Recent developments in biomaterials for long-bone segmental defect reconstruction: A narrative overview

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**Keywords:** Allograft, Artificial material, Biomaterial, Long-bone segmental defect reconstruction, Tissue engineering

**ABSTRACT**

Reconstruction of long-bone segmental defects (LBSDs) has been one of the biggest challenges in orthopaedics. Biomaterials for the reconstruction are required to be strong, osteoinductive, osteoconductive, and allowing for fast angiogenesis, without causing any immune rejection or disease transmission. There are four main types of biomaterials including autograft, allograft, artificial material, and tissue-engineered bone. Remarkable progress has been made in LBSD reconstruction biomaterials in the last ten years. The translational potential of this article: Our aim is to summarize recent developments in the divided four biomaterials utilized in the LBSD reconstruction to provide the clinicians with new information and comprehension from the biomaterial point of view.

**Background**

Long-bone segmental defects (LBSDs) are defined as the bone loss in length longer than one and half of the long-bone diameter, or longer than one-fifth to one-fourth of the long-bone length. LBSDs have always been a great challenge in orthopaedics. Congenital bone disease, arthritis, osteomyelitis, bone nonunion, bone exposure, trauma, and bone tumour excision can all be accompanied with an LBSD. An LBSD exists in any type of long-bone at any location, facing differing requirements for biomaterials applied in LBSD reconstruction.

In general, there are natural (bone, cartilage, corals, etc.) and synthetic (e.g., metallic, polymeric, ceramic, composite) biomaterials for the reconstruction: A narrative overview.
BMP-4), vascular endothelial growth factor (VEGF), basic activating factors including bone morphogenetic protein-2 and 4 (BMP-2 and BMP-4). After that, bone remodelling and angiogenesis starts, with the necrotic tissue disrupted, leading to acute necrosis and hypoxia, forming a haematoma. Osteoprogenitor and mesenchymal cells are recruited at the defect by the activated factors, e.g., growth factors and cytokines. Mesenchymal cells migrate to the defect region, and capillaries grow into the region from bone marrow and endosteum. Then, the granulation tissue is replaced by fibrocartilage, and an external callus is formed by the periosteum, which is mineralized from the inside to woven bone. After that, bone remodelling and angiogenesis starts, with the necrotic bone removed and the fracture callus replaced by lamellar bone [8–10]. The foregoing process is believed to be associated with quite a few activating factors including bone morphogenetic protein-2 and 4 (BMP-2 & BMP-4), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and fibroblast growth factor-2 (FGF-2). Among these, the VEGF is expressed in angioblasts, chondroblasts, chondrocytes, osteoprogenitor cells, and osteoblasts. VEGF can act with soluble receptor activator for nuclear factor-κ B ligand (sRANKL) to promote osteoclastogenesis and act as a substitute for a macrophage colony-stimulating factor (M-CSF) in osteoclastogenesis [11–13]. Fibroblast growth factor-2 is able to stimulate angiogenesis and the proliferation and differentiation of osteoblasts [14,15].

Testing methods for the LBSD reconstruction biomaterials

In vivo tests and clinical reporting (and various reviews) are frequently carried out in the research of LBSD reconstruction biomaterials. In animal tests, LBSDs are created in rabbits, dogs, sheep, goats, and pigs. Each animal type shows both advantages and disadvantages, while sheep are most frequently used large animal models to study LBSD reconstruction. Such a biomembrane could help to preclude resorption of the autograft and secrete growth factors. This biomembrane can also be applied in an LBSD reconstruction with other material types including allografts and artificial materials [27,28].

Based on the theory that mesenchymal stromal stem cells can produce fibroblastic cell lines with ossifying properties, the technique of percutaneous autologous bone marrow injection was newly applied to treat delayed and nonunion of long bones. This percutaneous injection technique is much less invasive and with much less morbidity than the traditional corticocancellous bone graft. The 8-year clinical follow-up of 45 cases (26 tibias, 16 femurs, and 3 humeri) of long-bone nonunion showed 69% healed tibiae and 63% healed femurs [29]. In another report, autologous bone marrow was injected in combination with an allograft decalcified bone matrix (DBM) in long-bone nonunions, and the union was attained after an average period of 8.1 months with a range of 2 months to 3 years [30]. In a similar report, 92% of patients showed union after a mean healing time of 15 ± 2.73 weeks (range: 12–22 weeks) [31]. This less-invasive percutaneous injection of osteogenic factors or medicine might be a developing direction for LBSD of a relatively small size.

Biomaterial types and recent developments

In the current narrative overview, we are concentrating on recent developments published from 2010 to 2018 in LBSD reconstruction biomaterials retrieved from the literature in PubMed and PubMed Central (summarized in Table 1).

Autograft

An autograft is a natural biomaterial that has always been the gold standard for the LBSD reconstruction. Autogenous bones exhibit superior osteoinductivity, osteoconductivity, and osteogenesis compared with other types of materials. Fast healing and avoidance of immune rejection are also the advantages of autografts. Vascularized autografts have showed good results in reconstructing an LBSD, frequently with a higher fusion rate than the nonvascularized autografts [18–20]. Normally, autologous cortical bone grafts are adopted for segmental defects of 5–6 cm in length, while vascularized cortical autografts are applied for defects longer than 6 cm [21] or in case of severe loss of vascularized soft tissue [22–24]. However, disadvantages do exist including limited availability of graft material, risk of comorbidity, insufficient integration into the damaged bone, prolonged anaesthetic periods, donor site morbidity, and predisposition to failure. Common sources for autografts are ilia, fibulae, and ribs, which are always low in strength and limit the application of autograft mostly to the upper limb with relatively low load-bearing needs [25].

Recent reports on autografts in the LBSD reconstruction show the trend to incorporate bioactive factors into autografts to promote osseointegration. For example, the combination of plateletrich plasma with autologous cancellous bone graft could improve bone healing compared with the sole graft of autologous bone [26]. One report described a technique using a biomembrane forming around the previously placed antibiotic spacer and the biomembrane could enclose the later placed cancellous autograft in the LBSD reconstruction. Such a biomembrane could help to prevent resorption of the autograft and secrete growth factors. This biomembrane can also be applied in an LBSD reconstruction with other material types including allografts and artificial materials [27,28].
Allograft

An allograft overcomes the limit of grafting resource accompanied with an autograft but brings concerns about rejection, disease transmission, delayed union, or nonunion. A nonunion situation is mostly due to the sterilization process before the allograft [32]. Common pre-treatment on xenogenic bones before usage include freezing, freeze-drying, chemical sterilization, irradiation, and decalcification. It is worth to notice that none of the aforementioned treatments can fully prevent immune rejection, while the antiinfection at the reconstruction site can be weak and antibiotics need to be applied. Some research studies have tested the effects of supercritical CO to degrease and sterilize bone allograft [33]. Treatments such as deproteinizing the allograft bone. This magnesium ion-activated xenogenic bone showed potential for faster union with an LBSD allograft [38]. Other bio-activating factors/cells include autologous concentrated bone marrow–derived cells [39], mesenchymal stem cells, osteogenic protein-1 [40], recombinant vascular endothelial growth factor-A (rVEGF-A) [41], and recombinant human bone morphogenetic protein-2 (rhBMP-2) [42], all showing positive healing results.

To sum up, recent developments in the LBSD allograft concentrate on overcoming some existing problems, especially delayed union or nonunion. In particular, combining the allograft with other more biocompatible materials such as an autograft or an osteocutaneous flap can promote biological incorporation. On the other hand, attempts such as incorporating bioactivating factors/cells to the xenogenic bones could enhance osteointegration to improve the therapeutic efficiency without destroying the original microstructure or compromising the mechanical properties of allografts.

Artificial materials

Recently, various artificial materials have become a hot research spot in LBSD treatments. Artificial materials can be designed, fine-tuned, and fabricated targeting at the intended clinical use, combining desired properties from each component. An artificial biomaterial could consist of bone (autogenous or allogenous), metal, ceramic (tricalcium phosphate, hydroxyapatite), polymer, hydrogel (alginate, chitosan), collagen, and silk fibroin. Problems that may accompany artificial materials in this LBSD reconstruction include insufficient bioactivity (such as insufficient osteocutaneous flap to mechanically enhance the implantation and achieve biological incorporation. The 9 lower-limb reconstruction amputated with partial weight-bearing at an average of 4.2 months and amputated with full weight-bearing at an average of 8.2 months [37].

Bioactivating the allograft bone with some bioactive factors/cells and thereby enhancing union is another research direction in an LBSD allografts. In a study by Wang et al. [38], coating xenogenic bone with magnesium plasma could upregulate the alkaline phosphatase (ALP) gene activity and viability of human tubal mesenchymal stem cells (hMSCs), while it maintained the original mechanical properties of an allograft bone. This magnesium ion-activated xenogenic bone showed potential for faster union with an LBSD allograft [38]. Other bioactivating factors/cells include autologous concentrated bone marrow–derived cells [39], mesenchymal stem cells, osteogenic protein-1 [40], recombinant vascular endothelial growth factor-A (rVEGF-A) [41], and recombinant human bone morphogenetic protein-2 (rhBMP-2) [42], all showing positive healing results.

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osteointegrative response, osteoconductivity, and osteogenesis), incomplete or even no degradation, too fast degradation before new bone consolidation, immune rejection, and possible adverse effects to cells. Given these, recent studies have concentrated on inducing bioactivity to the artificial material, in addition to maintaining its satisfying mechanical strength and durability. The components capable of inducing bioactivity include autograft, allograft, DBM, etc.

A clinical research study used a cylindrical titanium mesh cage, combined with cancellous bone allograft and DBM putty, to restore the LBSD. The cage held the cancellous bone and the bone matrix in place during therapy and provided mechanical support to a certain degree. The one-year follow-up showed satisfying limb alignment, stability, and bone healing. Immediate full weight-bearing was initiated, and early limb functional recovery was achieved [43].

More recently, poly(α-lactide)-tricalcium phosphate (TCP)–poly-caprolactone (PCL) and mPCL-TCP composite biomaterials were reported. These composites mimicked the inner microstructure of cancellous bones and allowed for coagulating blood retention and new bone ingrowth (Fig. 1). They can withstand the physiological cancellous bones and allowed for coagulating blood retention and were ported. These composites mimicked the inner microstructure of cancellous bones and allowed for coagulating blood retention and new bone ingrowth (Fig. 1). They can withstand the physiological cancellous bones and allowed for coagulating blood retention and new bone ingrowth [43].

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There have been a large number of studies on artificial materials for the LBSD reconstruction since 2010. Such biomaterials include oxidized-irradiated alginate hydrogel [45], β-tricalcium phosphate (β-TCP) combined with cancellous autograft [46], DBM plus autogenous bone [47], hydroxyapatite–DBM (allograft) composite [48], β-TCP plus collagen composite [49], chitosan hydrogel [50], porous poly(lactic acid) (PLA)/DBM composite [51,52], and bone-like hydroxyapatite/polyamino acid composite [53]. All the aforementioned artificial materials were assessed for their capability in LBSD reconstruction, and the results were promising.

Attention should be paid to the fact that the material may interfere with cellular processes, e.g., affecting bone formation. For instance, a study described the artificial biomaterial of a porous propylene fumarate (PPF) sleeve surrounding a solid porous propylene fumarate intramedullary rod for mechanical support. The results indicated that this structure, especially the nonporous intramedullary rod, may decrease bone formation, possibly because of its hindering effects [54].

Tissue-engineered bone

Tissue engineering in the LBSD reconstruction requires a cell source to amplify and build the new bone tissue and a scaffold to hold and protect the cells. In addition, the reconstruction should allow nutrients and metabolic wastes to diffuse through and degrade in the end [55]. Osteoblasts, exogenous stem/progenitor cells, and mesenchymal stem cells, especially bone marrow–derived mesenchymal stem cells and adipose-derived mesenchymal stem cells, are most frequently seeded in biocompatible and biodegradable scaffolds to build the tissue engineering reconstruction. Bone marrow, periosteum, fat, muscle, cord blood, and embryonic or induced pluripotent stem cells can be a cell source for bone tissue engineering [55–59]. In addition, growth factors, such as hepatocyte growth factor and rhBMP-2, can be incorporated to stimulate cell differentiation and amplification. Hydroxyapatite, TCP, polyethylene, collagen, chondroitin sulphate, calcium silicate, medical-grade polycaprolactone, silk, hydroxyapatite, chitosan, poly(l-lactide-co-D,L-lactide), polyglycolide, autologous bone graft, alumina, baghdadite (Ca₃ZrSi₂O₈), bioactive glass nanoparticles, and their combinations have been intensively studied as biocompatible scaffold materials [60–64]. Tissue engineering was demonstrated to be possible for the first time in 1930s, and since ca. 2010, growing amounts of research have been conducted on tissue engineering in LBSD reconstruction. A novel selective cell retention technology has been introduced in 2010: It allows for materials enriched with osteoprogenitors exerted from autologous bone marrow to substitute the traditional autografts [65]. This could be an attractive progress in the tissue engineering technique.

Ng et al [66] applied autologous mesenchymal stem cells (MSCs) and plasma-derived fibrin-impregnated ceramic block (combining ceramic block with osteogenic-induced mesenchymal stem cells and platelet-rich plasma, abbr. “TEB”) to restore segmental load-bearing bone defects. In the implanted composite, differentiated MSCs can express osteogenic genes and mineralize within the scaffold, while the plasma is a rich source of growth factors and the plasma-derived fibrin could promote osteogenic differentiation of MSCs in vitro and enhance osteogenesis in vivo. This in vivo study was conducted in rabbit tibias. Compared with the rabbits without implantation or implanted with fresh marrow—impregnated ceramic block and partially demineralized allogeneic bone block, the group implanted with TEB restored normal gait pattern faster, achieved the union faster, scored higher new bone percentage, and showed higher compressive strength (Figs. 2 and 3) [66].

In a study by Wang et al. [67], DBM scaffolds were seeded with expanded rabbit foetal bone marrow–derived mesenchymal stem cells

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**Figure 1.** Micro-CT 3D reconstructions of (A) a PDLLA-TCP-PCL and (B) mPCL-TCP composite (height 20 mm, diameter 18 mm). (C) Compressive stiffness values averaged 446 N/mm (SD = 66.3) for mPCL-TCP and 418 N/mm for PDLLA-TCP-PCL (SD = 88.1) scaffolds; (D) the elastic modulus 22.17 MPa (SD = 3.0) and 24.70 MPa (SD = 3.3); (E) Porosity was determined to be 70.55% for mPCL-TCP (SD = 3.78) composites and 43.76% for PDLLA-TCP-PCL (SD = 10.02) composites as determined by micro-CT analysis. Error bars represent standard deviations, n = 6 (Reichert et al., 2011) (No color used in print. Two-column fitting image.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) PCL = polycaprolactone; PDLLA = poly(ε-lactide); TCP = tricalcium phosphate;
(BMSCs) and cultivated in osteogenic media in vitro. The BMSC/DBM constructs were implanted in the prepared radial defects in rabbits and were compared with the groups implanted with DBM scaffolds alone. Significantly more new bone tissue was observed in the BMSC/DBM group.

In another study [68], β-TCP composite with rabbit adipose-derived mesenchymal stem cells (rADSCs) was constructed through tissue engineering. The cultivated composite biomaterial was then inserted in the 2-cm bone defect of radius in the middle and lower level in rabbits. Two control groups were treated with no implantation or implanted with solely β-TCP. After implantation, the rADSCs showed the potential to differentiate into osteoblast without provoking an immune rejection. The X-ray results on the 8th week showed superior healing situation in the rADSCs/β-TCP group compared with the control groups, with the bone connectivity and bone marrow cavity completely recovered. Six weeks after surgery, the rADSCs/β-TCP implant began to degrade and new bone growth could be observed in pores left after degradation. The degradation was significant 8 weeks after surgery, accompanied with recanalization of the bone marrow. In a similar study [69], allogenic adipose-derived stem cells (ADSCs) were combined with heterogeneous deproteinized bone (HDB) to repair segmental radial defects and obtained more desirable results compared with the heterogeneous deproteinized bone restored defects. In a clinical trial, the human autologous ADSCs were supplemented with the DBM and the autologous adipose-derived stem cell could fully differentiate into a 3D osteogenic-like implant [70].

One study compared the healing effects with a polycaprolactone–TCP composite material seeded with autologous BMSCs or rhBMP-7 and the gold standard autograft, all inserted in critical-sized tibial defects in sheep. Twelve months after surgery, the scaffold with rhBMP-7 showed...
Figure 3. Histological sections from the middle segment of the implants three months after implantation (H&E) (TEB: combining ceramic block with osteogenic-induced mesenchymal stem cells and platelet-rich plasma; MIC: fresh marrow-impregnated ceramic block; ALLO: partially demineralized allogeneic bone block) (A) Abundant new bone was found in TEB. The section reveals new bones (Nb) forming a trabecular network amidst infiltrated cells (Ic) while new compact bone (Nb) was found at the right periphery (40x). (B) Here, the peripheral bone appeared more mature with lamellar and osteon features (O) adjacent to the well-formed intramedullary canal filled with marrow element (Me) (100x). (C) Residual ceramic (Ce) was noted in MIC. Mineral deposits (Mi) (stained red) were seen around the ceramic (40x). (D) The section reveals new bones (Nb) that are undergoing mineralization amidst infiltrated marrow element (Me) (40x). (E) Significant fibrous tissues (Fb) were noted in ALLO. The section reveals new bones (Nb) forming a trabecular network amidst infiltrated cells (Ic) (40x). (F) An intact allograft bone (Allo) (100x) (Ng et al., 2014, Open Access) (Color used in print. Single-column fitting image.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
superior healing results compared with the autograft gold standard. The MSC-seeded biomaterial construct, with significantly more bone formation, higher strength, and more even axial bone distribution at the interface. The results suggested that rhBMP-7 is more bioactive than the MSCs in osteogenesis and bone remodelling in in vivo circumstances [71].

Moreover, a 3D scaffold of silk fibroin/chitosan/nano-hydroxyapatite (SF/CS/nHA) seeded with BMSCs [72], autologous MSCs plus rhBMP-2 incorporated in deproteinized bone [73], and adipose-derived stem cells seeded in hybrid baculovirus (BV) have been introduced to restore LBSDs. The biomechanical properties were shown to be comparable with living bones and autologous bones [74].

Compared and contrasted to the foregoing three biomaterials involving autograft, allograft, and artificial material, tissue-engineered bone is a relatively new biomaterial applied in the LBSD reconstruction. Most research studies of tissue engineering for LBSD reconstructions are still experiments. However, much progress has been achieved and bone tissue engineering has consolidated its role as a promising technique and a research hot spot in the LBSD restoration, giving much hope to patients.

Conclusions

This narrative overview has provided an insight into the most developments in biomaterials applied in the LBSD reconstruction since 2010. The previously described four biomaterial types, namely autograft, allograft, artificial material, and tissue-engineered bone, are overlapping. For example, the scaffold for tissue engineering is frequently an artificial biomaterial seeded with MSCs, and an artificial biomaterial can consist of autograft or allograft. Developments in one type of the biomaterials can simultaneously bring novel techniques to other biomaterials. Currently, autografts are still the gold standard for the LBSD reconstruction, because of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects. The usage of allografts has become more and more reliable. That said, an allograft is also an invaluable source of their satisfactory healing effects.

Future research in the LBSD reconstruction biomaterials may rely mainly on artificial biomaterials and bone tissue engineering, which possess flexibility in the material design and fabrication and may satisfy the requirements for the LBSD reconstruction to the maximum degree. In these said two fields, more work should definitely be carried out and more progress can be expected. Moreover, attaining a “perfect” LBSD reconstruction may not rely on just a single biomaterial type, but the union of two or more of them can be a wise choice.

Conflicts of interest statement

The author(s) have no conflicts of interest relevant to this article.

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