

Regenerative medicine in lower limb reconstruction

Josephine K McEwan¹, Howard C Tribe¹, Neal Jacobs², Nicholas Hancock³, Amir A Qureshi³, Douglas G Dunlop^{**3} & Richard OC Oreffo^{*,1}

¹Bone & Joint Research Group, Centre for Human Development, Stem Cell & Regeneration, Institute of Developmental Sciences, University of Southampton, Tremona Road, Southampton SO16 6YD, UK

²Salisbury NHS Foundation Trust, Salisbury, Wiltshire, UK

³Trauma & Orthopaedic Department, University Hospital Southampton, Southampton, UK

*Author for correspondence: richard.oreffo@soton.ac.uk

**Author for correspondence: doug.dunlop@uhs.nhs.uk

Bone is a highly specialized connective tissue and has a rare quality as one of the few tissues that can repair without a scar to regain pre-injury structure and function. Despite the excellent healing capacity of bone, tumor, infection, trauma and surgery can lead to significant bone loss requiring skeletal augmentation. Bone loss in the lower limb poses a complex clinical problem, requiring reconstructive techniques to restore form and function. In the past, amputation may have been the only option; however, there is now an array of reconstructive possibilities and cellular therapies available to salvage a limb. In this review, we will evaluate current applications of bone tissue engineering techniques in limb reconstruction and identify potential strategies for future work.

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Clinical need

Background

Major trauma is the leading cause of deaths in children and adults aged under 46 years old [1]. Major trauma describes serious injuries, often multiple, from which there is a high risk of death or disability. Major trauma can also be defined as patients with an Injury Severity Score greater than 15 [2], which is associated with a mortality of 10% [3]. The most common mechanism resulting in major trauma in the UK and USA is a road traffic accident. Other mechanisms include a fall from a height and physical violence. Major trauma also includes any single injury that is so complex that it exceeds the capabilities or expertise of a receiving trauma hospital. As such, open fractures, particularly those with bone defects, and or skin tissue loss, may constitute a major trauma and represent a limb threatening injury that could have life changing consequences.

Lower limb reconstruction

The complex management of severe lower limb injuries has led to the development of lower limb reconstruction as a subspecialty field within orthopedic surgery. Lower limb reconstruction aims to tackle clinical problems such as nonunion, limb deformities and critical-sized bone defects (CSDs) due to trauma, tumor or infection (<http://blrs.org.uk/>). A CSD relates to bone loss that will not heal within the patient's lifetime, generally accepted to be bone loss of greater than 1.5-times the diameter of the diaphysis of the bone [4].

The management of complex lower limb injuries and the sequelae of these injuries require a range of different surgical skills to restore form and function of a limb. Regenerative medicine aims to repair tissues through stem cell therapy, tissue engineering and manipulation of molecular biology to regenerate diseased or damaged tissue. Evaluating the role of regenerative medicine in limb reconstruction requires an understanding of the basic science of fracture healing, the current treatment modalities available and novel strategies that have already been implemented.

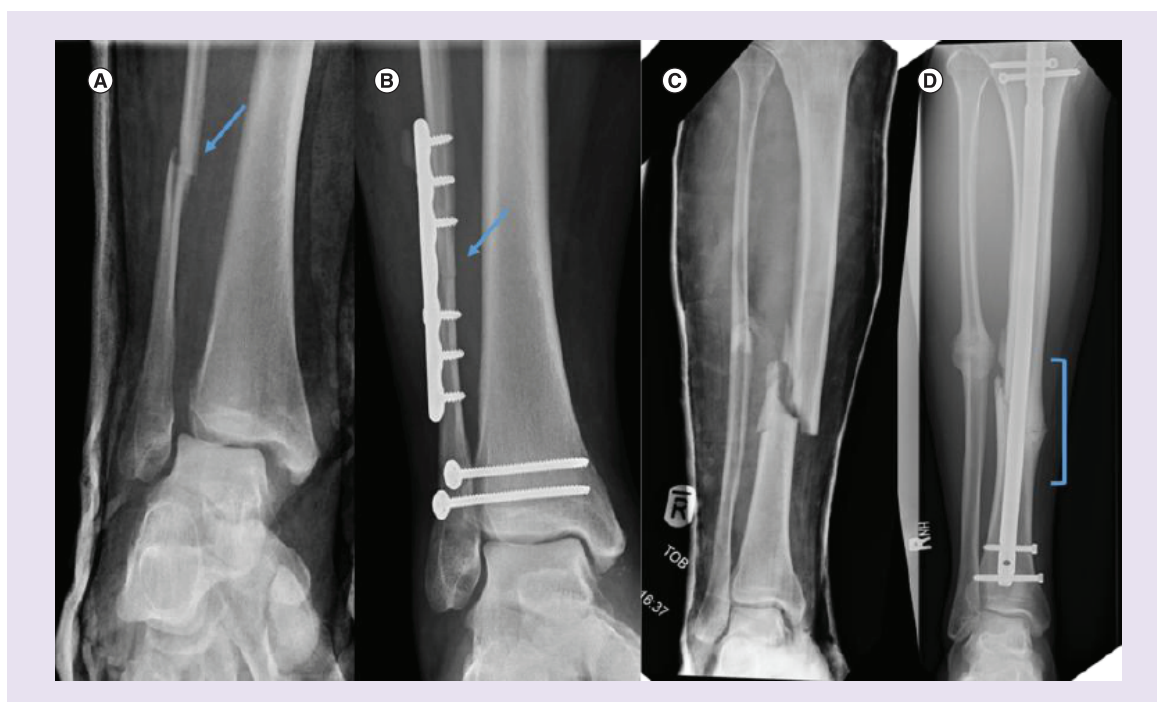


Figure 1. Primary and secondary bone healing. (A) Fibula fracture (see arrow). **(B)** Fibula fracture healed by primary bone healing following open reduction internal fixation (see arrow). **(C)** Tibia and fibula fracture. **(D)** Tibia and fibula fracture healed by secondary bone healing with callus formation (see bracket).

Bone healing

If bone is subjected to excessive force (stress or strain), it will fracture. Bone has an exceptional ability to heal and remodel, enabling restoration of physical and biological properties after an injury [5]. This clinical observation contributes to the rationale supporting the concept of a postnatal stem cell population residing within the bone marrow [6], which is responsible for bone regeneration and repair. Bone heals according to both the mechanical and biological environment in which the fracture exists [7], and as such, can be affected and influenced [5]. Recognizing the biological processes occurring in the context of the mechanical situation is crucial to understanding clinical scenarios and directing treatment strategies.

Primary (direct) fracture healing

Primary bone healing requires anatomical reduction of fracture fragments, which only usually occurs following operative open reduction and internal fixation (Figure 1A & B). Mechanically, this equates to a fracture gap of less than 10 μm and less than 5% strain at the fracture site, which will lead to intramembranous ossification [8]. Under these conditions, osteoclasts and osteoblasts are able to bridge the fracture gap and form a cutting cone in which the osteoclasts form the head of the cone to resorb bone, and the osteoblasts follow depositing new bone matrix.

Secondary (indirect) fracture healing

More commonly, bone healing is secondary bone healing; comprising of both intramembranous and endochondral bone formation and consists of four interlinked stages [9]. Following a bony injury, a hematoma forms which has osteogenic and angiogenic potential to create the environment for optimum healing [10]. The fracture hematoma and subsequent inflammation release numerous signaling molecules (IL-1, IL-6, TNF- α , FGF, IGF, PDGF, VEGF and TGF- β superfamily members) [5] that coordinate a tightly organized cascade of events to result in vascularized bone formation [11]. The fracture hematoma between the bone ends provides a mesh of fibrin that acts as an osteoconductive scaffold for soft cartilaginous callus to form. The soft callus then undergoes vascularization and calcification, with subsequent remodeling to resume normal bone architecture [12]. Clinically, endochondral ossification can be achieved by casting or functional bracing, or using operative techniques that allow micromotion at the fracture site (Figure 1C & D) to stimulate bone union [8,13].

Nonunion

A fracture that fails to heal is described as a nonunion. There is no globally agreed clinical or radiographic definition of a nonunion [14]. A generalized clinical description is the cessation of progressive fracture repair after 6 months, although there are many influencing factors that may affect the time scale in which a fracture is expected to heal. For example, high energy open fractures will take longer to heal than simple, low energy fractures, and patient age and bone site also play a role. An early definition of nonunion was reported by Weber and Cech in 1973, in which nonunions were categorized as active or inactive [15]. Nonunions are often categorized as hypertrophic or atrophic according to the volume of callus present on radiographic imaging. Hypertrophic nonunions are thought to represent biologically active vascular bone that lacks mechanical stability at the fracture site, which impedes progression to bony union. Atrophic nonunions are often thought to represent biologically inactive bone, with impaired vascularity [16] that are unable to heal despite the appropriate mechanical environment. However, it has been demonstrated that atrophic nonunions can be well vascularized [17] and a study that analyzed biopsies from hypertrophic and atrophic nonunion sites found there was no difference in the vascularity between the two groups [18]. Both forms of nonunion signify a serious complication and cause substantial morbidity to the patient; potentially requiring lengthy treatments, re-operation, restricted mobility and resultant poor quality of life. In addition, there is the economic impact of the cost of treatment to consider, which has been shown to range from approximately £6700–80,000 per nonunion [19], as well as the patient's sick leave from work [20].

Tibial diaphyseal fractures are the most common diaphyseal fractures encountered in orthopedics in adults [21] and may present as an isolated injury or as part of a polytrauma scenario. There is a high rate of tibial nonunion; the incidence of delayed union or nonunion for all fractures is estimated at 5–10% [22], yet for open tibial fractures, nonunion rates can rise to 60% [23]. The high prevalence of tibial nonunion is likely due to the lack of a soft tissue overlying the bone, contributing to the tibia's vulnerability to injury, as well as a relative decreased vascularity, compared with other long bones, leading to a subsequent high rate of impaired healing [24]. The management of complex tibial fractures and nonunion creates a significant clinical challenge requiring reconstructive strategies for successful treatment.

Lower limb reconstruction

Surgical strategies

Bone graft

Using autologous bone graft (ABG) or synthetic bone substitution to promote healing and fusion remains the simplest form of bone tissue engineering adopted in day to day practice [25]. The ideal graft material should provide three key properties: osteoconduction, osteoinduction and osteogenic capacity [25]. In addition, the graft material should be nonimmunogenic, nontoxic and degrade within an appropriate time frame [26]. Furthermore, in the austere environment, at least within the UK and the National Health Service, cost-effectiveness of new materials is also a pertinent consideration [27]. ABG is often considered the 'gold standard' graft material; however, harvest surgery has risks of operative complications, such as nerve injury, vascular injury, hernia formation and pain. Furthermore, the volume of graft harvested can be limited [28].

In the treatment of tibial nonunion, bone grafting and compression fixation has traditionally been the hallmark treatment of choice [16]. Yet this strategy is not always adequate in the management of CSDs, which require large volumes of bone with mechanical stability. Autologous bone harvest from the iliac crest can yield up to 20–50 cm³ of graft [29]; however, tibial defect volumes can range from 3.1 to 122.0 cm³ [30]. Vascularized bone grafts (typically rib or fibula transfer) are a highly specialized form of ABGs, illustrated in Figure 2. Vascularized bone grafts require microsurgical expertise and are technically demanding procedures, associated with nonunion rates reported from 5 to 23% [31,32], graft fracture rates of 15–20% [32,33] and lack of graft hypertrophy [32]. There are higher rates of complications using vascularized bone graft transfers in the lower limb compared with the upper limb [34], potentially as a consequence of greater load bearing and graft size mismatch, which is less of an issue in the upper limb.

Periosteal free flaps, originally popularized for treating avascular necrosis of the scaphoid by swinging tissue from the distal radius, can also be harvested from the medial femoral condyle, as a further microsurgical technique used in nonunion management. The periosteum and outer cortex is extracted on a vascular pedicle, the graft can then be shaped as required and the vascular supply is inset into the recipient site [35]. However, periosteal free flaps are primarily used for small bone defects <3 cm [36,37], rather than CSDs, and do not offer mechanical support. An

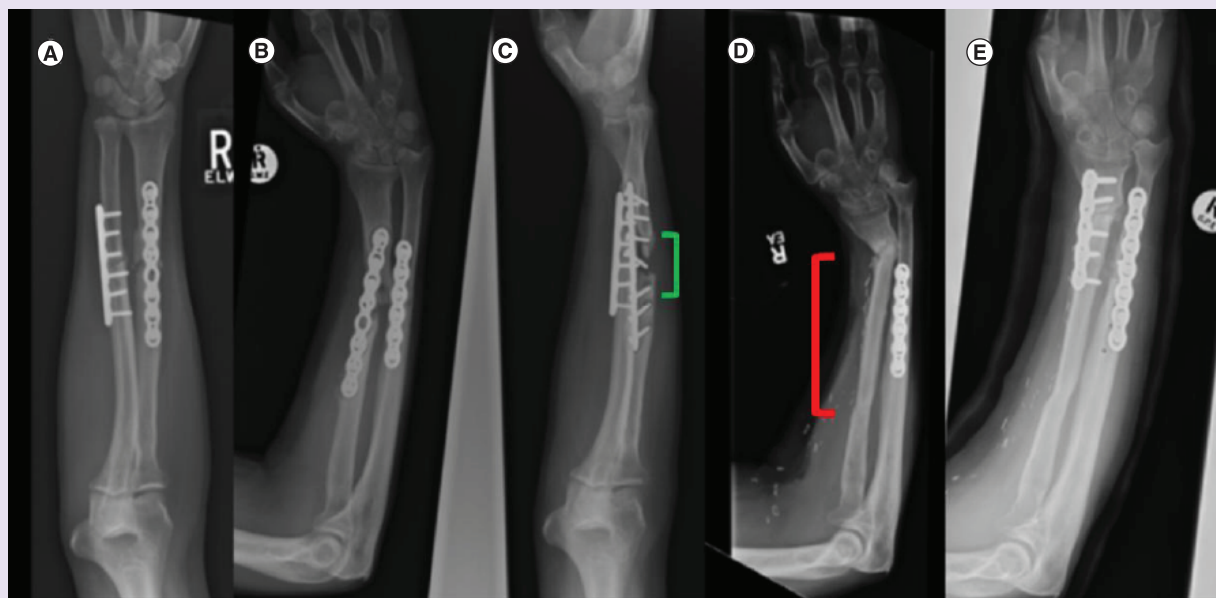


Figure 2. Vascularized fibula bone graft to reconstruct bone defect in the radius. Radiographs showing reconstruction using a fibular graft: (A) radius and ulnar fixation; (B) nonunion and deformity of the radius; (C) bone defect seen on a lateral view (bracket); (D) vascularized fibula graft used to reconstruct the defect (bracket); and (E) residual deformity at distal radius corrected with an osteotomy.

advancement of periosteal free flap has been developed in small animal studies to utilize the regenerative niche of the periosteum to functionalize decellularized bone matrix to prefabricate bone graft on a vascular pedicle that could then be transferred into a recipient site [38]. This technique has similarities to the Masquelet-induced membrane process discussed later.

Reamer-irrigator-aspirator (RIA) techniques have been utilized to harvest larger quantities of ABG from the medullary canal of long bones, as demonstrated in Figure 3. RIA graft has been found to have higher levels of gene expression associated with vascular, skeletal and hematopoietic tissues compared with ABG [39]. Furthermore, the filtrate of the irrigation has been shown to be a rich source of skeletal osteoprogenitor cells [40]. In a randomized control trial comparing ABG to RIA graft for the management of tibial nonunion, comparable results between the groups in the time to union were observed, but with fewer complications and less donor site morbidity in the RIA cohort [41]. However, the architecture of RIA graft is that of a liquid or semisolid [39] and, as such, provides limited mechanical integrity.

Allogenic graft is also widely available, although the manufacturing processes to allow simple storage and prevention of disease transmission requires removal of cellular material. This process also removes any osteoinductive capacity and can affect the mechanical properties. Tissue banks are a vital source of allogenic graft from living donors, for example, femoral head donation following total hip replacement. However, it is estimated that over 48% of potential donors are rejected following medical health screening [42]. Cadaveric samples can also be used; however, the issues regarding screening for infection or communicable disease can become difficult to manage due to the absence of an interval blood test to detect seroconversion postretrieval. Fresh-frozen allografts as well as having storage issues have been found to be potential sources of viral infection [43] and may provoke immunogenic incompatibility (reduced by washing away donor marrow) and thus, are less commonly used.

Distraction osteogenesis

An option that minimizes the need for any graft material in the management of bone tissue loss is distraction osteogenesis (DO). DO was first pioneered by Codivilla in 1905, by means of a surgical osteotomy in combination with skeletal traction to lengthen congenitally short limbs (reviewed in a re-publication in 2008) [44]. DO was further developed by Gavriil Ilizarov, practicing in Western Siberia in post-Soviet Russia, and brought to Europe

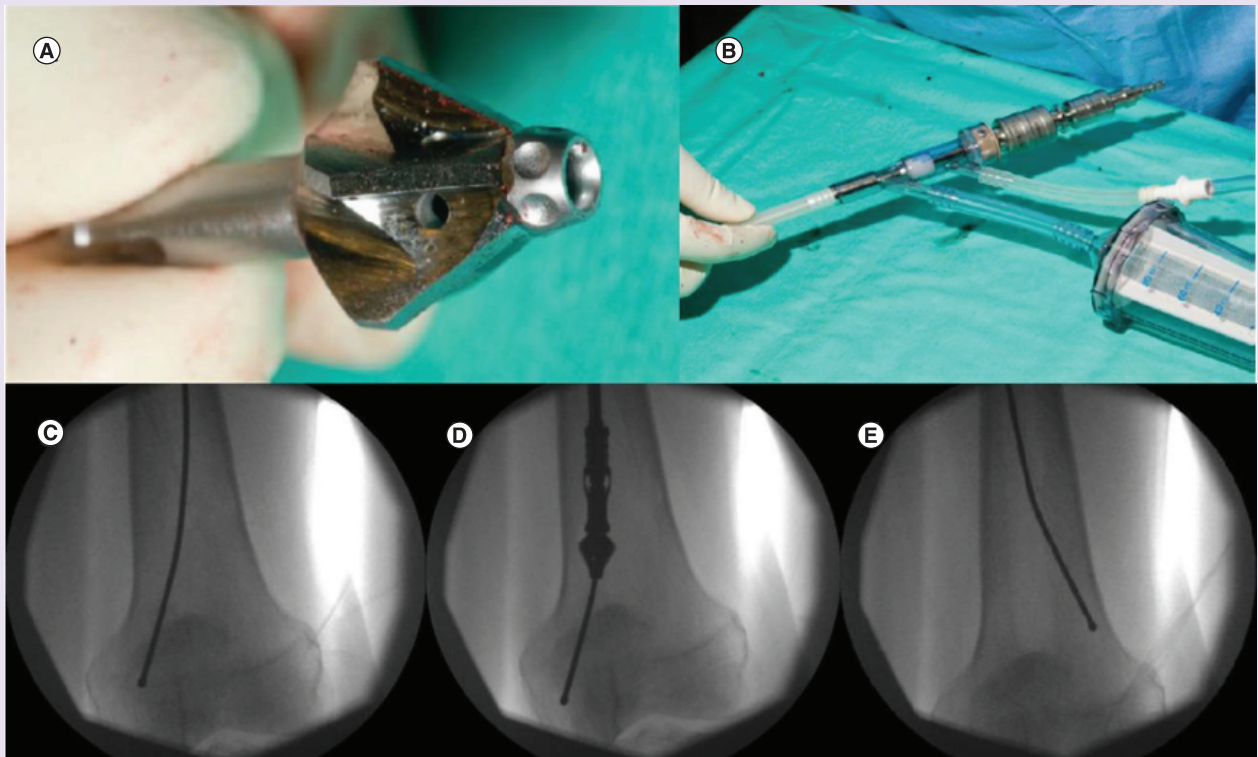


Figure 3. Reamer-irrigator-aspirator system. (A & B) RIA equipment. **(C–E)** Fluoroscopic images demonstrating RIA intramedullary bone graft harvest in the distal femur over a guidewire.
RIA: Reamer-irrigator-aspirator.

in the 1980s. The optimal regimen for bone lengthening or bone transport has been well established: a stable fixation construct, an atraumatic osteotomy, a latent period to initiate the repair process, gradual distraction and a consolidation phase to allow the new bone to mature [45], as illustrated in Figure 4.

DO harnesses the regenerative capacity of bone using a mechanical stimulus and has a number of clinical indications including deformity correction, limb lengthening, management of nonunions and bone defects [46]. The development of reliable bone transport through bifocal DO has reshaped the field of limb reconstruction as large quantities of new vascularized bone, formed locally, can be generated [47]. However, DO and bone transport require lengthy treatment periods. For example, the standard regimen suggests 1 mm per day of transport or lengthening [48] and additional time for bone consolidation. Therefore, this technique requires a highly motivated and compliant patient cohort. Furthermore, DO has a high complication rate, with pin site infection the most common complication, which has been reported in almost every case [49]. Furthermore, docking site nonunions occur in 44% [50], often requiring further surgery with supplemental bone graft. Therefore, adjuncts to accelerate bone formation and consolidation to decrease the length of treatments would be beneficial.

In preclinical models of DO, rhBMP-2 has been used successfully, leading to significantly enhanced bone consolidation in rats [51] and even premature fusion, as the rate of ossification was faster than the distraction protocol [52]. BMP-7 has also been shown to accelerate ossification of the bone regenerate in DO [53], but these studies have not been undertaken in clinical trials. The addition of bone marrow aspirate concentrate (BMAC) and platelet-rich plasma (PRP) in conjunction with DO has been performed in several preclinical studies with varying results; accelerated formation of higher quality bone was observed in a canine study [54], mechanically stronger bone was found in a rabbit study [55], yet no difference was demonstrated in an ovine femoral segmental defect model [56]. In a human study, results showed that although the overall length of time in a circular frame was not reduced, there was significantly enhanced cortical healing time in the BMAC/PRP treatment group permitting

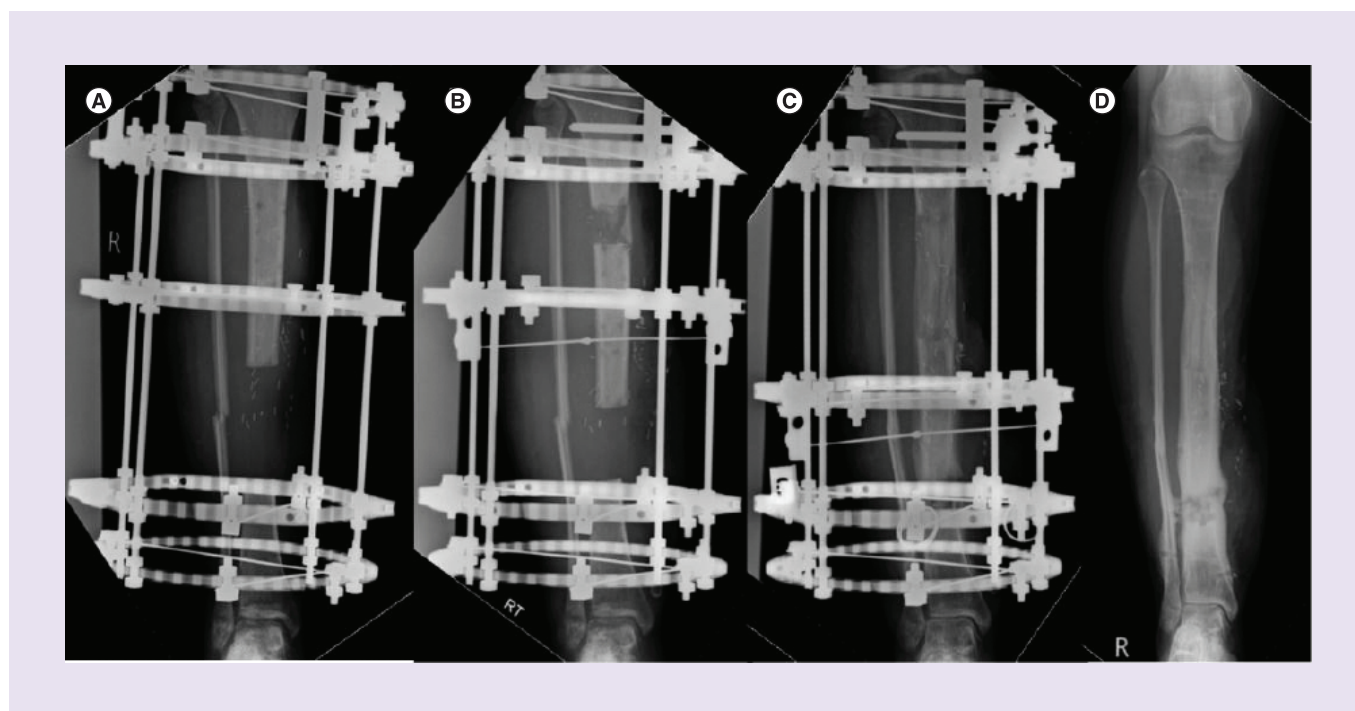


Figure 4. Bone transport through distraction osteogenesis for a tibial defect. Radiographs showing bone transport through distraction osteogenesis: (A) bone defect in the tibia; (B) proximal osteotomy to commence bone transport; (C) regenerate bone filling the defect; and (D) final outcome following removal of the circular frame.

earlier full weight bearing [57], which consequently has the additional benefit of maximizing the positive effects of full weight bearing on bone consolidation [45].

Bone transport over an intramedullary nail using an external fixator is an established technique [58], which has the advantage of reducing the time in a circular frame [59]. The disadvantages of this technique are similar to the disadvantages of treatment in a circular frame alone, which include: failed docking at the end of the transport process requiring further surgery [60]; premature consolidation [61]; and high rates of infection [59]. Importantly, there has been no significant increase in the risk of deep infection using this combination treatment method.

Masquelet technique

The Masquelet technique, or induced membrane technique, was first introduced in 1986 describing the management of diaphyseal bone defects of 4–25 cm [62], see Figure 5. Briefly, the technique involves insertion of a polymethylmethacrylate cement spacer into a bone defect and skeletal fixation, internal or external. The cement spacer provides mechanical stability and also initiates a foreign body reaction resulting in a biologically active vascularized membrane that secretes VEGF, TGF- β 1 and BMP-2 [63] and envelops the cement spacer [64]. Approximately 2 months later, the spacer is removed, while preserving the membrane, and autograft is inserted with concurrent skeletal fixation. The membrane facilitates incorporation of the bone graft to consolidate the bone defect. A meta-analysis of the Masquelet technique reported that successful bone union was achieved in up to 90% of cases; however, bone union was only accomplished following at least one additional surgical procedure in 36.3% of cases [65]. One study reported a mean number of 11.5 surgical interventions to facilitate 86% union rate in the tibia [66]. Overall, several studies have highlighted mixed results following Masquelet and unpredictable time frames to consolidation and bone union [67,68].

There have been several attempts to improve the induced-membrane technique developed by Masquelet. In preclinical studies, the Masquelet technique had been assessed using synthetic scaffolds with the addition of BMP-7 and a systemic bisphosphonate, suggesting improved bone formation when all three were used compared with BMP-7 or scaffold or both [69]. However, in a clinical case series of 11 patients undergoing a Masquelet procedure with the addition of BMP-7, radiolucent changes and areas of bone resorption after 2 months were

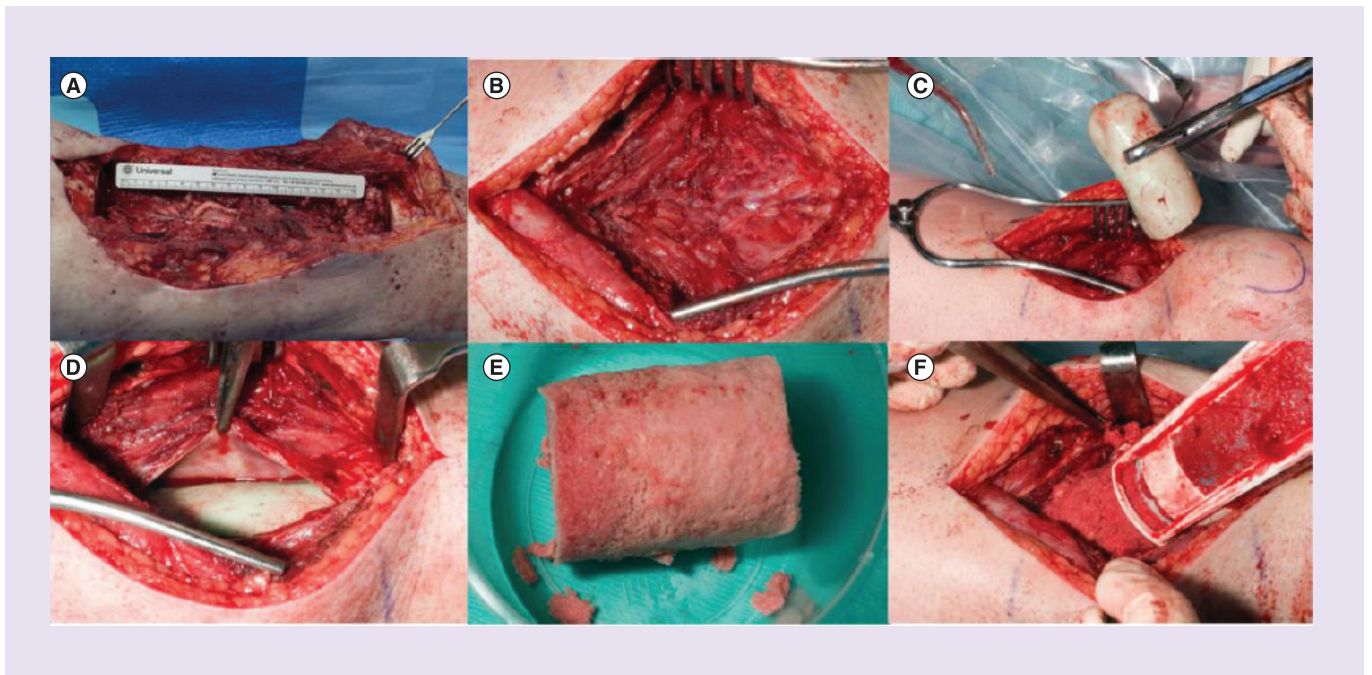


Figure 5. Masquelet technique to reconstruct a tibial defect. Clinical photographs demonstrating Masquelet technique: (A) bone defect > 15 cm; (B) healthy wound following debridement; (C) cement spacer inserted into the defect; (D) biological-induced membrane formed around the cement spacer; (E) autologous bone graft; and (F) autologous bone graft inserted into the defect, contained by the induced membrane.

noted. This finding was markedly different to experiences prior to the introduction of BMP-7 and has led the investigators to abandon the additional application of BMP-7 [63]. Attempts to reduce the volume of cancellous bone graft required during the Masquelet procedure have also been described; a case series using absorbable gelatin sponges in the medullary canal suggested favourable outcomes [70]. As acknowledged by the authors, longer term follow-up is required and additionally a randomized control trial to compare outcomes between this new technique and standard practice would be valuable. A recent retrospective study comparing Ilizarov bone transport to Masquelet technique, for reconstruction of infected bone defects, concluded that Ilizarov methods were superior when deformity correction was required, whereas Masquelet may be preferential in short bones or in periarticular defects [71]. The number of case series reporting on the Masquelet technique are comparatively few, with each center adapting the technique resulting in a combination of different fixation modalities, different bone graft compositions and different postoperative managements leading to heterogeneous patient cohorts that are difficult to compare [68].

Regenerative augmentation

Osteogenic potential: cell-based treatments

Cellular treatment strategies that are used in orthopedics include BMAC, which contains a fraction of mononuclear cells including osteoprogenitor cells and the use of RIA systems that harvest cells from the medullary canal of long bones. Both techniques aim to harvest a population of skeletal stem cells from the bone marrow to provide an osteogenic graft material. Percutaneous BMAC injections have been used for a number of different clinical indications: tibial nonunion [72], osteoarthritis [73], avascular necrosis [74], osteonecrosis [75] and DO [57,76,77]. In a tibial nonunion case series, BMAC injection was used to obtain a union rate of 95%, and an important correlation between higher colony forming unit–fibroblastic count and decreased time to union was demonstrated [72]. All of these studies aim to use the presence of skeletal stem cells to promote enhanced healing and repair; however, as these reports represent small clinical series rather than randomized control trials, their impact, to date, has been limited.

Induced pluripotent stem cells (iPSCs) are derived from adult somatic cells reprogrammed to a pluripotent state [78]. The advantages of iPSCs include a personalized renewable pluripotent cell source without the ethical concerns and restrictions associated with embryonic stem cells [79]. Applications of iPSCs are varied and new concepts continue to

emerge from this developing field, including *in vitro* disease modeling, drug development and organ development, which are all areas of intense research. The ability to reprogram a somatic cell creates the potential for an individual's own cells to be used for patient specific tissue regeneration and individualized treatments, which holds great interest in regenerative medicine. However, there are concerns regarding the immunogenicity of iPSCs [80], as well as potential mutations in gene expression and DNA methylation. It is thought that genetic variations that have been detected in iPSCs are due to the somatic cell itself, rather than the induction process [81]. This raises concern as the patient's cell source may contain unknown mutations that could become harmful in a clonal iPSC population. A handful of clinical cases using iPSCs exist, although, at the time of writing, none related to bone regeneration [82,83].

Autologous blood derived growth factors have also been investigated in isolation, such as PDGF, and in the form of PRP. PRP contains a number of chemotactic and mitogenic growth factors to promote healing (PDGF, IGF, FGF, TGF, VEGF and EGF) [84]. There has been an increase in the number of studies and clinical trials using growth factors and mesenchymal stem cells. However, due to the heterogeneity in study design, cell characterization and reporting measures, it is difficult to draw meaningful conclusions. To rectify this, a suggested checklist has been developed to standardize reporting measures and enable comparisons across studies, which will improve evaluations of their efficacy [85].

Osteoinduction: bone morphogenic proteins

In 1965, Urist discovered a bone autoinductive agent, later termed bone morphogenic protein, crucial in bone healing and development [86]. BMPs enhance recruitment of skeletal progenitor cells and promote osteogenic differentiation by encoding a ligand of the TGF- β superfamily, which subsequently activates the SMAD family transcription factors that regulate gene expression, potentially influencing osteoblastic proliferation [87]. There are two commercially available BMP formulations that are in use in clinical practice. rhBMP-2 (dibotermine alfa; Infuse/InductOs[®] Medtronic BioPharma B.V., Harleen, Netherlands) and rhBMP-7 (OP-1 Implant/OP-1 Putty, Stryker, MI, USA), which are licenced for the management of open tibial diaphyseal fractures and for single level lumbar spinal fusion (www.medicines.org.uk).

Freidlaender *et al.* found comparable results between patients treated with BMP-7 compared with autograft for tibial nonunion [88]. Further encouraging results followed the BESTT study (BMP-2 Evaluation in Surgery for Tibial Trauma), which demonstrated a significant risk reduction by 44% of secondary surgery for nonunion in the treatment cohort using rhBMP-2 in a large study of 450 patients [23]. In a clinical study comparing BMP-7 with PRP application in the treatment of long bone nonunion at various sites, BMP-7 was also found to be superior [89]. There is an on-going need for randomized control trials to evaluate the effect of BMPs as an additive agent in conjunction with other surgical techniques such as grafting with autologous bone. Most of the published literature describes small patient cohorts, without control groups, making it difficult to draw concrete conclusions.

Novel 'off licence' applications of BMPs have been reported as experimental procedures. For example, BMP-7 was applied to bone mineral blocks in conjunction with autologous bone marrow to reconstruct a mandibular defect. The BMP-7 was used to induce heterotopic bone formation within a titanium mesh case which was implanted in a patient's latissimus dorsi for maturation, followed by a subsequent successful free bone-muscle flap [90].

Although there is significant interest in the role of BMPs to augment skeletal repair and fusion, a plethora of adverse effects have been reported, which has led to concern and, subsequently, a more cautious approach to the uptake of BMPs in clinical practice [91]. The adverse effects including life-threatening swelling with airway compromise [92], heterotopic ossification [93] and osteolysis [94]. These side effects are thought to be due to the supraphysiological dose of BMP in the current preparations [91]. Therefore, techniques to localize the target area of the BMP and reduce the dose are currently areas of intense investigation [95] in order to optimize dosage and delivery.

Osteoconduction: matrix & biomaterials

There are vast numbers of extracellular matrix products available commercially, both decellularized and cellularized. Decellularized bone matrix products can be obtained as putties, pastes, granules, powders, gels, sponges and chips, with or without carrier substances such as bovine collagen, according to the clinical scenario and surgical preference. An in-depth discussion of decellularized bone matrix products and the relevant considerations for selecting a product are reviewed elsewhere [96]. Viable allografts or cellularized bone matrix are an interesting development to offer osteogenic, osteoinductive and osteoconductive properties in a graft material. Viable allografts contain mesenchymal

stem cells within an extracellular matrix, some with the addition of angiogenic growth factors. However, the current evidence to evaluate their use is limited to a few small case series and industry funded studies.

Given the disadvantages of autograft and allogenic grafting option, there is a clinical pressure to fabricate suitable engineered biocomposite replacements [97]. Bioactive ceramics, including hydroxyapatite, tricalcium phosphate, bioactive glass and calcium silicate, have all been investigated due to their capacity to promote bony on-growth. Hydroxyapatite is commonly used as a surface coating on orthopedic implants to enhance osseointegration. However, as materials in isolation, they are brittle and not suitable to withstand mechanical load [98], which is vital in the management of CSDs.

Three-dimensional printing of scaffold materials can create reproducible biomimetic scaffolds using a range of different materials including hydrogels, polydimethylsiloxane, polycaprolactone, polyurethane and some metals, such as tantalum or titanium custom implants. There are also exciting developments in the refinement of bioinks enabling 3D cell printing [99–101]. The limitations of additive manufacturing techniques relate to the damage to cells that occurs during the printing process, which affects cell viability and proliferation. An additional issue is the often lack of internal porosity in the materials unless specifically manufactured, which consequently inhibits the delivery of nutrients to the cells. This is increasingly relevant as the size of the material increases, moving cells further away from any nutrient source. Kang *et al.* present a novel technique describing an integrated tissue-organ printer that can fabricate bone, cartilage and skeletal muscle. The printed tissue contained microchannels to facilitate nutrient diffusion, which may contribute to their high cell viability (91%) and their observation of neoangiogenesis around the printed tissue *in vivo* [102]. Although these approaches hold tremendous potential for the future, to date, studies are limited to *in vitro* and small animal *in vivo* investigations. As such, additive manufacturing applications are still some years from routine clinical translation as patient treatments.

Conclusion

Bone tissue loss remains a significant clinical challenge in orthopedics, impacting on a range of clinical scenarios including trauma, limb reconstruction, tumor excision and revision arthroplasty [97]. It is recognized that for optimal bone formation and bone healing four key elements, as highlighted by The Diamond Concept are pivotal: an osteoconductive scaffold; osteogenic cells; growth factors; and an appropriate mechanical environment [5]. This principle is applicable from simple fracture healing through to complex limb reconstruction strategies. A challenge within the field is a lack of robust clinical evidence in the form of randomized control trials, to draw firm conclusions with regard to specific treatment strategies for lower limb reconstruction. Critically, much of the reported literature relates to small case series, case reports and observational studies, which is understandable, given the clinical cases are rare [103]. Furthermore, the management strategies utilized often involve highly specialized procedures and usually a surgeon has preference for one particular technique due to their training exposure or surgical ethos. Thus, it remains difficult to directly compare the outcomes for each technique as there are a number of variables in play. Consequently, each patient case requires an individualistic approach rather than standardization of care.

There are several avenues of research within regenerative medicine that offer significant value to lower limb reconstruction surgery. The enhanced delivery of osteoinductive molecules within a biomimetic scaffold to improve target localization and clinical efficacy as well enhanced stem cell strategies are at the forefront for clinical translation. Furthermore, the field of orthopedics is receptive to emerging computer-based technologies, with the development of computer aided design and computer aided manufacturing to fabricate patient-specific implants, as well as custom designed instrumentation and 3D models to facilitate pre-operative planning in complex clinical cases [104–106]. Bespoke titanium cages have been 3D printed to successfully salvage nonreconstructable defects [106,107]. Individualized 3D printed scaffold materials combined with bone grafting have already been successfully utilized in titanium constructs [107,108]. Progression of this technique using patient-tailored cellular printing methods to engineer patient-specific grafts custom designed for a specific bone defect with the capacity to provide a mechanically stable environment offer exciting new vistas to address central elements of limb reconstruction and advance the future of limb reconstruction.

Future perspective

Bone tissue loss in orthopedics is a complex clinical challenge. Bone tissue is a vascularized osteogenic environment which also provides mechanical support. Currently, there is no single engineered material that addresses all these various elements. Combined tissue engineering strategies utilizing 3D-printed biomaterials primed with osteogenic stem and progenitor cells are an exciting prospect as these offer innovative reproducible biomaterials with the

capacity to integrate with the host tissue. However, it is essential that any biomaterial is developed with attention to the mechanical functionality if they are to be successfully translated into clinical practice, particularly in the management of CSDs.

Lower limb reconstruction cases are rare, each with different challenges in terms of biological, mechanical and host issues. Individualized treatment strategies are required to tackle each case and this is likely to extend into the future with personalized iPSC therapy to deliver the host's own reprogrammed stem cells to enhance the healing potential. Further extrapolation of individualized medicine would be through genomic profiling enabling gene targeted therapies in patients with poor biological potential.

Executive summary

Clinical need

- Trauma to a limb can result in soft tissue and bone loss.
- Reconstruction techniques have been developed to preserve limbs and avoid amputation.
- Regenerative medicine strategies are needed to augment surgical techniques and enhance bone healing.

Lower limb reconstruction surgeries

- Bone grafting and skeletal fixation is often the hallmark procedure; however, large bone defects may not be amenable to this approach.
- Distraction osteogenesis uses mechanical stress to promote bone formation over a prolonged period of time and has a high incidence of complication.
- Induced membrane Masquelet technique has shown promising outcomes; however, Masquelet requires a two-stage operation and outcomes are not reliable.

Regenerative medicine strategies

- Stem cell and growth factor therapies are gaining popularity but need standardized reporting measures to enable critical evaluation in clinical studies.
- Bone morphogenetic proteins have been implemented in clinical practice to induce bone healing; however, off licence use has led to serious adverse side effects.
- Biomaterials encompass a range of scaffolds and products for bone tissue engineering. None as yet provide the ideal solution for limb reconstruction; however, 3D-printed reproducible biomimetic scaffolds with osteoinductive agents are promising.

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