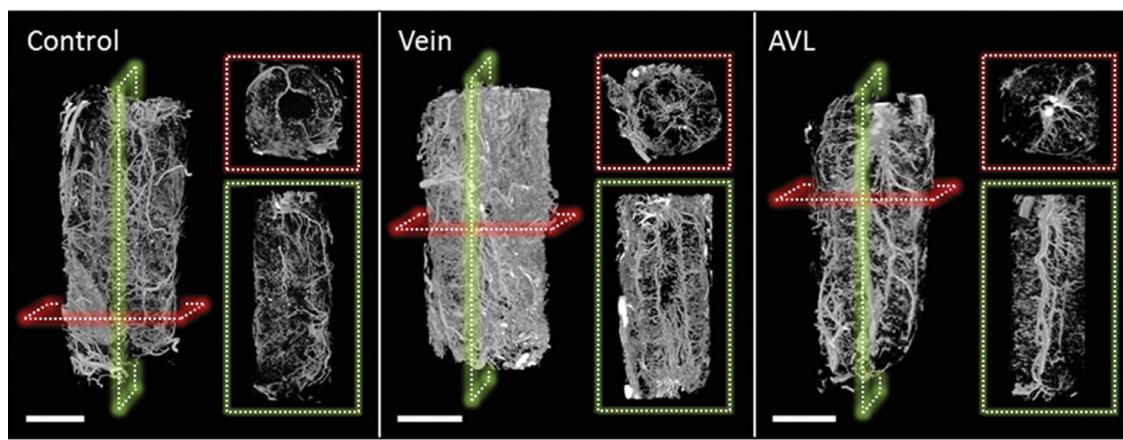


Fig. 8. Comparison of vascular networks developed by extrinsic capillary invasion (left), a flow through vein (center) and a microsurgically created AVL, as shown by contrast agent perfusion and micro-CT (scale bar = 2 mm) Reproduced with permission from Charbonnier et al. [252].

cessible tissues such as peripheral blood and dental pulp. These developments are reviewed in detail elsewhere e.g. [255–257].

3.2.1. Use of endothelial cells

Techniques developed to create constructs resulting in the formation of microcapillary-like structures resembling bone tissues in vitro are documented [258,259]. They aim to improve bone formation and integration in vivo by recreating or accelerating the formation of the bone vascular microenvironment [258,260]. It has been demonstrated in vitro that the combination of these different cells may have a synergistic effect resulting in 2D and 3D constructs (organoids or scaffolds), and an augmented number of microvessel-like structures, mineralized tissue, and/or proangiogenic (VEGF, TGF, PDGF, etc.) [258,261,262] and osteogenic factors [263]. Direct addition of those factors to cultures has also been also been proven to increase both angiogenesis and osteogenesis [264]. Whether ectopically or in orthotopic models (subcutaneous, calvarial and femur artificial defects), different cells type combinations have resulted in an increased osteogenesis and bone formation when compared to single cell-type experiments [265–272]. Overall, the reported increases in bone resulting from these experiments is about 1.2 to 3 times higher with EC at the end of the experiment (4 to 8 weeks) and the resulting bone formation is found to be homogeneous in small defects [268,270,273] but more concentrated on the edges of larger defects [267,269]. However, Shah et al. [274] and Koob et al. [275] demonstrated no improvement of osteogenesis with coculture when compared to monoculture after in vivo implantation, and hypothesized that this might be the result of absence of culture optimization. However, a meta-analysis of pre-clinical in vivo studies of cell co-transplantation for vascularized craniofacial bone tissue engineering by Shanbhag et al. [276] concluded that co-transplantation had a statistically significant benefit when compared to monoculture in term of new bone formation measured radiographically, but histomorphometry remained inconclusive. As is often the case with in vivo studies, extrapolating a statistical significance in preclinical studies to a clinical significance is challenging.

3.2.2. Pre-vascularisation

Perhaps unexpectedly pre-vascularization results in higher number of microvascular structures was often reported in vivo (1.2 to 20 times more) [258,277–287]. The resulting vessel length has been shown to be influenced by both time of pre-vascularization and time of implantation [278,281]. Researchers reported an anastomosis of the construct with the host vasculature, sometimes exhibiting chimeric vessels in studies using cells from different species

than the host (Fig. 9) [279,281]. Host vasculature invasion is limited to the outside of the implanted constructs. The earliest time for anastomosis found in literature was three days [282] for cell spheroids in an ectopic site. For larger scaffolds anastomosis appear to occur at a later point and was limited to the edges of the scaffold. Efficiency of blood perfusion after anastomosis has been shown to be relatively low (30% of the entire scaffold at 2 weeks) [281]. However the meta-analysis by Shanbhag et al. [276] concluded that there was no statistically significant benefit of a coculture versus monoculture in terms of new vessel formation when looking at the broad range of the existing models that have been studied, although this conclusion requires a cautious interpretation due to the heterogeneity of studies, and their biological and methodological factors. Interestingly, Zigdon-Giladi et al. [272] and Seebach et al. [270] demonstrated an increase of the blood vessel density for short timepoints and then a decrease of this density when looking at longer timepoints. This would imply an instability of immature new vessels and it is known that early stage angiogenesis of small vessels is often followed by a regression if the vessels are not functional [288]. It is worth noting that marrow aspirate, a commonly used clinical osteogenic cell source contains 20 vol% microvessels [289], compared to 2% in cortical bone [290] and it seems probable that the anastomosis of these capillaries can occur following transplantation. Recent studies on other marrow subpopulations have been extremely illuminating. It was demonstrated that when CD14+ monocytes were removed from marrow, bone formation ability was lost [130] indicating no unique role for MSC.

3.2.3. Recapitulating endochondral bone formation

In embryonic development, the formation of bone occurs via two main mechanisms i) intramembranous ossification giving rise to flat bones such as the cranium and the pelvis and ii) endochondral ossification, giving rise to long bones [77–79]. During endochondral ossification, the cartilage grows in length, with the division of chondrocytes and the secretion of extracellular matrix, and is accompanied by new chondroblasts that develop from the perichondrium. The perichondrium becomes the periosteum which contains a layer of osteoprogenitor cells which later become osteoblasts that secrete osteoid against the shaft of the cartilage that serves support for the new bone. Chondrocytes in the primary center of ossification undergo hypertrophy and secrete alkaline phosphatase leading to the calcification of the matrix used by the osteoprogenitor as a scaffold to secrete osteoid and to form trabecular bone. Osteoclasts then break down spongy bone to form the medulla.

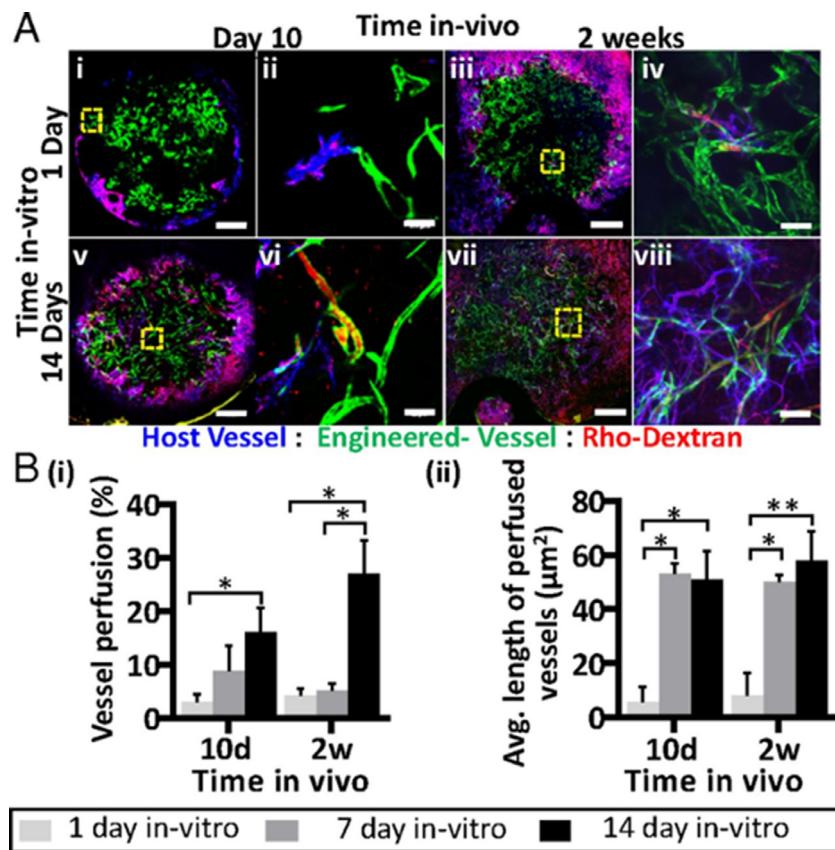


Fig. 9. Demonstration of Host-graft vasculature anastomosis and functionality following implantation of fibrin gel implants containing GFP-expressing HUVEC and HNDF. (A) Representative intravital confocal images of host-graft vasculature, as viewed through the dorsal skinfold window on days 10 and 14 after implantation; grafts had been cultured in vitro for either 1 d (Upper) or 14 d (Lower) before implantation. Implanted cells are shown in green, and host vessels are shown in blue, blood flow in red. The images clearly show the interconnection of host graft vasculature. (B) Graph exhibiting the percentage of perfused engineered vessels in the graft cultured for 1, 7, or 14 d in vitro prior to implantation. Scale bar in (i, iii, v, viii) 1 mm, (ii, iv, vi, viii) 100 μ m. Reproduced from Ben-Shaul et al. [281].

In the adult, fracture healing may occur via endochondral ossification [291] which suggests a therapeutic role for cartilage tissue in bone regeneration strategies. It has been suggested that cell-based regeneration including endochondral ossification approaches comprise four major components: stem cells, growth factors/morphogens, scaffolds, and mechanical stimulation [291,292]. It has been shown in vitro that mineralization could be induced in chondrocytes [291,293–297] through the use of various protocols aiming to optimize priming times, growth factors and processing [298,299]. The lack of vascularization is a major problem in tissue engineering. As such, inducing vascularization of these constructs through various means has been studied [295]. Cell coculture has been shown to improve vascularization in vivo [300–302]. Several studies have succeeded to form ossicles or bone in vivo [296,299,303,304], Bernhard and al [305] compared critical sized bone defect healing using hypertrophic chondrocytes, osteoblasts and acellular scaffolds. They reported that hypertrophic chondrocyte grafts bridged 7/8 defects, as compared to only 1/8 for osteoblast grafts and 3/8 in acellular scaffolds.

Endochondral recapitulation has been used to repair large defects resulting in improved bone formation in cranial defects [306] and in critical sized rat femoral defects [302,307,308] and even in a massive bone defect [309], and by controlling TGF β , and loading in chondral tissue scaffolds [310], but reports on transfer to larger animal models are more scarce [299]. Very encouraging results were obtained by Eamon et al. [311] for the formation of vascularized and mineralized engineered phalanx. Of the preclinical approaches under investigation for segmental bone defect regener-

ation, endochondral approaches seem the most poised to make a large impact clinically in the future.

4. Concluding remarks

It is clear then that a variety of approaches are beginning to yield new reconstructive approaches. It is notable that many are surgically driven rather than scientifically driven. Often, possibly due to the complexity of whole skeletal tissues scientists focus too narrowly on one or two cell types or interactions. However, especially for large bone defects, the problem of regeneration often involves multiple tissue types and processes. Indeed, as described in Section 2.1, revascularization of massive grafts seems to be driven by the vascularity of the surrounding soft tissue. Relatively little progress has been made on accelerating or enhancing soft tissue vascularity, and it is generally assumed to be an anatomy and patient specific fixed parameter, this may be a target for future therapies.

Infection is a common concomitant issue associated with orthopedic trauma. When self-setting inorganic cements were first introduced clinically, the vision was for a moldable material that could be sculpted to match complex anatomy that would be resorbed and replaced by host bone. Long term follow-up data indicate that these materials are not fully remodeled and more importantly have unacceptable rates of infection. If supply of blood borne phagocytes is initially insufficient, bacterial contaminant colonization can take hold, not only preventing bone formation, but destroying overlying soft tissue and once the skin barrier is compromised, failure is somewhat inevitable [312,313].

It is noteworthy that one of the most recent developments in massive allograft reconstruction post tumor resection [82] does not attempt to mimic a vascularized bone flap. Instead, the focus is on osteointegration of the allograft bone segment ends and the bulk of the graft is shielded with PMMA to prevent graft resorption. If the graft does fail it is protected from catastrophic brittle failure by the PMMA core. While simple, this solution builds on decades of prior knowledge, is safe, cheap and most importantly effective. Similarly, the Masquelet technique, stages healing by blocking healing zones, again with PMMA, a material that is neither biomimetic nor bioactive.

At the other spectrum, biological or cellular engineering approaches have yet to make a significant impact. A notable exception is the *in vivo* bioreactor approach- essentially using the patient's body as a sterile culture environment to engineer tissues ectopically for subsequent transplantation. Again, the key hurdle is the lack of control over neovascularization by microsurgically anastomosable vessels (>1 mm diameter). Additive manufacturing has a key advantage in being able to easily recapitulate the complex geometries of the skeleton. Beyond that, advantages are less clear, often the proposed solutions are crude analogues of bone tissue into which various channels and openings at the macro (>500micron) scale have been 'designed'. Often approaches seem misguided by anthropomorphic heuristics and assumptions not supported by data, such as cells choosing certain micro-environments, or cells having whole organism like requirements for oxygen and nutrition that will apparently summon blood vessels, and result in a regenerative cascade [314,315]. These unconscious biases have arguably slowed progress as researchers have struggled to reconcile with their own data contradicting what seemed to be reasonable hypotheses. For example, the role of MSC in bone formation is not a topic on which the community has full consensus [130]. In many techniques dedicated to reconstructing bone, a lot of effort is often made in maintaining or inducing vascularization. Fewer complications are observed with vascularized bone grafts, yet the presence of an adequate vascularization does not necessarily lead to success. While success rates of large avascular grafts is lower than for vascularized ones, it is not zero. Indeed, these grafts do succeed, albeit not with high reliability. The mechanisms behind failures are not, to the authors' knowledge, elucidated, yet this seems important to understand better.

If osteodistraction teaches us that proper vessel growth is necessary for healing to succeed, the Masquelet technique shows that one of the most important elements of recreating bone is to first repair surrounding soft tissues and potential vascularization. Moreover, the use of bone filler in osteodistraction or the Masquelet techniques indicate that creating bone is not the major issue in bone healing, rather it is the vascularization. Experiments clearly show that dead bone can be repopulated if blood vessels are present. On one hand, for replacing bone or just healing bone, the major requirement is an intrinsic vascularization. On the other hand, the intrinsic vascularization of "an empty" scaffold is not required for *de novo* bone formation.

While cancer therapeutics are a high-profile priority for many developed countries, improving reconstruction of cancer survivors has fallen woefully behind. We have identified this patient group and their currently poorly served needs as an emerging and growing priority for bone regeneration that can only be addressed by surgically led multidisciplinary approaches that are pragmatic and feasible within the increasingly resource limited setting of the healthcare system. The ideal reconstruction strategy for tumor surgery reconstruction is not yet clear. Confounding factors such as radiation damage to vascular supply of the surrounding soft tissue, systemic chemotherapy suppression of healing [316–319], are not fully elucidated. This poorly served area would appear to be an important priority for future scientific discovery.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] C. Mauffrey, B.T. Barlow, W. Smith, Management of segmental bone defects, *JAAOS - J. Am. Acad. Orthop. Surgeons* 23 (3) (2015) 143.
- [2] A. Bigham-Sadegh, A. Oryan, Selection of animal models for pre-clinical strategies in evaluating the fracture healing, bone graft substitutes and bone tissue regeneration and engineering, *Connect. Tissue Res.* 56 (3) (2015) 175–194.
- [3] P. Garcia, T. Histing, J.H. Holstein, M. Klein, M.W. Laschke, R. Matthys, A. Ignatius, B. Wildemann, J. Lienau, A. Peters, B. Willig, G. Duda, L. Claes, T. Pohleman, M.D. Menger, Rodent animal models of delayed bone healing and non-union formation: a comprehensive review, *Eur. Cells Mater.* 26 (2013) 1–12 discussion 12–4.
- [4] Y. Li, S.-K. Chen, L. Li, L. Qin, X.-L. Wang, Y.-X. Lai, Bone defect animal models for testing efficacy of bone substitute biomaterials, *J. Orthop. Transl.* 3 (3) (2015) 95–104.
- [5] D.S. Sparks, S. Saifzadeh, F.M. Savi, C.E. Dlaska, A. Berner, J. Henkel, J.C. Reichert, M. Wüllschleger, J. Ren, A. Cipitria, J.A. McGovern, R. Steck, M. Wagels, M.A. Woodruff, M.A. Schuetz, D.W. Hutmacher, A preclinical large-animal model for the assessment of critical-size load-bearing bone defect reconstruction, *Nat. Protoc.* 15 (3) (2020) 877–924.
- [6] J.C. Reichert, A. Berner, S. Saifzadeh, D.W. Hutmacher, Preclinical animal models for segmental bone defect research and tissue engineering, 2011.
- [7] L. Helbig, T. Guehring, N. Titze, D. Nurjadi, R. Sonntag, J. Armbruster, B. Wildemann, G. Schmidmaier, A.P. Gruetzner, H. Freischmidt, A new sequential animal model for infection-related non-unions with segmental bone defect, *BMC Musculoskelet. Disord.* 21 (1) (2020) 329.
- [8] J.C. Reichert, S. Saifzadeh, M.E. Wüllschleger, D.R. Epari, M.A. Schütz, G.N. Duda, H. Schell, M. van Grienden, H. Redl, D.W. Hutmacher, The challenge of establishing preclinical models for segmental bone defect research, *Biomaterials* 30 (12) (2009) 2149–2163.
- [9] J.F. Keating, A.H.R.W. Simpson, C.M. Robinson, The management of fractures with bone loss, *J. Bone Joint Surg. British volume* 87-B (2) (2005) 142–150.
- [10] A.R. Elniel, P.V. Giannoudis, Open fractures of the lower extremity: current management and clinical outcomes, *EFORT Open Rev.* 3 (5) (2018) 316–325.
- [11] T.H. Tosounidis, P.V. Giannoudis, Biological Facet of Segmental Bone Loss Reconstruction, *J. Orthop. Trauma* 31 (5) (2017) S27–S31 Suppl.
- [12] P.S. Pipitone, S. Rehman, Management of traumatic bone loss in the lower extremity, *Orthop. Clin. North Am.* 45 (4) (2014) 469–482.
- [13] S. Eccles, B. Handley, U. Khan, I. McFadyen, J. Nanchahal, S. Nayagam, Standards For the Management of Open Fractures, Oxford University Press, 2020.
- [14] B.L. Norris, M. Vanderkarr, C. Sparks, A.S. Chitnis, B. Ray, C.E. Holy, Treatments, cost and healthcare utilization of patients with segmental bone defects, *Injury* (2021).
- [15] R.G. Burwell, History of bone grafting and bone graft substitutes with special reference to osteogenic induction, Chapter 1, in: *Bone Grafts Derivatives and Substitutes*, Butterworth Heinemann Ltd., Oxford, 1994, pp. 3–102.
- [16] K.M. Alghazali, Z.A. Nima, R.N. Hamzah, M.S. Dhar, D.E. Anderson, A.S. Biris, Bone-tissue engineering: complex tunable structural and biological responses to injury, drug delivery, and cell-based therapies, *Drug Metab. Rev.* 47 (4) (2015) 431–454.
- [17] D.B. Raina, I. Qayoom, D. Larsson, M.H. Zheng, A. Kumar, H. Isaksson, L. Lidgren, M. Tagil, Guided tissue engineering for healing of cancellous and cortical bone using a combination of biomaterial based scaffolding and local bone active molecule delivery, *Biomaterials* 188 (2019) 38–49.
- [18] T. Winkler, F.A. Sass, G.N. Duda, K. Schmidt-Bleek, A review of biomaterials in bone defect healing, remaining shortcomings and future opportunities for bone tissue engineering: the unsolved challenge, *Bone Joint Res.* 7 (3) (2018) 232–243.
- [19] G. Levander, An experimental study of the role of the bone marrow in bone regeneration, *Acta Chir. Scand.* 83 (1940) 545–560.
- [20] D. Andreev, M. Liu, D. Weidner, K. Kachler, M. Faas, A. Gruneboom, U. Schlotzer-Schrehardt, L.E. Munoz, U. Steffen, B. Grotzsch, B. Killy, G. Kronke, A.M. Luebke, A. Niemeier, F. Wehrhan, R. Lang, G. Schett, A. Bozec, Osteocyte necrosis triggers osteoclast-mediated bone loss through macrophage-inducible C-type lectin, *J. Clin. Invest.* 130 (9) (2020) 4811–4830.
- [21] M. De Martinis, L. Ginaldi, M.M. Sirufo, G. Pioggia, G. Calapai, S. Gangemi, C. Mannucci, Alarms in Osteoporosis, RAGE, IL-1, and IL-33 Pathways: a Literature Review, *Medicina (Kaunas)* 56 (3) (2020).
- [22] R.G. Burwell, E. Rouholamin, L.J. Papineau, W.W. Tomford, H.J. Mankin, in: *Bone Grafts Derivatives and Substitutes*, Butterworth Heinemann Ltd., Oxford, 1994, pp. 3–192.
- [23] G.I. Taylor, G.D. Miller, F.J. Ham, The free vascularized bone graft, a clinical extension of microvascular techniques, *Plast. Reconstr. Surg.* 55 (5) (1975) 533–544.
- [24] M.R. Urist, B.S. Strates, Bone morphogenetic protein, *J. Dent. Res.* 50 (6) (1971) 1392–1406.

- [25] A.J. Friedenstein, Precursor cells of mechanocytes, *Int. Rev. Cytol.* 47 (1976) 327–359.
- [26] D. Gothard, E.L. Smith, J.M. Kanczler, H. Rashidi, O. Qutachi, J. Henstock, M. Rotherham, A. El Haj, K.M. Shakesheff, R.O. Oreffo, Tissue engineered bone using select growth factors: a comprehensive review of animal studies and clinical translation studies in man, *Eur. Cell Mater.* 28 (2014) 166–207 discussion 207–8.
- [27] Q. Cui, A.S. Dighe, J.N. Irvine Jr., Combined angiogenic and osteogenic factor delivery for bone regenerative engineering, *Curr. Pharm. Des.* 19 (19) (2013) 3374–3383.
- [28] K.D. Hankenson, K. Gagne, M. Shaughnessy, Extracellular signaling molecules to promote fracture healing and bone regeneration, *Adv. Drug. Deliv. Rev.* 94 (2015) 3–12.
- [29] A.I. Caplan, Mesenchymal Stem Cells: time to Change the Name!, *STEM CELLS Transl. Med.* 6 (6) (2017) 1445–1451.
- [30] T. Hu, S.A. Abbah, M. Wang, S.Y. Toh, R.W. Lam, M. Naidu, G. Bhakta, S.M. Cool, K. Bhakoo, J. Li, J.C. Goh, H.K. Wong, Novel Protamine-Based Polyelectrolyte Carrier Enhances Low-Dose rhBMP-2 in Posteriorlateral Spinal Fusion, *Spine (Phila Pa 1976)* 40 (9) (2015) 613–621.
- [31] M. Wang, S.A. Abbah, T. Hu, R.W. Lam, S.Y. Toh, T. Liu, S.M. Cool, K. Bhakoo, J. Li, J.C. Goh, H.K. Wong, Polyelectrolyte Complex Carrier Enhances Therapeutic Efficiency and Safety Profile of Bone Morphogenetic Protein-2 in Porcine Lumbar Interbody Fusion Model, *Spine (Phila Pa 1976)* 40 (13) (2015) 964–973.
- [32] S. Zwingenberger, R. Langanke, C. Vater, G. Lee, E. Niederlohmann, M. Sensenschmidt, A. Jacobi, R. Bernhardt, M. Muders, S. Rammelt, S. Knack, M. Gelinsky, K.P. Günther, S.B. Goodman, M. Stiehler, The effect of SDF-1 α on low dose BMP-2 mediated bone regeneration by release from heparinized mineralized collagen type I matrix scaffolds in a murine critical size bone defect model, *J. Biomed. Mater. Res. A* 104 (9) (2016) 2126–2134.
- [33] S. Injamuri, M.N. Rahaman, Y. Shen, Y.W. Huang, Relaxin enhances bone regeneration with BMP-2-loaded hydroxyapatite microspheres, *J. Biomed. Mater. Res. A* 108 (5) (2020) 1231–1242.
- [34] X. Zhang, Q. Yu, Y.A. Wang, J. Zhao, Dose reduction of bone morphogenetic protein-2 for bone regeneration using a delivery system based on lyophilization with trehalose, *Int. J. Nanomed.* 13 (2018) 403–414.
- [35] M.C. Walsh, N. Takegahara, H. Kim, Y. Choi, Updating osteoimmunology: regulation of bone cells by innate and adaptive immunity, *Nature Rev. Rheumatol.* 14 (3) (2018) 146–156.
- [36] H. Kobayashi, T. Ukai, C. Shiraishi, Y. Ozaki, A. Yoshimura, Y. Hara, T cell and periosteum cooperation in osteoclastogenesis induced by lipopolysaccharide injection in transplanted mouse tibia, *J. Dental Sci.* 13 (3) (2018) 226–233.
- [37] M. Roser, War and Peace. <https://ourworldindata.org/war-and-peace>. 09/13 2020.
- [38] S. Reinberg, Worldwide War Deaths Underestimated, 2009. <https://abcnews.go.com/Health/Healthday/story?id=5207645&page=1>. (Accessed 09/13/2020).
- [39] V.T. DeVita Jr., The 'War on Cancer' and its impact, *Nature clinical practice, Oncology* 1 (2) (2004) 55.
- [40] S.M. Ott, Cortical or Trabecular Bone: what's the Difference? *Am. J. Nephrol.* 47 (6) (2018) 373–375.
- [41] A. Grüneboom, I. Hawwari, D. Weidner, S. Culemann, S. Müller, S. Henneberg, A. Brenzel, S. Merz, L. Bornemann, K. Zec, M. Wuelling, L. Kling, M. Hasenberg, S. Voortmann, S. Lang, W. Baum, A. Ohs, O. Kraff, H.H. Quick, M. Jäger, S. Landgraebel, M. Dudda, R. Danuser, J.V. Stein, M. Rohde, K. Gelse, A.I. Garbe, A. Adamczyk, A.M. Westendorf, D. Hoffmann, S. Christiansen, D.R. Engel, A. Vortkamp, G. Krönke, M. Herrmann, T. Kamradt, G. Schett, A. Hasenberg, M. Gunzer, A network of trans-cortical capillaries as mainstay for blood circulation in long bones, *Nat. Metab.* 1 (2) (2019) 236–250.
- [42] G.S. Travlos, Normal Structure, Function, and Histology of the Bone Marrow, *Toxicol. Pathol.* 34 (5) (2006) 548–565.
- [43] S.K. Ramasamy, Structure and Functions of Blood Vessels and Vascular Niches in Bone, *Stem Cells Int.* 2017 (2017) 5046953–5046953.
- [44] A.P. Kusumbe, S.K. Ramasamy, R.H. Adams, Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone, *Nature* 507 (7492) (2014) 323–328.
- [45] J.A. Spencer, F. Ferraro, E. Roussakis, A. Klein, J. Wu, J.M. Runnels, W. Zaher, L.J. Mortensen, C. Alt, R. Turcotte, R. Yusuf, D. Côté, S.A. Vinogradov, D.T. Scadden, C.P. Lin, Direct measurement of local oxygen concentration in the bone marrow of live animals, *Nature* 508 (7495) (2014) 269–273.
- [46] B. Thompson, D.A. Towler, Arterial calcification and bone physiology: role of the bone-vascular axis, *Nat. Rev. Endocrinol.* 8 (9) (2012) 529–543.
- [47] C. Owen-Woods, A. Kusumbe, Fundamentals of bone vasculature: specialization, interactions and functions, *Semin. Cell Dev. Biol.* (2021).
- [48] M. Hendriks, S.K. Ramasamy, Blood Vessels and Vascular Niches in Bone Development and Physiological Remodeling, *Front. Cell Dev. Biol.* 8 (2020) 602278.
- [49] E.C. Watson, R.H. Adams, Biology of Bone: the Vasculature of the Skeletal System, *Cold Spring Harb. Perspect. Med.* 8 (7) (2018).
- [50] S. Stegen, G. Carmeliet, The skeletal vascular system - Breathing life into bone tissue, *Bone* 115 (2018) 50–58.
- [51] A. Grosso, M.G. Burger, A. Lunger, D.J. Schaefer, A. Banfi, N. Di Maggio, It Takes Two to Tango: coupling of Angiogenesis and Osteogenesis for Bone Regeneration, *Front. Bioeng. Biotechnol.* 5 (2017) 68.
- [52] M.H. Lafage-Proust, B. Roche, M. Langer, D. Cleret, A. Vandenh Bossche, T. Olivier, L. Vico, Assessment of bone vascularization and its role in bone remodeling, *Bonekey Rep.* 4 (2015) 662.
- [53] C.E. Baker, S.N. Moore-Lotridge, A.A. Hysong, S.L. Posey, J.P. Robinette, D.M. Blum, M.A. Benvenuti, H.A. Cole, S. Egawa, A. Okawa, M. Saito, J.R. McCarthy, J.S. Nyman, M. Yuasa, J.G. Schoenecker, Bone Fracture Acute Phase Response—A Unifying Theory of Fracture Repair: clinical and Scientific Implications, *Clin. Rev. Bone Mineral Metabol.* 16 (4) (2018) 142–158.
- [54] R. Marsell, T.A. Einhorn, The biology of fracture healing, *Injury* 42 (6) (2011) 551–555.
- [55] R.T. Steffen, N.A. Athanasou, H.S. Gill, D.W. Murray, Avascular necrosis associated with fracture of the femoral neck after hip resurfacing, *J. Bone Joint Surgery. British volume* 92-B (6) (2010) 787–793.
- [56] J. Moya-Angeler, A.L. Gianakos, J.C. Villa, A. Ni, J.M. Lane, Current concepts on osteonecrosis of the femoral head, *World J. Orthop.* 6 (8) (2015) 590–601.
- [57] G.M. Calori, E. Mazza, A. Colombo, S. Mazzola, M. Colombo, Core decompression and biotechnologies in the treatment of avascular necrosis of the femoral head, *EJORT Open Rev.* 2 (2) (2017) 41–50.
- [58] R.K. Sen, Management of avascular necrosis of femoral head at pre-collapse stage, *Indian J. Orthop.* 43 (1) (2009) 6–16.
- [59] S.Y. Chang, J.J. Huang, C.K. Tsao, A. Nguyen, K. Mittakanti, C.Y. Lin, M.H. Cheng, Does ischemia time affect the outcome of free fibula flaps for head and neck reconstruction? a review of 116 cases, *Plast. Reconstr. Surg.* 126 (6) (2010) 1988–1995.
- [60] Y. Tamura, G. Inoue, T. Miura, N. Ishiguro, Reperfusion Injury in Bone: effects of CV-3611, a Free Radical Scavenger, on Ischemic Revascularized Bone Grafts in Rats, *J. Reconstr. Microsurg.* 8 (6) (1992) 471.
- [61] H. Yuan, W. Jiang, Y. Chen, B.Y.S. Kim, Study of Osteocyte Behavior by High-Resolution Intravital Imaging Following Photo-Induced Ischemia, *Molecules* 23 (11) (2018) 2874.
- [62] J. Michalova, F. Savvulidi, L. Sefc, K. Forgacova, E. Necas, Cadaveric bone marrow as potential source of hematopoietic stem cells for transplantation, *Chimerism* 2 (3) (2011) 86–87.
- [63] M. Descheppe, K. Oudina, B. David, V. Myrtil, C. Collet, M. Bensidhoum, D. Logeart-Avramoglou, H. Petite, Survival and function of mesenchymal stem cells (MSCs) depend on glucose to overcome exposure to long-term, severe and continuous hypoxia, *J. Cell. Mol. Med.* 15 (7) (2011) 1505–1514.
- [64] A. Moya, J. Paquet, M. Descheppe, N. Larochette, K. Oudina, C. Denoeud, M. Bensidhoum, D. Logeart-Avramoglou, H. Petite, Human Mesenchymal Stem Cell Failure to Adapt to Glucose Shortage and Rapidly Use Intracellular Energy Reserves Through Glycolysis Explains Poor Cell Survival After Implantation, *Stem Cells* 36 (3) (2018) 363–376.
- [65] F. Lau, B. Dalisson, Y.L. Zhang, J. Zhao, N. Eliopoulos, J.E. Barralet, Effects of oxygen and glucose on bone marrow mesenchymal stem cell culture, *Adv. Biosyst.* 4 (2020) 11 e2000094.
- [66] X.-S. He, Y. Ma, L.-W. Wu, W.-Q. Ju, J.-L. Wu, R.-D. Hu, G.-H. Chen, J.-F. Huang, Safe time to warm ischemia and posttransplant survival of liver graft from non-heart-beating donors, *World J. Gastroenterol.* 10 (21) (2004) 3157–3160.
- [67] T.F.T. Khan, N. Ahmad, A.S. Serageldeen, K. Fourtounas, Implantation Warm Ischemia Time in Kidney Transplant Recipients: defining Its Limits and Impact on Early Graft Function, *Ann. Transplant.* 24 (2019) 432–438.
- [68] J. Valentin, Basic anatomical and physiological data for use in radiological protection: reference values ICRP Publication 89: approved by the Commission in September 2001, *Ann. ICRP* 32 (3–4) (2002) 1–277.
- [69] M. Mukisi-Mukaza, A. Gomez-Brouchet, M. Donkerwolcke, M. Hinsenkamp, F. Burny, Histopathology of aseptic necrosis of the femoral head in sickle cell disease, *Int. Orthop.* 35 (8) (2011) 1145–1150.
- [70] K.-c. Hua, X.-g. Yang, J.-t. Feng, F. Wang, L. Yang, H. Zhang, Y.-c. Hu, The efficacy and safety of core decompression for the treatment of femoral head necrosis: a systematic review and meta-analysis, *J. Orthop. Surg. Res.* 14 (1) (2019) 306.
- [71] T.P. Pierce, J.J. Jauregui, R.K. Elmallah, C.J. Lavernia, M.A. Mont, J. Nace, A current review of core decompression in the treatment of osteonecrosis of the femoral head, *Curr. Rev. Musculoskelet. Med.* 8 (3) (2015) 228–232.
- [72] T. Sunagawa, A.T. Bishop, K. Muramatsu, Role of conventional and vascularized bone grafts in scaphoid nonunion with avascular necrosis: a canine experimental study, *J. Hand Surg. [Am]* 25 (5) (2000) 849–859.
- [73] S.G. Pneumaticos, G.K. Triantafyllopoulos, E.K. Basdra, A.G. Papavassiliou, Segmental bone defects: from cellular and molecular pathways to the development of novel biological treatments, *J. Cell. Mol. Med.* 14 (11) (2010) 2561–2569.
- [74] P.J. Girard, K.M. Kuhn, J.R. Bailey, J.A. Lynott, M.T. Mazurek, Bone transport combined with locking bridge plate fixation for the treatment of tibial segmental defects: a report of 2 cases, *J. Orthop. Trauma* 27 (9) (2013) e220–e226.
- [75] M. Chimutengwende-Gordon, A. Mbogo, W. Khan, R. Wilkes, Limb reconstruction after traumatic bone loss, *Injury* 48 (2) (2017) 206–213.
- [76] M.A. Pogrel, S. Podlesh, J.P. Anthony, J. Alexander, A comparison of vascularized and nonvascularized bone grafts for reconstruction of mandibular continuity defects, *J. Oral Maxillofac. Surg.* 55 (11) (1997) 1200–1206.
- [77] F. Fang, K.C. Chung, An evolutionary perspective on the history of flap reconstruction in the upper extremity, *Hand Clin.* 30 (2) (2014) 109–v.
- [78] G.I. Taylor, R.J. Corlett, M.W. Ashton, The evolution of free vascularized bone transfer: a 40-year experience, *Plast. Reconstr. Surg.* 137 (4) (2016) 1292–1305.
- [79] M. Peled, I.A. El-Naaj, Y. Lipin, L. Ardekian, The use of free fibular flap for functional mandibular reconstruction, *J. Oral Maxillofac. Surg.* 63 (2) (2005) 220–224.

- [80] A. Ham, S. Gordon, The origin of bone that forms in association with cancellous chips transplanted into muscle, *Br. J. Plast. Surg.* 5 (3) (1952) 154–160.
- [81] L. Klein, S. Stevenson, J.W. Shaffer, D. Davy, V.M. Goldberg, Bone mass and comparative rates of bone resorption and formation of fibular autografts: comparison of vascular and nonvascular grafts in dogs, *Bone* 12 (5) (1991) 323–329.
- [82] S. Gupta, L.A. Kafchinski, K.R. Gundle, K. Saidi, A.M. Griffin, J.S. Wunder, P.C. Ferguson, Intercalary allograft augmented with intramedullary cement and plate fixation is a reliable solution after resection of a diaphyseal tumour, *Bone Joint J.* 99-b (7) (2017) 973–978.
- [83] C.H. Gerrard, A.M. Griffin, A.M. Davis, A.E. Gross, R.S. Bell, J.S. Wunder, Large segment allograft survival is improved with intramedullary cement, *J. Surg. Oncol.* 84 (4) (2003) 198–208.
- [84] P.C. Dell, H. Burchardt, J.F.P. Glowczewskie, A roentgenographic, biomechanical, and histological evaluation of vascularized and non-vascularized segmental fibular canine autografts, *J. Bone Joint Surg. Am.* 67 (1) (1985) 105–112.
- [85] J.B. Moore, J.M. Mazur, D. Zehr, P.K. Davis, E.G. Zook, A biomechanical comparison of vascularized and conventional autogenous bone grafts, *Plast. Reconstr. Surg.* 73 (3) (1984) 382–386.
- [86] P.K. Davis, J.M. Mazur, G.N. Coleman, A torsional strength comparison of vascularized and nonvascularized bone grafts, *J. Biomech.* 15 (11) (1982) 875–880.
- [87] R.W.H. Pho, Bone transplant in reconstructive orthopaedic surgery, in: R.W.H. Pho (Ed.), *Microsurgical Technique in orthopaedics*, Butterworths, Sevenoaks, 1988, pp. 128–178.
- [88] A.C. Hatchell, A. Aoude, S. Aldebeyan, C.D. McKenzie, P. Lewkonia, W. de Haas, Use of an Omental Flow-Through Flap for Recipient Vessels in the Reconstruction of a Lumbar Spine Defect: a Case Report, *JBJS Case Connect* 10 (4) (2020) e2000156.
- [89] P. Feltri, L. Solaro, C. Errani, G. Schiavon, C. Candrian, G. Filardo, Vascularized fibular grafts for the treatment of long bone defects: pros and cons. A systematic review and meta-analysis, *Arch. Orthop. Trauma Surg.* (2021).
- [90] C. Hirche, L. Xiong, C. Heffinger, M. Münzberg, S. Fischer, U. Kneser, T. Kremer, Vascularized versus non-vascularized bone grafts in the treatment of scaphoid non-union: a clinical outcome study with therapeutic algorithm, *J. Orthop. Surg. (Hong Kong)* 25 (1) (2017) 2309499016684291.
- [91] W. Ahmed, M.A. Asim, A. Ehsan, Q. Abbas, Non-Vascularized Autogenous Bone Grafts for Reconstruction of Maxillofacial Osseous Defects, *J. Coll. Phys. Surgeons-Pakistan: JCSP* 28 (1) (2018) 17–21.
- [92] R.D. Foster, J.P. Anthony, A. Sharma, M.A. Pogrel, Vascularized bone flaps versus nonvascularized bone grafts for mandibular reconstruction: an outcome analysis of primary bony union and endosseous implant success, *HED Head Neck* 21 (1) (1999) 66–71.
- [93] B.J. Allsopp, D.J. Hunter-Smith, W.M. Rozen, Vascularized versus Nonvascularized Bone Grafts: what Is the Evidence? *Clin. Orthop. Relat. Res. Clin. Orthop. Rel. Res.* 474 (5) (2016) 1319–1327.
- [94] S.K. Devireddy, M. Senthil Murugan, R.V. Kishore Kumar, R. Gali, S.R. Kanubady, M. Sunayana, Evaluation of Non-vascular Fibula Graft for Mandibular Reconstruction, *J. Maxillofacial Oral Surg.* 14 (2) (2015) 299–307.
- [95] N.G. Lasanianos, N.K. Kanakaris, P.V. Giannoudis, Current management of long bone large segmental defects, *Orthop. Trauma* 24 (2) (2010) 149–163.
- [96] A.J. Weiland, T.W. Phillips, M.A. Randolph, Bone grafts: a radiologic, histologic, and biomechanical model comparing autografts, allografts, and free vascularized bone grafts, *Plast. Reconstr. Surg.* 74 (3) (1984) 368–379.
- [97] E.P. Estrella, E.H.M. Wang, A Comparison of Vascularized Free Fibular Flaps and Nonvascularized Fibular Grafts for Reconstruction of Long Bone Defects after Tumor Resection, *J. Reconstr. Microsurg.* 33 (3) (2017) 194–205.
- [98] J.S. Brown, D. Lowe, A. Kanatas, A. Schache, Mandibular reconstruction with vascularised bone flaps: a systematic review over 25 years, *Br. J. Oral Maxillofac. Surg.* 55 (2) (2017) 113–126.
- [99] B. Triquet, P. Ruffieux, C. Mainetti, D. Salomon, J.H. Saurat, Topical Haemotherapy for Leg Ulcers, *Dermatology* 189 (4) (1994) 418–420.
- [100] M. Iwayama-Hibino, K. Sugiura, Y. Muro, Y. Tomita, Successful topical hemotherapy with a new occlusive dressing for an intractable ulcer on the toe, *J. Dermatol.* 36 (4) (2009) 245–248.
- [101] J. Asai, H. Takenaka, K. Ichihashi, E. Ueda, N. Katoh, S. Kishimoto, Successful treatment of diabetic gangrene with topical application of a mixture of peripheral blood mononuclear cells and basic fibroblast growth factor, *J. Dermatol.* 33 (5) (2006) 349–352.
- [102] K. Mifugi, M. Ishikawa, N. Kamei, R. Tanaka, K. Arita, H. Mizuno, T. Asahara, N. Adachi, M. Ochi, Angiogenic conditioning of peripheral blood mononuclear cells promotes fracture healing, *Bone Joint Res.* 6 (8) (2017) 489–498.
- [103] N. Hopper, J. Wardale, R. Brooks, J. Power, N. Rushton, F. Henson, Peripheral Blood Mononuclear Cells Enhance Cartilage Repair in vivo Osteochondral Defect Model, *PLoS One* 10 (8) (2015) e0133937-e0133937.
- [104] J. Ju, L. Li, R. Zhou, R. Hou, Combined application of latissimus dorsi myocutaneous flap and iliac bone flap in the treatment of chronic osteomyelitis of the lower extremity, *J. Orthop. Surg. Res.* 13 (1) (2018) 117–117.
- [105] J. Ogawa, H. Inoue, A. Shohotsu, T. Tajima, R. Tanino, S. Yamazaki, Reconstruction of sternal defects with autologous bone grafts and myocutaneous flap of the latissimus dorsi muscle, *Tokai J. Exp. Clin. Med.* 7 (1) (1982) 63–68.
- [106] S. Jaisinghani, N.S. Adams, R.J. Mann, J.W. Polley, J.A. Girotto, Virtual Surgical Planning in Orthognathic Surgery, *Eplasty* 17 (2017).
- [107] J.L. Mayo, H.S. Hilaire, *Craniomaxillofacial Free Flap Reconstruction Using Virtual Surgical Planning, Planning, Digital Technologies in Craniomaxillo facial Surgery*, Springer, New York, NY, 2018, pp. 331–350.
- [108] A. Modabber, N. Ayoub, A. Bock, S.C. Möhlenrich, B. Lethaus, A. Ghassemi, D.A. Mitchell, F. Hözle, Medial approach for minimally-invasive harvesting of a deep circumflex iliac artery flap for reconstruction of the jaw using virtual surgical planning and CAD/CAM technology, *Br. J. Oral Maxillofac. Surg.* 55 (9) (2017) 946–951.
- [109] W. Ren, L. Gao, S. Li, C. Chen, F. Li, Q. Wang, Y. Zhi, J. Song, Z. Dou, L. Xue, K. Zhi, Virtual Planning and 3D printing modeling for mandibular reconstruction with fibula free flap, *Med. Oral Patol. Oral Cir. Bucal* 23 (3) (2018) e359–e366.
- [110] Z.H. Lee, T. Avraham, C. Monaco, A.A. Patel, D.L. Hirsch, J.P. Levine, Optimizing functional outcomes in mandibular condyle reconstruction with the free fibula flap using computer-aided design and manufacturing technology, *J. Oral Maxillofac. Surg.* 76 (5) (2018) 1098–1106.
- [111] C. Marchetti, Mandibular reconstruction: our experience with the use of computer-aided design/computer-aided manufacturing technology, *Int. J. Oral Maxillofac. Surg.* 46 (2017) 34.
- [112] K.A. Raskin, F. Hornicek, *Allograft Reconstruction in Malignant Bone Tumors: indications and Limits*, in: P.-U. Tunn (Ed.), *Treatment of Bone and Soft Tissue Sarcomas*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2009, pp. 51–58.
- [113] T. van Isacker, O. Barbier, A. Traore, O. Cornu, F. Mazzeo, C. Delloye, Forearm reconstruction with bone allograft following tumor excision: a series of 10 patients with a mean follow-up of 10 years, *Orthop. Traumatol.: Surg. Res.* 97 (8) (2011) 793–799.
- [114] G. Han, Y. Wang, W. Bi, J. Jia, W. Wang, M. Xu, X. Zheng, L. Mei, M. Yang, Reconstruction using massive allografts after resection of extremity osteosarcomas the study design: a retrospective cohort study, *Int. J. Surg.* 21 (2015) 108–111.
- [115] D.P. Mastorakos, J.J. Disa, E. Athanasian, P. Boland, J.H. Healey, P.G. Cordeiro, Soft-Tissue Flap Coverage Maximizes Limb Salvage after Allograft Bone Extremity Reconstruction, *Plast. Reconstr. Surg.* 109 (5) (2002).
- [116] V. Struckmann, G. Schmidmaier, T. Ferbert, U. Kneser, T. Kremer, Reconstruction of Extended Bone Defects Using Massive Allografts Combined with Surgical Angiogenesis: a Case Report, *JBJS Case Connector* 7 (1) (2017).
- [117] D.J. Costain, R.W. Crawford, Fresh-frozen vs. irradiated allograft bone in orthopaedic reconstructive surgery, *Injury* 40 (12) (2009) 1260–1264.
- [118] R. Pathak, A. Amarpal, H. Aithal, P. Kinjavdevkar, A. Pawde, T. Paramasivam Rashmi, N. Sharma, K. Dhamo, Bone Tissue Engineering: latest Trends and Future Perspectives, *Adv. Anim. Veter. Sci.* Vol. 3 (2015) 9–22.
- [119] F. Mahyudin, D.N. Utomo, H. Suroto, T.W. Martanto, M. Edward, I.I. Gaol, Comparative Effectiveness of Bone Grafting Using Xenograft Freeze-Dried Cortical Bovine, Allograft Freeze-Dried Cortical New Zealand White Rabbit, Xenograft Hydroxyapatite Bovine, and Xenograft Demineralized Bone Matrix Bovine in Bone Defect of Femoral Diaphysis of White Rabbit: experimental Study In Vivo, *Int. J. Biomater.* 2017 (2017) 7571523–7571523.
- [120] A.S. Herford, E. Stoffella, C.M. Stanford, E. Stoffella, Chapter 5 - Bone Grafts and Bone Substitute Materials, in: M. Torabinejad, M.A. Sabeti, C.J. Goodacre (Eds.), *Principles and Practice of Single Implant and Restorations*, W.B. Saunders, Saint Louis, 2014, pp. 75–86.
- [121] J.M. Rummelhart, J.T. Mellong, J.L. Gray, H.J. Towle, A comparison of freeze-dried bone allograft and demineralized freeze-dried bone allograft in human periodontal osseous defects, *J. Periodontol.* 60 (12) (1989) 655–663.
- [122] S. van der Donk, T. Weernink, P. Buma, P. Aspenberg, T.J.J.H. Slooff, B.W. Schreurs, Rinsing Morselized Allografts Improves Bone and Tissue Ingrowth, *Clin. Orthop. Rel. Res.* 408 (2003).
- [123] C. Delloye, P. Simon, C. Nyssen-Behets, X. Banse, F. Bresler, D. Schmitt, Perforations of Cortical Bone Allografts Improve Their Incorporation, *Clinical Orthop. Rel. Res.* (1976–2007) 396 (2002).
- [124] E. Goujon, Recherches experimentales sur les propriétés physiologiques de la moelle des os, *J. de l'Anatomie et de la Physiologie Normales et Pathologiques de l'Homme et des Animaux* 6 (1869) 399.
- [125] R.G. Burwell, in: *The Function of Bone Marrow in the Incorporation of a Bone Graft*, Clinical Orthopaedics and Related Research, 1985, pp. 125–141.
- [126] B. Charbonnier, A. Baradaran, D. Sato, O. Alghamdi, Z. Zhang, Y.-L. Zhang, U. Gbureck, M. Gilardino, E. Harvey, N. Makhoul, Material-Induced Venosome-Supported Bone Tubes, *Adv. Sci.* (2019) 1900844.
- [127] P.H. Warneke, I.N.G. Springer, J. Wiltfang, Y. Acil, H. Eufinger, M. Wehmöller, P.A.J. Russo, H. Bolte, E. Sherry, E. Behrens, Growth and transplantation of a custom vascularised bone graft in a man, *Lancet North Am. Ed.* 364 (9436) (2004) 766–770.
- [128] R.G. Burwell, Studies in the transplantation of bone. vii. the fresh composite homograft-autograft of cancellous bone; an analysis of factors leading to osteogenesis in marrow transplants and in marrow-containing bone grafts, *J. Bone. Joint Surg. Br.* 46 (1964) 110–140.
- [129] M. Cushing, Autogenous Red Marrow Grafts: their Potential for Induction of Osteogenesis, *J. Periodontol.-Periodontics* 40 (8) (1969) 492–497.
- [130] D. Henrich, C. Seebach, R. Verboket, A. Schaible, I. Marzi, H. Bonig, The osteo-inductive activity of bone-marrow-derived mononuclear cells resides within the CD14+ population and is independent of the CD34+ population, *Eur. Cell Mater.* 35 (2018) 165–177.

- [131] R. Burwell, Studies in the Transplantation of Bone VII: the Fresh Composite Homograft-Autograft of Cancellous Bone. An Analysis of Factors Leading to Osteogenesis in Marrow Transplants and in Marrow-Containing Bone Grafts, *J. Bone Joint Surg. British volume* 46 (1964) 110–140.
- [132] K. Lin, J. Vandenberg, S.M. Putnam, C.D. Parks, A. Spragg-Hughes, C.M. McAndrew, W.M. Ricci, M.J. Gardner, Bone marrow aspirate concentrate with cancellous allograft versus iliac crest bone graft in the treatment of long bone nonunions, *OTA International* 2 (1) (2019) e012.
- [133] D.N. Utomo, K.D. Hernugrahanto, M. Edward, L. Widhiyanto, F. Mahyudin, Combination of bone marrow aspirate, cancellous bone allograft, and platelet-rich plasma as an alternative solution to critical-sized diaphyseal bone defect: a case series, *Int. J. Surg. Case Rep.* 58 (2019) 178–185.
- [134] S.C. Lavareda Corrêa, J. Elias de Sousa, P.J. Pasquali, L.G. Scavone de Macedo, A.C. Aloise, M.L. Teixeira, A.A. Pelegrine, Use of bone allograft with or without bone marrow aspirate concentrate in appositional reconstructions: a tomographic and histomorphometric study, *Implant Dent.* 26 (6) (2017) 915–921.
- [135] A.A. Pelegrine, M.L. Teixeira, M. Sperandio, T.S. Almada, K.E. Kahnberg, P.J. Pasquali, A.C. Aloise, Can bone marrow aspirate concentrate change the mineralization pattern of the anterior maxilla treated with xenografts? a preliminary study, *Contemp. Clin. Dent.* 7 (1) (2016) 21–26.
- [136] D. Keskin, C. Gündoğdu, A.C. Atac, Experimental comparison of bovine-derived xenograft, xenograft-autologous bone marrow and autogenous bone graft for the treatment of bony defects in the rabbit ulna, *Med. Princ. Pract.* 16 (4) (2007) 299–305.
- [137] Fa.O.o.t.U. Nations, Global livestock production systems chapter 7: applications of global livestock production systems, <http://www.fao.org/3/i2414e/i2414e0.htm>, 2011.
- [138] K. Alraei, J. Sharqawi, S. Harcher, I. Ghita, Efficacy of the Combination of rhBMP-2 with Bone Marrow Aspirate Concentrate in Mandibular Defect Reconstruction after a Pindborg Tumor Resection, *Case Rep. Dentistry* 2020 (2020) 8281741.
- [139] M. Schlund, R. Nicot, A. Depeyre, J. Alkasbi, J. Ferri, Reconstruction of a Large Posttraumatic Mandibular Defect Using Bone Tissue Engineering With Fresh-Frozen Humeral Allograft Seeded With Autologous Bone Marrow Aspirate and Vascularized With a Radial Forearm Flap, *J. Craniofac. Surg.* 30 (7) (2019) 2085–2087.
- [140] S. Rigal, P. Merloz, D. Le Nen, H. Mathevon, A.C. Masquelet, Bone transport techniques in posttraumatic bone defects, *Orthop. Traumatol.: Surg. Res.* 98 (1) (2012) 103–108.
- [141] P. Merloz, Y. Tourné, J. Dayez, S. Plawiecki, I. Soued, C. Faure, J. Butel, P. Pauget, Value of Ilizarov's method in the treatment of long bone pseudarthroses. Apropos of a ASAMIF series of 87 cases, *J. Chir. (Paris)* 127 (4) (1990) 199–208.
- [142] R. Cattaneo, M. Catagni, E.E. Johnson, The treatment of infected nonunions and segmental defects of the tibia by the methods of Ilizarov, *Clin. Orthop. Relat. Res.* 280 (1992) 143–152.
- [143] G.A. Ilizarov, V.I. Ledyayev, The replacement of long tubular bone defects by lengthening distraction osteotomy of one of the fragments, *Clin. Orthop. Rel. Res.* 280 (1992) 7–10.
- [144] T.R. Madhusudhan, B. Ramesh, K.S. Manjunath, H.M. Shah, D.C. Sundareswaran, N. Krishnappa, Outcomes of Ilizarov ring fixation in recalcitrant infected tibial non-unions—a prospective study, *J. Trauma Manage. Outcomes* 2 (1) (2008) 6.
- [145] G.A. Ilizarov, The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation, *Clin. Orthop. Relat. Res.* 238 (1989) 249–281.
- [146] G.A. Ilizarov, The tension-stress effect on the genesis and growth of tissues: part II. The influence of the rate and frequency of distraction, *Clin. Orthop. Relat. Res.* 239 (1989) 263–285.
- [147] R.B. Gustilo, J.T. Anderson, Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses, *JBJS* 58 (4) (1976) 453–458.
- [148] M. Salai, H. Horoszowski, M. Pritsch, Y. Amit, Primary reconstruction of traumatic bony defects using allografts, *Arch. Orthop. Trauma Surg.* 119 (7–8) (1999) 435–439.
- [149] J.F. Keating, A. Simpson, C.M. Robinson, The management of fractures with bone loss, *J. Bone Joint Surg. British volume* 87 (2) (2005) 142–150.
- [150] P.V. Giannoudis, O. Faour, T. Goff, N. Kanakaris, R. Dimitriou, Masquelet technique for the treatment of bone defects: tips-tricks and future directions, *Injury* 42 (6) (2011) 591–598.
- [151] R.C. Hamdy, J.S. Rendon, M. Tabrizian, Distraction Osteogenesis and Its Challenges in Bone Regeneration, *Bone Regeneration*, InTech2012.
- [152] J. Aronson, Current concepts review-limb-lengthening, skeletal reconstruction, and bone transport with the Ilizarov method, *JBJS* 79 (8) (1997) 1243–1258.
- [153] C.-W. Oh, H.-R. Song, J.-Y. Roh, J.-K. Oh, W.-K. Min, H.-S. Kyung, J.-W. Kim, P.-T. Kim, J.-C. Ihn, Bone transport over an intramedullary nail for reconstruction of long bone defects in tibia, *Arch. Orthop. Trauma Surg.* 128 (8) (2008) 801–808.
- [154] B. Spiegelberg, T. Parratt, S.K. Dherendra, W.S. Khan, R. Jennings, D.R. Marsh, Ilizarov principles of deformity correction, *Ann. R. Coll. Surg. England* 92 (2) (2010) 101–105.
- [155] G. Bertele, M. Mercanti, F. Stella, M. Albanese, D.S. De, Osteodistraction in the craniofacial region, *Minerva Stomatol.* 54 (4) (2005) 179–198.
- [156] B.C. Cho, S.K. Hwang, K.I. Uhm, Distraction Osteogenesis of the Cranial Vault for the Treatment of Craniofacial Synostosis, *J. Craniofac. Surg.* 15 (1) (2004) 135.
- [157] G. Gasparini, C.D. Rocco, G. Tamburini, S. Pelo, External craniofacial osteodistraction in complex craniosynostoses, *Childs Nerv. Syst.* 28 (9) (2012) 1565–1570.
- [158] A.A. Heggie, R. Kumar, J.M. Shand, The role of distraction osteogenesis in the management of craniofacial syndromes, *Ann Maxillofac Surg* 3 (1) (2013) 4–10.
- [159] C.C. Snyder, G.A. Levine, H.M. Swanson, E.Z. Browne Jr, Mandibular lengthening by gradual distraction: preliminary report, *Plast. Reconstr. Surg.* 51 (5) (1973) 506–508.
- [160] A.M. Makhdom, L. Nayef, M. Tabrizian, R.C. Hamdy, The potential roles of nanobiomaterials in distraction osteogenesis, *Nanomed. Nanotechnol. Biol. Med.* 11 (1) (2015) 1–18.
- [161] R.S. Carvalho, T.A. Einhorn, W. Lehmann, C. Edgar, A. Al-Yamani, A. Apazidis, D. Pacicca, T.L. Clemens, L.C. Gerstenfeld, The role of angiogenesis in a murine tibial model of distraction osteogenesis, *Bone* 34 (5) (2004) 849–861.
- [162] T.D. Fang, A. Salim, W. Xia, R.P. Nacamuli, S. Guccione, H.M. Song, R.A. Carano, E.H. Filvaroff, M.D. Bednarski, A.J. Giaccia, Angiogenesis is required for successful bone induction during distraction osteogenesis, *J. Bone Miner. Res.* 20 (7) (2005) 1114–1124.
- [163] D.M. Pacicca, N. Patel, C. Lee, K. Salisbury, W. Lehmann, R. Carvalho, L.C. Gerstenfeld, T.A. Einhorn, Expression of angiogenic factors during distraction osteogenesis, *Bone* 33 (6) (2003) 889–898.
- [164] N.M. Rowe, B.J. Mehrara, J.S. Luchs, M.E. Dudziak, D.S. Steinbrech, P.B. Ille, G.J. Fernandez, G.K. Gittes, M.T. Longaker, Angiogenesis during mandibular distraction osteogenesis, *Ann. Plast. Surg.* 42 (5) (1999) 470–475.
- [165] R. Agarwal, Unfavourable results with distraction in craniofacial skeleton, *Indian J. Plast. Surg.* 46 (2) (2013) 194–203.
- [166] D. Paley, Problems, Obstacles, and Complications of Limb Lengthening by the Ilizarov Technique, *Clin. Orthop. Rel. Res.* NA (250) (1990) 81??–104.
- [167] G.F. Kewitt, J.E. Van Sickels, Long-term effect of mandibular midline distraction osteogenesis on the status of the temporomandibular joint, teeth, periodontal structures, and neurosensory function, *J. Oral Maxillofac. Surg.* 57 (12) (1999) 1419–1425.
- [168] H.R. Song, S.H. Cho, K.H. Koo, S.T. Jeong, Y.J. Park, J.H. Ko, Tibial bone defects treated by internal bone transport using the Ilizarov method, *Int. Orthop.* 22 (5) (1998) 293–297.
- [169] E. García-Cimbrelo, J.C. Martí-González, Circular external fixation in tibial nonunions, *Clin. Orthop. Rel. Res.* 419 (2004) 65–70.
- [170] J. Mahaluxmivala, R. Nadarajah, P.W. Allen, R.A. Hill, Ilizarov external fixator: acute shortening and lengthening versus bone transport in the management of tibial non-unions, *Injury* 36 (5) (2005) 662–668.
- [171] B. El-Alfy, H. El-Mowafy, N. El-Moghzay, Distraction osteogenesis in management of composite bone and soft tissue defects, *Int. Orthop.* 34 (1) (2010) 115–118.
- [172] A. Ali, Y. Ren, C.H. Zhou, J. Fang, C.H. Qin, Unprecedented tibial bone lengthening of 33.5 cm by distraction osteogenesis for the reconstruction of a subtotal tibial bone defect. a case report and literature review, *BMC Musculoskeletal Disord.* 22 (1) (2021) 88.
- [173] T.A. Malkova, D.Y. Borzunov, International recognition of the Ilizarov bone reconstruction techniques: current practice and research (dedicated to 100(th) birthday of G. A. Ilizarov), *World J. Orthop.* 12 (8) (2021) 515–533.
- [174] K. Aktuglu, K. Erol, A. Vahabi, Ilizarov bone transport and treatment of critical-sized tibial bone defects: a narrative review, *J. Orthop. Traumatol.* 20 (1) (2019) 22.
- [175] J. Dabis, O. Templeton-Ward, A.E. Lacey, B. Narayan, A. Trompeter, The history, evolution and basic science of osteotomy techniques, *Strategies Trauma Limb Reconstr.* 12 (3) (2017) 169–180.
- [176] S.M. Quinnan, Segmental Bone Loss Reconstruction Using Ring Fixation, *J. Orthop. Trauma* 31 (5) (2017) S42–s46 Suppl.
- [177] K. Klaue, U. Knothe, A.C. Masquelet, Effet biologique des membranes à corps étranger induites in situ sur la consolidation des greffes d'os spongieux, *Rev. Chir. Orthop.* (1995) Suppl 70 (e r kunion annuelle) 109–110.
- [178] M. Tarchala, E.J. Harvey, J. Barralet, Biomaterial-Stabilized Soft Tissue Healing for Healing of Critical-Sized Bone Defects: the Masquelet Technique, *Adv. Healthc. Mater.* 5 (6) (2016) 630–640.
- [179] S. McBride-Gagyi, Z. Toth, D. Kim, V. Ip, E. Evans, J.T. Watson, D. Nicolaou, Altering spacer material affects bone regeneration in the Masquelet technique in a rat femoral defect, *J. Orthop. Res.* (2018).
- [180] N. Gaio, A. Martino, Z. Toth, J.T. Watson, D. Nicolaou, S. McBride-Gagyi, Masquelet technique: the effect of altering implant material and topography on membrane matrix composition, mechanical and barrier properties in a rat defect model, *J. Biomech.* 72 (2018) 53–62.
- [181] I. Morelli, L. Drago, D.A. George, D. Romanò, C.L. Romanò, Managing large bone defects in children: a systematic review of the 'induced membrane technique', *J. Pediatric Orthop. B* 27 (5) (2018) 443.
- [182] M. Durand, Masquelet Induced Membrane Technique for The Surgical Treatment of Large Bone Defects: the Reasons for Successes and Failures, *American J. Biomed. Sci. Res.* 2 (2019).
- [183] C. Christou, R.A. Oliver, Y. Yu, W.R. Walsh, The Masquelet Technique for Membrane Induction and the Healing of Ovine Critical Sized Segmental Defects, *PLoS One* 9 (12) (2014) e114122.
- [184] M. Powerski, B. Maier, J. Frank, I. Marzi, Treatment of severe osteitis after elastic intramedullary nailing of a radial bone shaft fracture by using cancellous bone graft in Masquelet technique in a 13-year-old adolescent girl, *J. Pediatr. Surg.* 44 (8) (2009) e17–e19.

- [185] A.J. Micev, D.M. Kalainov, A.P. Soneru, Masquelet technique for treatment of segmental bone loss in the upper extremity, *J. Hand Surg. [Am]* 40 (3) (2015) 593–598.
- [186] V. Viateau, M. Bensidhoum, G.v. Guillemin, H. Petite, D. Hannouche, F. Anagnostou, P. Pélassier, Use of the induced membrane technique for bone tissue engineering purposes: animal studies, *Orthop. Clin. North Am.* 41 (1) (2010) 49–56.
- [187] C.Y.-L. Woon, K.-W. Chong, M.-K. Wong, Induced membranes—a staged technique of bone-grafting for segmental bone loss: a report of two cases and a literature review, *JBJS* 92 (1) (2010) 196–201.
- [188] O.-M. Aho, P. Lehenkari, J. Ristiniemi, S. Lehtonen, J. Risteli, H.-V. Leskelä, The mechanism of action of induced membranes in bone repair, *JBJS* 95 (7) (2013) 597–604.
- [189] A.C. Masquelet, T. Begue, The concept of induced membrane for reconstruction of long bone defects, *Orthop. Clinics* 41 (1) (2010) 27–37.
- [190] B.C. Taylor, B.G. French, T.T. Fowler, J. Russell, A. Poka, Induced Membrane Technique for Reconstruction To Manage Bone Loss, *JAAOS - J. Am. Acad. Orthop. Surgeons* 20 (3) (2012) 142.
- [191] R. Gouron, L. Petit, C. Boudot, I. Six, M. Brazier, S. Kamel, R. Mentaverri, Osteoclasts and their precursors are present in the induced-membrane during bone reconstruction using the Masquelet technique, *J. Tissue Eng. Regener. Med.* 11 (2) (2017) 382–389.
- [192] P.H. Pelissier, A.C. Masquelet, R. Bareille, S.M. Pelissier, J. Amedee, Induced membranes secrete growth factors including vascular and osteoinductive factors and could stimulate bone regeneration, *J. Orthop. Res.* 22 (1) (2004) 73–79.
- [193] M. Durand, L. Barbier, L. Mathieu, T. Poyot, T. Demoures, J.-B. Souraud, A.-C. Masquelet, J.-M. Collombet, Towards Understanding Therapeutic Failures in Masquelet Surgery: first Evidence that Defective Induced Membrane Properties Are Associated with Clinical Failures, *J. Clin. Med.* 9 (2) (2020) 450.
- [194] M. Tarchala, V. Engel, J. Barralet, E.J. Harvey, A pilot study: alternative biomaterials in critical sized bone defect treatment, *Injury* (2017).
- [195] A.I. Alford, D. Nicolaou, M. Hake, S. McBride-Gagyi, Masquelet's induced membrane technique: review of current concepts and future directions, *J. Orthop. Res.* 39 (4) (2021) 707–718.
- [196] P. Andrzejowski, A. Masquelet, P.V. Giannoudis, Induced Membrane Technique (Masquelet) for Bone Defects in the Distal Tibia, Foot, and Ankle: systematic Review, Case Presentations, Tips, and Techniques, *Foot Ankle Clin.* 25 (4) (2020) 537–586.
- [197] L. Mathieu, M. Durand, J.M. Collombet, A. de Rousiers, N. de l'Escalopier, A.C. Masquelet, Induced membrane technique: a critical literature analysis and proposal for a failure classification scheme, *Eur. J. Trauma Emerg. Surg.* (2020).
- [198] R. Pereira, W.C. Perry, P.A. Crisologo, M.D. Liette, B. Hall, S.G. Hafez Hassn, S. Masadeh, Membrane-Induced Technique for the Management of Combined Soft Tissue and Osseous Defects, *Clin. Podiatr. Med. Surg.* 38 (1) (2021) 99–110.
- [199] C. Klein, M. Monet, V. Barbier, A. Vanlaeys, A.C. Masquelet, R. Gouron, R. Mentaverri, The Masquelet technique: current concepts, animal models, and perspectives, *J. Tissue Eng. Regen. Med.* 14 (9) (2020) 1349–1359.
- [200] N.L.P. Preston, T.E. Black, R.C. Thomas, Reconstruction of a Traumatic Partial First-Ray Amputation with the Use of an Induced Pseudosynovial Membrane and Corticocancellous Autograft, *J. Am. Podiatr. Med. Assoc.* 110 (2) (2020).
- [201] M. Mi, C. Papakostidis, X. Wu, P.V. Giannoudis, Mixed results with the Masquelet technique: a fact or a myth? *Injury* 51 (2) (2020) 132–135.
- [202] S. Careri, R. Vitiello, M.S. Oliva, A. Ziranu, G. Maccauro, C. Perisano, Masquelet technique and osteomyelitis: innovations and literature review, *Eur. Rev. Med. Pharmacol. Sci.* 23 (2) (2019) 210–216 Suppl.
- [203] W. Han, J. Shen, H. Wu, S. Yu, J. Fu, Z. Xie, Induced membrane technique: advances in the management of bone defects, *Int. J. Surg.* 42 (2017) 110–116.
- [204] R.S. Shaw, Reconstructive arterial surgery in upper-extremity injuries, *JBJS* 41 (4) (1959) 665–673.
- [205] H.E. Kleinert, M.L. Kasdan, J.L. Romero, Small Blood-Vessel Anastomosis for Salvage of Severely Injured Upper Extremity, *JBJS* 45 (4) (1963) 788.
- [206] Y. Hori, S. Tamai, H. Okuda, H. Sakamoto, T. Takita, K. Masuhara, Blood vessel transplantation to bone, *J. Hand Surg. [Am]* 4 (1) (1979) 23–33.
- [207] Y. Tanaka, K.-C. Sung, A. Tsutsumi, S. Ohba, K. Ueda, W.A. Morrison, Tissue engineering skin flaps: which vascular carrier, arteriovenous shunt loop or arteriovenous bundle, has more potential for angiogenesis and tissue generation? *Plast. Reconstr. Surg.* 112 (6) (2003) 1636–1644.
- [208] C.-H. Lin, S. Mardini, Y.-T. Lin, J.-T. Yeh, F.-C. Wei, H.-C. Chen, Sixty-five clinical cases of free tissue transfer using long arteriovenous fistulas or vein grafts, *J. Trauma Acute Care Surg.* 56 (5) (2004) 1107–1117.
- [209] B. Lind, W. McCarthy, G. Derman, C. Jacobs, Arteriovenous loop grafts for free tissue transfer, *Vasc. Endovascular Surg.* 46 (1) (2012) 30–33.
- [210] A.M. Eweida, W. Lang, M. Schmitz, R.E. Horch, Salvage of a free radial forearm flap by creation of an arteriovenous fistula at the distal arterial pedicle, *Microsurgery* 33 (5) (2013) 391–395.
- [211] A. Meyer, K. Goller, R.E. Horch, J.P. Beier, C.D. Taeger, A. Arkudas, W. Lang, Results of combined vascular reconstruction and free flap transfer for limb salvage in patients with critical limb ischemia, *J. Vasc. Surg.* 61 (5) (2015) 1239–1248.
- [212] R.E. Horch, J.P. Beier, U. Kneser, A. Arkudas, Successful human long-term application of in situ bone tissue engineering, *j cell mol med.* 18 (7) (2014) 1478–1485.
- [213] H. Kokemueller, S. Spalthoff, M. Nolff, F. Tavassol, H. Essig, C. Stuehmer, K.H. Bormann, M. Rücker, N.C. Gellrich, Prefabrication of vascularized biartificial bone grafts in vivo for segmental mandibular reconstruction: experimental pilot study in sheep and first clinical application, *Int. J. Oral Maxillofac. Surg.* 39 (4) (2010) 379–387.
- [214] R.E. Horch, J.P. Beier, U. Kneser, A. Arkudas, Successful human long-term application of in situ bone tissue engineering, *J. Cell. Mol. Med.* 18 (7) (2014) 1478–1485.
- [215] P.H. Warnke, I.N. Springer, J. Wiltfang, Y. Acil, H. Eufinger, M. Wehmöller, P.A. Russo, H. Bolte, E. Sherry, E. Behrens, H. Terheyden, Growth and transplantation of a custom vascularised bone graft in a man, *Lancet* 364 (9436) (2004) 766–770.
- [216] P.H. Warnke, J. Wiltfang, I. Springer, Y. Acil, H. Bolte, M. Kosmahl, P.A. Russo, E. Sherry, U. Lützen, S. Wolfart, H. Terheyden, Man as living bioreactor: fate of an exogenously prepared customized tissue-engineered mandible, *Biomaterials* 27 (17) (2006) 3163–3167.
- [217] J. Wiltfang, M. Rohen, J.H. Egberts, U. Lützen, H. Wieker, Y. Acil, H. Naujokat, Man as a Living Bioreactor: prefabrication of a Custom Vascularized Bone Graft in the Gastrocolic Omentum, *Tissue Eng. Part C Methods* 22 (8) (2016) 740–746.
- [218] A.M. Eweida, A.S. Nabawi, M.K. Marei, M.R. Khalil, H.A. Elhammady, Mandibular reconstruction using an axially vascularized tissue-engineered construct, *Ann. Surg. Innov. Res.* 5 (1) (2011) 2.
- [219] K.A. Raskin, F. Hornicek, Allograft reconstruction in malignant bone tumors: indications and limits, *Recent Results Cancer Res.* 179 (2009) 51–58.
- [220] B. Fung, G. Hoit, E. Schemitsch, C. Godbout, A. Nauth, The induced membrane technique for the management of long bone defects, *Bone Joint J.* 102-b (12) (2020) 1723–1734.
- [221] F. Chotel, L. Nguiabanda, P. Braillon, R. Kohler, J. Bérard, K. Abelin-Genevois, Induced membrane technique for reconstruction after bone tumor resection in children: a preliminary study, *Orthop. Traumatol. Surg. Res.* 98 (3) (2012) 301–308.
- [222] J.C. Aurégan, T. Bégué, G. Rigoulot, C. Glorion, S. Pannier, Success rate and risk factors of failure of the induced membrane technique in children: a systematic review, *Injury* 47 (6) (2016) S62–S67 Suppl.
- [223] S.A. Green, Skeletal defects. A comparison of bone grafting and bone transport for segmental skeletal defects, *Clin. Orthop. Relat. Res.* 301 (1994) 111–117.
- [224] H. Tsuchiya, T. Shirai, A.F. Morsy, K. Sakayama, T. Wada, K. Kusuzaki, T. Sugita, K. Tomita, Safety of external fixation during postoperative chemotherapy, *J. Bone. Joint Surg. Br.* 90 (7) (2008) 924–928.
- [225] K. Tong, Z. Zhong, Y. Peng, C. Lin, S. Cao, Y. Yang, G. Wang, Masquelet technique versus Ilizarov bone transport for reconstruction of lower extremity bone defects following posttraumatic osteomyelitis, *Injury* 48 (7) (2017) 1616–1622.
- [226] H. Naujokat, Y. Acil, A. Gülses, F. Birkenfeld, J. Wiltfang, Man as a living bioreactor: long-term histological aspects of a mandibular replacement engineered in the patient's own body, *Int. J. Oral Maxillofac. Surg.* 47 (11) (2018) 1481–1487.
- [227] J. Matschke, R. Armbruster, C. Reeps, J. Weitz, A. Dragu, AV loop free flap: an interdisciplinary approach for perineal and sacral defect reconstruction after radical oncological exenteration and radiation in a colorectal cancer patient, *World J. Surg. Oncol.* 17 (1) (2019) 154.
- [228] R. Knackstedt, R. Aliotta, J. Gatherwright, R. Djohan, B. Gastman, G. Schwarz, M. Hendrickson, R. Gurunluoglu, Single-stage versus two-stage arteriovenous loop microsurgical reconstruction: a meta-analysis of the literature, *Microsurgery* 38 (6) (2018) 706–717.
- [229] H. Bezstarosti, W.J. Metsemakers, E.M.M. van Lieshout, L.W. Voskamp, K. Kortram, M.A. McNally, L.C. Marais, M.H.J. Verhofstad, Management of critical-sized bone defects in the treatment of fracture-related infection: a systematic review and pooled analysis, *Arch. Orthop. Trauma Surg.* 141 (7) (2021) 1215–1230.
- [230] Y. Tanaka, A. Tsutsumi, D.M. Crowe, S. Tajima, W.A. Morrison, Generation of an autologous tissue (matrix) flap by combining an arteriovenous shunt loop with artificial skin in rats: preliminary report, *Br. J. Plast. Surg.* 53 (1) (2000) 51–57.
- [231] R. Mian, W.A. Morrison, J.V. Hurley, A.J. Penington, R. Romeo, Y. Tanaka, K.R. Knight, Formation of New Tissue from an Arteriovenous Loop in the Absence of Added Extracellular Matrix, *Tissue Eng.* 6 (6) (2000) 595–603.
- [232] M.M. Stevens, R.P. Marini, D. Schaefer, J. Aronson, R. Langer, V.P. Shastri, In vivo engineering of organs: the bone bioreactor, *Proc. Natl. Acad. Sci.* 102 (32) (2005) 11450–11455.
- [233] G.E. Holt, J.L. Halpern, T.T. Dovan, D. Hamming, H.S. Schwartz, Evolution of an in vivo bioreactor, *J. Orthop. Res.* 23 (4) (2005) 916–923.
- [234] R.-L. Huang, E. Kobayashi, K. Liu, Q. Li, Bone graft prefabrication following the in vivo bioreactor principle, *EBioMedicine* 12 (2016) 43–54.
- [235] T. Rademakers, J.M. Horvath, C.A. Blitterswijk, V.L.S. LaPointe, Oxygen and nutrient delivery in tissue engineering: approaches to graft vascularization, *J. Tissue Eng. Regener. Med.* (2019).
- [236] J. Fan, L. Bi, D. Jin, K. Wei, B. Chen, Z. Zhang, G. Pei, Microsurgical techniques used to construct the vascularized and neurotized tissue engineered bone, *Biomed. Res. Int.* 2014 (2014) 281872–281872.
- [237] A. Weigand, J.P. Beier, A. Arkudas, M. Al-Abdoobi, E. Polykandriotis, R.E. Horch, A.M. Boos, The Arteriovenous (AV) Loop in a Small Animal Model to Study Angiogenesis and Vascularized Tissue Engineering, *J. Vis. Exp.* 117 (2016).

- [238] D.H.R. Kempen, L. Lu, A. Heijink, T.E. Hefferan, L.B. Creemers, A. Maran, M.J. Yaszemski, W.J.A. Dhert, Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration, *Biomaterials* 30 (14) (2009) 2816–2825.
- [239] J.R. García, A.Y. Clark, A.J. García, Integrin-specific hydrogels functionalized with VEGF for vascularization and bone regeneration of critical-size bone defects, *J. Biomed. Mater. Res. Part A* 104 (4) (2016) 889–900.
- [240] W. Wang, L. Guo, Y. Yu, Z. Chen, R. Zhou, Z. Yuan, Peptide REDV-modified polysaccharide hydrogel with endothelial cell selectivity for the promotion of angiogenesis, *J. Biomed. Mater. Res. Part A* 103 (5) (2015) 1703–1712.
- [241] D. Kaigler, Z. Wang, K. Horger, D.J. Mooney, P.H. Krebsbach, VEGF Scaffolds Enhance Angiogenesis and Bone Regeneration in Irradiated Osseous Defects, *J. Bone Miner. Res.* 21 (5) (2006) 735–744.
- [242] J.Kent Leach, D. Kaigler, Z. Wang, P.H. Krebsbach, D.J. Mooney, Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration, *Biomaterials* 27 (17) (2006) 3249–3255.
- [243] E. Wernike, M.O. Montjovent, Y. Liu, D. Wismeijer, E.B. Hunziker, K.A. Siebenrock, W. Hofstetter, F.M. Klenke, VEGF incorporated into calcium phosphate ceramics promotes vascularisation and bone formation in vivo, *Eur. Cells Mater.* 19 (2010) 30–40.
- [244] S. Young, Z.S. Patel, J.D. Kretlow, M.B. Murphy, P.M. Mountzaris, L.S. Baggett, H. Ueda, Y. Tabata, J.A. Jansen, M. Wong, A.G. Mikos, Dose Effect of Dual Delivery of Vascular Endothelial Growth Factor and Bone Morphogenetic Protein-2 on Bone Regeneration in a Rat Critical-Size Defect Model, *Tissue Eng. Part A* 15 (9) (2009) 2347–2362.
- [245] Q. Yang, K.P. McHugh, S. Patnitrapong, X. Gu, L. Wunderlich, P.V. Hauschka, VEGF enhancement of osteoclast survival and bone resorption involves VEGF receptor-2 signaling and beta3-integrin, *Matrix Biol.: J. Int. Soc. Matrix Biol.* 27 (7) (2008) 589–599.
- [246] S. Niida, M. Kaku, H. Amano, H. Yoshida, H. Kataoka, S. Nishikawa, K. Tanne, N. Maeda, S. Nishikawa, H. Kodama, Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption, *J. Exp. Med.* 190 (2) (1999) 293–298.
- [247] C.G. Maes C, Vascular and Nonvascular Roles of VEGF in Bone Development, Madame Curie Bioscience Database, 2021 [Internet]. (Accessed 03/16).
- [248] M. Nakagawa, T. Kaneda, T. Arakawa, S. Morita, T. Sato, T. Yonada, K. Hanada, M. Kumegawa, Y. Hakeda, Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts, *FEBS Lett.* 473 (2) (2000) 161–164.
- [249] U. Helmrich, N. Di Maggio, S. Güven, E. Groppe, L. Melly, R.D. Largo, M. Heberer, I. Martin, A. Scherberich, A. Banfi, Osteogenic graft vascularization and bone resorption by VEGF-expressing human mesenchymal progenitors, *Biomaterials* 34 (21) (2013) 5025–5035.
- [250] S. Spalthoff, P. Juhn, R. Zimmerer, U. Möllmann, N.C. Gellrich, H. Kokemueller, Heterotopic bone formation in the musculus latissimus dorsi of sheep using β-tricalcium phosphate scaffolds: evaluation of an extended prefabrication time on bone formation and matrix degeneration, *Int. J. Oral Maxillofac. Surg.* 44 (6) (2015) 791–797.
- [251] A.M. Tatara, J.D. Kretlow, P.P. Spicer, S. Lu, J. Lam, W. Liu, Y. Cao, G. Liu, J.D. Jackson, J.J. Yoo, A. Atala, J.J.P. van den Beucken, J.A. Jansen, F.K. Kasper, T. Ho, N. Demian, M.J. Miller, M.E. Wong, A.G. Mikos, Autologously generated tissue-engineered bone flaps for reconstruction of large mandibular defects in an ovine model, *Tissue Eng. Part A* 21 (9–10) (2015) 1520–1528.
- [252] B. Charbonnier, S. Maillard, O. Sayed, A. Baradaran, H. Mangat, B. Dalisson, Z. Zhang, Y.-L. Zhang, S.N.A. Hussain, D. Mayaki, H. Seitz, E.J. Harvey, M. Gillardino, U. Gbureck, N. Makhlouf, J. Barralet, Biomaterial-Induction of a Transplantable Angiosome, *Adv. Funct. Mater.* 30 (1) (2020) 1905115.
- [253] B.M. Roux, M.-H. Cheng, E.M. Brey, Engineering clinically relevant volumes of vascularized bone, *J. Cell. Mol. Med.* 19 (5) (2015) 903–914.
- [254] M. Csobonyeiova, S. Polak, R. Zamborsky, L. Danisovic, iPS cell technologies and their prospect for bone regeneration and disease modeling: a mini review, *J. Adv. Res.* 8 (4) (2017) 321–327.
- [255] C. Li, Z. Mills, Z. Zheng, Novel cell sources for bone regeneration, *MedComm* 2 (2) (2021) 145–174.
- [256] D. Marolt Presen, A. Traweger, M. Gimona, H. Redl, Mesenchymal Stromal Cell-Based Bone Regeneration Therapies: from Cell Transplantation and Tissue Engineering to Therapeutic Secretomes and Extracellular Vesicles, *Front. Bioeng. Biotechnol.* 7 (2019) 352.
- [257] J. Cao, Z. Yang, R. Xiao, B. Pan, Regenerative potential of pluripotent non-tumorigenic stem cells: multilineage differentiating stress enduring cells (MuSe cells), *Regen. Ther.* 15 (2020) 92–96.
- [258] R.E. Unger, E. Dohle, C.J. Kirkpatrick, Improving vascularization of engineered bone through the generation of pro-angiogenic effects in co-culture systems, *Adv. Drug. Deliv. Rev.* 94 (2015) 116–125.
- [259] B. Hafen, S. Wiesner, K. Schlegelmilch, A. Keller, L. Seefried, R. Ebert, H. Walles, F. Jakob, N. Schütze, Physical contact between mesenchymal stem cells and endothelial precursors induces distinct signatures with relevance to the very early phase of regeneration, *J. Cell. Biochem.* 119 (11) (2018) 9122–9140.
- [260] M. Grellier, L. Bordenave, J. Amédée, Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering, *Trends Biotechnol.* 27 (10) (2009) 562–571.
- [261] C.J. Kirkpatrick, S. Fuchs, R.E. Unger, Co-culture systems for vascularization – Learning from nature, *Adv. Drug. Deliv. Rev.* 63 (4) (2011) 291–299.
- [262] S. Zhang, M. Zhou, Z. Ye, Y. Zhou, W.-S. Tan, Fabrication of viable and functional pre-vascularized modular bone tissues by coculturing MSCs and HUVECs on microcarriers in spinner flasks, *Biotechnol. J.* 12 (8) (2017) 1700008.
- [263] A. Hayrapetyan, S. Surjandi, E.E.P.J. Lemsom, M.M.W. Wolters, J.A. Jansen, J.J.P. van den Beucken, Coculture effects on the osteogenic differentiation of human mesenchymal stromal cells, *Tissue Eng. Regen. Med.* 13 (6) (2016) 713–723.
- [264] J. Ng, K. Spiller, J. Bernhard, G. Vunjak-Novakovic, Biomimetic Approaches for Bone Tissue Engineering, *Tissue Eng. Part B: Rev.* 23 (5) (2016) 480–493.
- [265] J. Tao, Y. Sun, Q. Wang, C. Liu, Induced Endothelial Cells Enhance Osteogenesis and Vascularization of Mesenchymal Stem Cells, *Cells Tissues Organs* 190 (4) (2009) 185–193.
- [266] H. Yu, P.J. Vandevord, W. Gong, B. Wu, Z. Song, H.W. Matthew, P.H. Wooley, S.-Y. Yang, Promotion of osteogenesis in tissue-engineered bone by pre-seeding endothelial progenitor cells-derived endothelial cells, *J. Orthop. Res.* 26 (8) (2008) 1147–1152.
- [267] J. Zhou, H. Lin, T. Fang, X. Li, W. Dai, T. Uemura, J. Dong, The repair of large segmental bone defects in the rabbit with vascularized tissue engineered bone, *Biomaterials* 31 (6) (2010) 1171–1179.
- [268] M. Grellier, P.L. Granja, J.-C. Fricain, S.J. Bidarra, M. Renard, R. Bareille, C. Bourget, J. Amédée, M.A. Barbosa, The effect of the co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect, *Biomaterials* 30 (19) (2009) 3271–3278.
- [269] A.R. Amini, T.O. Xu, R.M. Chidambaram, S.P. Nukavarapu, Oxygen Tension-Controlled Matrices with Osteogenic and Vasculogenic Cells for Vascularized Bone Regeneration In Vivo, *Tissue Eng. Part A* 22 (7–8) (2016) 610–620.
- [270] C. Seebach, D. Henrich, C. Häling, K. Wilhelm, A.E. Tami, M. Alini, I. Marzi, Endothelial Progenitor Cells and Mesenchymal Stem Cells Seeded onto β-TCP Granules Enhance Early Vascularization and Bone Healing in a Critical-Sized Bone Defect in Rats, *Tissue Eng. Part A* 16 (6) (2010) 1961–1970.
- [271] L. Li, J. Li, Q. Zou, Y. Zuo, B. Cai, Y. Li, Enhanced bone tissue regeneration of a biomimetic cellular scaffold with co-cultured MSCs-derived osteogenic and angiogenic cells, *Cell Prolif.* 52 (5) (2019) e12658–e12658.
- [272] H. Zigdon-Giladi, T. Bick, D. Lewinson, E.E. Machtei, Co-Transplantation of Endothelial Progenitor Cells and Mesenchymal Stem Cells Promote Neovascularization and Bone Regeneration, *Clin. Implant Dent. Relat. Res.* 17 (2) (2015) 353–359.
- [273] X. Liu, W. Chen, C. Zhang, W. Thein-Han, K. Hu, M.A. Reynolds, C. Bao, P. Wang, L. Zhao, H.H.K. Xu, Co-Seeding Human Endothelial Cells with Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells on Calcium Phosphate Scaffold Enhances Osteogenesis and Vascularization in Rats, *Tissue Eng. Part A* 23 (11–12) (2017) 546–555.
- [274] A.R. Shah, A. Cornejo, D.E. Sahar, S.M. Stephenson, S. Chang, N.K. Krishnegowda, H.T. Wang, T. Guda, R. Sharma, Differentiated adipose-derived stem cell cocultures for bone regeneration in polymer scaffolds *in vivo*, *J. Craniofac. Surg.* 25 (4) (2014) 1504–1509.
- [275] S. Koob, N. Torio-Padron, G.B. Stark, C. Hannig, Z. Stankovic, G. Finkenzeller, Bone formation and neovascularization mediated by mesenchymal stem cells and endothelial cells in critical-sized calvarial defects, *Tissue Eng. Part A* 17 (3–4) (2011) 311–321.
- [276] S. Shanbhag, N. Pandis, K. Mustafa, J.R. Nyengaard, A. Stavropoulos, Cell Co-transplantation Strategies for Vascularized Craniofacial Bone Tissue Engineering: a Systematic Review and Meta-Analysis of Preclinical In Vivo Studies, *Tissue Eng. Part B: Rev.* 23 (2) (2016) 101–117.
- [277] W. Chen, W. Thein-Han, M.D. Weir, Q. Chen, H.H.K. Xu, Prevascularization of biofunctional calcium phosphate cement for dental and craniofacial repairs, *Dent. Mater.* 30 (5) (2014) 535–544.
- [278] R. Mishra, B.M. Roux, M. Posukonis, E. Bodamer, E.M. Brey, J.P. Fisher, D. Dean, Effect of prevascularization on *in vivo* vascularization of poly(propylene fumarate)/fibrin scaffolds, *Biomaterials* 77 (2016) 255–266.
- [279] R.E. Unger, S. Ghanaati, C. Orth, A. Sartoris, M. Barbeck, S. Halstenberg, A. Motta, C. Migliaresi, C.J. Kirkpatrick, The rapid anastomosis between pre-vascularized networks on silk fibroin scaffolds generated *in vitro* with cocultures of human microvascular endothelial and osteoblast cells and the host vasculature, *Biomaterials* 31 (27) (2010) 6959–6967.
- [280] J. Rouwkema, J.D. Boer, C.A.V. Blitterswijk, Endothelial Cells Assemble into a 3-Dimensional Prevascular Network in a Bone Tissue Engineering Construct, *Tissue Eng.* 12 (9) (2006) 2685–2693.
- [281] S. Ben-Shaul, S. Landau, U. Merdler, S. Levenberg, Mature vessel networks in engineered tissue promote graft-host anastomosis and prevent graft thrombosis, *Proc. Natl. Acad. Sci. U. S. A.* 116 (8) (2019) 2955–2960.
- [282] R. Walser, W. Metzger, A. Görg, T. Pohlmann, M.D. Menger, M.W. Laschke, Generation of co-culture spheroids as vascularisation units for bone tissue engineering, *Eur. Cells Mater.* (2013) 222–233.
- [283] S. Fuchs, S. Ghanaati, C. Orth, M. Barbeck, M. Kolbe, A. Hofmann, M. Eblenkamp, M. Gomes, R.L. Reis, C.J. Kirkpatrick, Contribution of outgrowth endothelial cells from human peripheral blood on *in vivo* vascularization of bone tissue engineered constructs based on starch polycaprolactone scaffolds, *Biomaterials* 30 (4) (2009) 526–534.
- [284] S. Ghanaati, S. Fuchs, M.J. Webber, C. Orth, M. Barbeck, M.E. Gomes, R.L. Reis, C.J. Kirkpatrick, Rapid vascularization of starch-poly(caprolactone) *in vivo* by outgrowth endothelial cells in co-culture with primary osteoblasts, *J. Tissue Eng. Regen. Med.* 5 (6) (2011) e136–e143.
- [285] O.P. Torbjorn, L.B. Anna, X. Ying, X. Zhe, S. Yang, F.-W. Anna, B.L. James, F. Inge, N.L. Knut, M. Kamal, Mesenchymal stem cells induce endothelial cell quiescence and promote capillary formation, *Stem Cell Res. Therapy* (2014) 23.

- [286] J. Ma, F. Yang, S.K. Both, H.-J. Prins, M.N. Helder, J. Pan, F.-Z. Cui, J.A. Jansen, J.J.P. van den Beucken, In vitro and in vivo angiogenic capacity of BM-MSCs/HUVECs and AT-MSCs/HUVECs cocultures, *Biofabrication* 6 (1) (2014) 015005.
- [287] T.M. McFadden, G.P. Duffy, A.B. Allen, H.Y. Stevens, S.M. Schwarzmaier, N. Plesnila, J.M. Murphy, F.P. Barry, R.E. Guldberg, F.J. O'Brien, The delayed addition of human mesenchymal stem cells to pre-formed endothelial cell networks results in functional vascularization of a collagen-glycosaminoglycan scaffold in vivo, *Acta Biomater.* 9 (12) (2013) 9303–9316.
- [288] E.C. Watson, Z.L. Grant, L. Coultras, Endothelial cell apoptosis in angiogenesis and vessel regression, *Cell. Mol. Life Sci.* 74 (24) (2017) 4387–4403.
- [289] A. Gomariz, P.M. Helbling, S. Isringhausen, U. Süessbier, A. Becker, A. Boss, T. Nagasawa, G. Paul, O. Goksel, G. Székely, S. Stoma, S.F. Nørrelykke, M.G. Manz, C. Nombela-Arrieta, Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy, *Nat. Commun.* 9 (1) (2018) 2532.
- [290] P.H. Wu, M. Gibbons, S.C. Foreman, J. Carballido-Gamio, M. Han, R. Krug, J. Liu, T.M. Link, G.J. Kazakia, Cortical bone vessel identification and quantification on contrast-enhanced MR images, *Quant. Imaging Med. Surg.* 9 (6) (2019) 928–941.
- [291] J.R. Perez, D. Kouroupis, D.J. Li, T.M. Best, L. Kaplan, D. Correa, Tissue Engineering and Cell-Based Therapies for Fractures and Bone Defects, *Front. Bioeng. Biotechnol.* 6 (2018) 105–105.
- [292] S.C. Dennis, C.J. Berkland, L.F. Bonewald, M.S. Detamore, Endochondral ossification for enhancing bone regeneration: converging native extracellular matrix biomaterials and developmental engineering in vivo, *Tissue Eng. Part B Rev.* 21 (3) (2015) 247–266.
- [293] S.M. Oliveira, I.F. Amaral, M.A. Barbosa, C.C. Teixeira, *Tissue Eng. Part A* 15 (3) (2008) 625–634.
- [294] J. Sasaki, T. Matsumoto, H. Egusa, M. Matsusaki, A. Nishiguchi, T. Nakano, M. Akashi, S. Imazato, H. Yatani, In vitro reproduction of endochondral ossification using a 3D mesenchymal stem cell construct, *Integr. Biol.: Quant. Biosci. Nano to Macro* 4 (10) (2012) 1207–1214.
- [295] F.E. Freeman, L.M. McNamara, Endochondral Priming: a Developmental Engineering Strategy for Bone Tissue Regeneration, *Tissue Eng. Part B Rev.* 23 (2) (2017) 128–141.
- [296] E. Farrell, S.K. Both, K.I. Odörfer, W. Koevoet, N. Kops, F.J. O'Brien, R.J. Baatenburg de Jong, J.A. Verhaar, V. Cuijpers, J. Jansen, R.G. Erben, G.J.V.M. van Osch, In-vivo generation of bone via endochondral ossification by in-vitro chondrogenic priming of adult human and rat mesenchymal stem cells, *BMC Musculoskelet. Disord.* 12 (2011) 31–31.
- [297] F.E. Freeman, M.G. Haugh, L.M. McNamara, Investigation of the optimal timing for chondrogenic priming of MSCs to enhance osteogenic differentiation in vitro as a bone tissue engineering strategy, *J. Tissue Eng. Regen. Med.* 10 (4) (2016) E250–E262.
- [298] E. Farrell, O.P. van der Jagt, W. Koevoet, N. Kops, C.J. van Manen, C.A. Hellingman, H. Jahr, F.J. O'Brien, J.A. Verhaar, H. Weinans, G.J. van Osch, Chondrogenic priming of human bone marrow stromal cells: a better route to bone repair? *Tissue Eng. Part C, Methods* 15 (2) (2009) 285–295.
- [299] I. Papantoniou, G. Nilsson Hall, N. Loverdou, R. Lesage, T. Herpelink, L. Mendes, L. Geris, Turning Nature's own processes into design strategies for living bone implant biomanufacturing: a decade of Developmental Engineering, *Adv. Drug. Deliv. Rev.* 169 (2021) 22–39.
- [300] F.E. Freeman, H.Y. Stevens, P. Owens, R.E. Guldberg, L.M. McNamara, Osteogenic Differentiation of Mesenchymal Stem Cells by Mimicking the Cellular Niche of the Endochondral Template, *Tissue Eng. Part A* 22 (19–20) (2016) 1176–1190.
- [301] F.E. Freeman, A.B. Allen, H.Y. Stevens, R.E. Guldberg, L.M. McNamara, Effects of in vitro endochondral priming and pre-vascularisation of human MSC cellular aggregates in vivo, *Stem Cell Res. Therapy* 6 (2015) 218–218.
- [302] F.E. Freeman, M.Á. Brennan, D.C. Browne, A. Renaud, J. De Lima, D.J. Kelly, L.M. McNamara, P. Layrolle, A Developmental Engineering-Based Approach to Bone Repair: endochondral Priming Enhances Vascularization and New Bone Formation in a Critical Size Defect, *Front. Bioeng. Biotechnol.* 8 (2020) 230–230.
- [303] W. Yang, S.K. Both, G.J.V.M. van Osch, Y. Wang, J.A. Jansen, F. Yang, Effects of in vitro chondrogenic priming time of bone-marrow-derived mesenchymal stromal cells on in vivo endochondral bone formation, *Acta Biomater.* 13 (2015) 254–265.
- [304] C. Scotti, B. Tonnarelli, A. Papadimitropoulos, A. Scherberich, S. Schaeren, A. Schauererte, J. Lopez-Rios, R. Zeller, A. Barbero, I. Martin, Recapitulation of endochondral bone formation using human adult mesenchymal stem cells as a paradigm for developmental engineering, *Proc. Natl. Acad. Sci.* 107 (16) (2010) 7251.
- [305] J. Bernhard, J. Ferguson, B. Rieder, P. Heimel, T. Nau, S. Tangl, H. Redl, G. Vunjak-Novakovic, Tissue-engineered hypertrophic chondrocyte grafts enhanced long bone repair, *Biomaterials* 139 (2017) 202–212.
- [306] E.M. Thompson, A. Matsiko, D.J. Kelly, J.P. Gleeson, F.J. O'Brien, An Endochondral Ossification-Based Approach to Bone Repair: chondrogenically Primed Mesenchymal Stem Cell-Laden Scaffolds Support Greater Repair of Critical-Sized Cranial Defects Than Osteogenically Stimulated Constructs In Vivo, *Tissue Eng. Part A* 22 (5–6) (2016) 556–567.
- [307] G.M. Cunniffe, T. Vinardell, J.M. Murphy, E.M. Thompson, A. Matsiko, F.J. O'Brien, D.J. Kelly, Porous decellularized tissue engineered hypertrophic cartilage as a scaffold for large bone defect healing, *Acta Biomater.* 23 (2015) 82–90.
- [308] J. van der Stok, M.K. Koolen, H. Jahr, N. Kops, J.H. Waarsing, H. Weinans, O.P. van der Jagt, Chondrogenically differentiated mesenchymal stromal cell pellets stimulate endochondral bone regeneration in critical-sized bone defects, *Eur. Cells Mater.* 27 (2014) 137–148 discussion 148.
- [309] N. Harada, Y. Watanabe, K. Sato, S. Abe, K. Yamanaka, Y. Sakai, T. Kaneko, T. Matsushita, Bone regeneration in a massive rat femur defect through endochondral ossification achieved with chondrogenically differentiated MSCs in a degradable scaffold, *Biomaterials* 35 (27) (2014) 7800–7810.
- [310] A.M. McDermott, S. Herberg, D.E. Mason, J.M. Collins, H.B. Pearson, J.H. Dawaahre, R. Tang, A.N. Patwa, M.W. Grinstaff, D.J. Kelly, E. Alsberg, J.D. Boerckel, Recapitulating bone development through engineered mesenchymal condensations and mechanical cues for tissue regeneration, *Sci. Transl. Med.* 11 (495) (2019) eaav7756.
- [311] E.J. Sheehy, T. Mesallati, L. Kelly, T. Vinardell, C.T. Buckley, D.J. Kelly, Tissue Engineering Whole Bones Through Endochondral Ossification: regenerating the Distal Phalanx, *Biores. Open Access* 4 (1) (2015) 229–241.
- [312] F. Pourdanesh, N. Latifi, F. Latifi, Complications after craniofacial reconstruction with calcium phosphate cements: a case report and review of the literature, *J. Korean Assoc. Oral Maxillofacial Surgeons* (2018) 207–211.
- [313] M.S. Gilardino, D.S. Cabiling, S.P. Bartlett, Long-Term Follow-Up Experience with Carbonated Calcium Phosphate Cement (Norian) for Cranioplasty in Children and Adults, *Plast. Reconstr. Surg.* 123 (3) (2009) 983–994.
- [314] L.G. Sullivan, Myth, Metaphor and Hypothesis: how Anthropomorphism Defeats Science, *Philos. Trans.: Biol. Sci.* 349 (1328) (1995) 215–218.
- [315] L.M. Osbeck, N.J. Nersessian, Affective problem solving: emotion in research practice, *Mind Soc.: Cogn. Stud. Econ. Social Sci.* 10 (1) (2011) 57–78.
- [316] R.T. Beyene, S.L. Derryberry Jr., A. Barbul, The Effect of Comorbidities on Wound Healing, *Surg. Clin. North Am.* 100 (4) (2020) 695–705.
- [317] H. Khalil, M. Cullen, H. Chambers, M. McGrail, Medications affecting healing: an evidence-based analysis, *Int. Wound J.* 14 (6) (2017) 1340–1345.
- [318] P. Perrot, J. Rousseau, A.L. Bouffaut, F. Rédati, E. Cassagnau, F. Deschaseaux, M.F. Heymann, D. Heymann, F. Duteille, V. Trichet, F. Gouin, Safety concern between autologous fat graft, mesenchymal stem cell and osteosarcoma recurrence, *PLoS One* 5 (6) (2010) e10999.
- [319] A. Nazarian, D. von Stechow, D. Zurakowski, R. Müller, B.D. Snyder, Bone volume fraction explains the variation in strength and stiffness of cancellous bone affected by metastatic cancer and osteoporosis, *Calcif. Tissue Int.* 83 (6) (2008) 368–379.