Journal of
Musculoskeletal Disorders and Treatment

REVIEW ARTICLE

Survey of Current and Prospective Approaches in Bone Grafting Technology

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Abstract

It is estimated that more than 500,000 bone grafting surgeries occur annually in the US to repair or replace defects. Significant progress has been made in this field in recent years, thus making it opportune to survey the technologies currently available and highlight promising future strategies. Here, we offer a timely summarization of the field separated into three areas: Autografts-where bone tissue is harvested from the patient; allografts- taken from cadavers or animals; and intelligently-engineered bone graft substitutes. Additionally, we view innovative strategies to augment bone grafting, namely 3D printing, incorporation of diverse stem cell populations or growth factors, and engineered biomaterial scaffolds. Our work serves to orient the reader to the field of bone grafting and will assist clinicians and scientists in choosing and/or designing appropriate treatment strategies for patients requiring bone grafts.

Keywords

Bone graft, Osteoinductive, Osteoconductive, Osteogenic, Mesenchymal stems cells

Introduction

Bone organs have a remarkable capacity for self-healing and this ability is highly conserved across evolution. Some bone defects, however, heal poorly due an individual's health status or by exceeding a critical size and require intervention for successful repair, such as the placement of a bone graft [1]. It is estimated that more than 500,000 bone grafting surgeries occur annually in the US to repair or replace defects caused by trauma, tumor resection, pathological degeneration and con-

genital malformations [2].

The process of graft incorporation is akin to natural fracture healing and is reminiscent of endochondral ossification that occurs during embryonic bone development [3]. The steps are generally outlined as i) Inflammation, ii) Formation of a cartilaginous callus, iii) Formation of a bony callus, and iv) Architectural remodelling [3]. During the initial inflammatory phase, signaling molecules such as platelet-derived growth factor (PDGF) initiate revascularization by promoting infiltration of small perforating arteries into the site. Revascularization allows for the delivery of nutrients and growth factors that promote differentiation of osteochondral progenitor cells into cartilage-secreting chondrocytes, which form a cartilaginous callus that stabilizes the bone fragments [4]. In contrast, defects repaired through absolute stability fixation may bypass cartilage formation and heal via intramembranous ossification to directly from laminar bone. The cartilaginous callus is subsequently replaced by bone matrix via the action of osteoblasts and osteoclasts and, over time, the callus is remodeled into a shape that generally resembles the original shape of the bone organ [3].

Significant progress has been made in recent years to increase the technologies available for treating bone defects. Choosing a successful type of graft depends on numerous factors such as defect size, the mechanical stresses of the defect site, patient health, graft availability, and cost [5,6]. Available grafting technologies may

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Table 1: Summary of select current and prospective technologies for bone grafting applications.

Materials	Living bone (e.g., autograft)
	Non-living bone (e.g., allograft)
	Scaffold
	- Natural polymer (e.g., collagen, chitosan, alginate)
	- Synthetic polymer (e.g., polycaprolactone triol)
	- Bioactive ceramic (e.g., tricalcium phosphate)
	- Bioactive glass
	- Hydrogels
	- Metals (e.g., steel, gold, titanium)
Cells	Bone marrow-derived mesenchymal stem cells
	Adipose-derived mesenchymal stem cells
	Induced pluripotent stem cells
	Others
Biologics	Bone morphogenetic protein 2 (BMP2)
	Bone morphogenetic protein 7 (BMP7, OP1)
	Fibroblast growth factor 2 (FGF2)
	Vascular endothelial growth factor (VEGF)
	Platelet-rich plasma (PRP)

be categorized into autografts, where the graft is taken from the recipient; allografts, where the graft is from a donor, or engineered grafts which range from modified natural scaffolds to fully synthetic bone substitutes. These technologies have a range of characteristics that make them suited to a particular application and are generally described in terms of osteoconductive and/or osteoinductive properties, which relate to a graft's ability to serve as a scaffold for bone repair or to induce stem cells to differentiate into mature bone cells, respectively [7]. These properties may be conceptualized hierarchically. First, a graft's osteoconductive properties lay the groundwork for bone healing to occur. The internal architecture of the graft must be porous enough to encourage the infiltration of vasculature and stem cells but strong enough to withstand the mechanical forces required of bone. Since natural sources of bone (autograft and allograft) already have the biological scaffold needed to promote the later steps of graft union they are typically highly osteoconductive [6]. Artificial scaffolds attempt to imitate the natural bone but often struggle to accurately mimic the architecture necessary for the later stages of bone healing [8]. Second, a graft's osteoinductive properties are largely related to growth factors that promote recruitment or differentiation of progenitor cells. While this is characteristic of autografts, growth factors may be incorporated into engineered or synthetic graft substitutes to increase their osteoinductivity [5,9].

The autograft has repeatedly proven to be the gold standard for repairing critical sized bone defects and defects incurred as a result of certain bone diseases [10]. Autografts are the first choice in most cases due to their superior osteoconductive, osteoinductive, and osteogenic properties [10]. Additionally, they have no antigenicity and therefore do not induce an immune response and carry no risk of viral transmission [5]. However, since autografts must be harvested from

the patient (iliac crest, tibia, distal radius), donor site morbidities and systemic disease can limit its use in situations where the patient is not healthy enough for a second operation [11]. In such cases, allografts, which are typically harvested from a cadaver, may be useful. That said, allografts are osteoconductive but tend to lack sufficient cell viability to be highly osteoinductive or osteogenic. For these reasons, their recovery times tend to be slower, and their union rates are lower [10]. Furthermore, since they typically come from a donor via a bone bank, allografts may also come with a risk, albeit minimal, of disease transmission [12]. Due to these reasons and others, tissue engineering has explored the use of growth factors, diverse cell type with osteogenic potential, natural and synthetic scaffolding materials, and numerous combinations thereof to provide alternative grafting technologies (Table 1). The field of engineered bone grafting technologies is vast and rapidly advancing; therefore we feel it is opportune to survey the strategies currently available and orient clinicians, engineers, and basic scientists to particularly promising future strategies. Where appropriate, we direct the reader to comprehensive reviews of particular topics beyond the scope of the present survey. In the following sections, we organize the field hierarchically, first detailing scaffolding materials then describing results of incorporating relevant cell types and/or growth factors.

Scaffolding Materials

A successful bone grafting scaffold must achieve a balance of three main characteristics: osteoconductivity, mechanical strength, and the ability to be resorbed. That is, a material must possess sufficient mechanical strength to meet the durability requirements of bone while also providing a structure porous enough to allow the infiltration of small perforating arteries. These arteries provide an avenue for the movement of the MSCs and growth factors that are crucial to graft incorporation [13]. Finally, an ideal scaffold would be remodeled and replaced with native bone over time. Thus, the material cannot possess metabolites which are toxic or which adversely affect the balance of new bone formation. The major types of scaffolds which are currently in use are: i) Natural or synthetic polymers, ii) Bioactive ceramics and glass, iii) Hydrogels, and iv) Metals. While each will be surveyed individually below, it should be noted that many strategies employ a combination of these materials- termed "composites" or "hybrids" - in an attempt to mitigate their individual limitations. For more comprehensive information on this topic, the reader is directed to several extensive reviews of bone scaffold materials and the biomechanical/biophysical constraints thereof [14-16].

Polymers

Polymer scaffolds can be either natural or synthetic in origin. Both consist of a repeating molecular sequence of proteins or polysaccharides. The natural polymers

used for bone grafting tend to have greater overall biocompatibility. Synthetic polymers, on the other hand, are more versatile, more readily availability, and can be better manipulated for specific situational needs.

Collagen is the most popular biologic material used in the production of natural polymer tissue grafts [5]. It is a popular choice due to its high abundance, easy processing, and its ability to be combined with other materials [17]. It is also a highly porous, endogenous compound, which lends to its high degree of biocompatibility; i.e. it is readily resorbed with low antigenicity [17]. The major drawbacks of collagen matrices are its high cost of manufacturing, *in-vivo* swelling (due to its hydrophilicity), and poor mechanical strength when used alone and not combined with other substrates [17]. Collagen is used in many different forms to facilitate grafting. A recent review focusing on collagen categorized grafts into the following: Injectable gels, membranes and films, sponges, scaffolds, microspheres, and nanospheres [17]. Collagen gels and films are generally not used not to improve mechanical strength, but instead for their osteoinductivity, osteoconductivity, and biocompatibility characteristics. Collagen's ability to enhance osteogenesis is, at least in part, due to its unique hexapeptide sequence GFOGER. Moreover, simply coating a polymeric scaffold with this collagen sequence has been shown to result in significant acceleration and production of bone in previously non-healed rat femoral defects [18]. The observed acceleration of osteoblastic differentiation is independent of exogenous cells and growth factor impregnation and is instead dependent on the binding of $\alpha2\beta1$ endogenous, integrin receptors to the hexapeptide sequence found on natural collagen [18]. Some commonly used natural collagens are chitosan, alginates, hyaluronan, and chondroitin sulphate.

Chitosan is a natural polymer of linked β (1→4)-glucosamine and N-acetyl-D-glucosamine that is created from alkylating chitin, a structural polysaccharide commonly found in the exoskeleton of insects, shells of crustaceans, and cell walls of fungi [19]. Chitosan provides good mechanical strength and adhesion in grafts while also maintaining a high degree of absorbability. Human enzymes readily metabolize chitosan and it also readily dissolves in acidic environments [19]. Due to its physiologic and biologic properties, chitosan has been used as a candidate for bone regeneration techniques and numerous studies demonstrate its efficacy [19].

Alginates are naturally-derived polysaccharide block copolymers of specific repeating sequences that are linked non-consecutively [19]. They are harvested from 3 species of marine brown algae, and are one of the algae's main structural components- up to 40% dry weight [20]. Alginates can undergo reversible gelation in the presence of water and divalent cations such as calcium, which facilitates cell migration and allows alginates to be effective as a cell delivery mechanism [19]. Alginates also have good

adhesive properties, porosity, mechanical strength, cell adhesion, and biocompatibility and support osteogenic differentiation [20,21].

Hyaluronan is an endogenous glycosaminoglycan (GAG) that is one of the main components of the extracellular matrix. It is a linear polysaccharide made up of a repeating disaccharide (α-1,4-D-glucuronic acid and β-1,3-N-acetyl-D-glucosamine). One of its main functions is to attract water, and thus provide an environment that facilitates cellular movement and the diffusion of molecules. Hyaluronan is well studied and is also readily available commercially [19]. For these reasons, hyaluronan is a commonly used substrate in bone tissue engineering.

Chondroitin sulphate is another GAG commonly used in tissue engineering [19]. One specific use of this GAG is in combination with chitosan to form a delivery system for growth factors [19]. The negatively charged nature of GAGs attracts positively charged platelet derived growth factor (PDGF) enabling its transport [22].

There have been many attempts to mimic natural collagen, but to date synthetic collagens have been unable to reproduce the unique characteristics of the natural product [17]. Synthetic polymers range from those that mimic natural polymers to polymers constructed as single walled carbon nanotubes [23]. Unfortunately, none mimic the biocompatibility of natural polymers at present. To bridge this gap, researchers have created natural-synthetic polymer hybrids. Two synthetic polymers, which are gaining popularity, are Poly-l-lactide and polycaprolactone triol, the latter of which is especially popular due to its recent success in being able to create anatomically shaped tissue engineered cartilage [24].

Bioactive ceramics, bioactive glass, and hydrogels

Bioactive ceramics are composed of calcium phosphate in varying molecular structures [25]. Their unique promise lies in the fact that they are composed of the same basic material as the extracellular bone matrix and thus have good resorptive capabilities. The major substrates are hydroxyapatite, tricalcium phosphate, and biphasic calcium phosphate [26]. While capable of inducing osteoblastic differentiation *in-vitro*, they are typically too brittle and resorb too slowly to be practical for bone grafting applications [27].

Bioactive glasses are composed of phosphate and calcium laden silica [28,29]. They have similar properties to ceramics and are useful in both periodontal and orthopedic applications. Since it is made from a silica matrix, it is more susceptible to fracture with load bearing [26]. Of note, PerioGlas is approved in the US for several periodontal applications [5] and is superior to open debridement and equivalent to allograft in dental applications [30,31]. Additionally, in a head-to-head study against hydroxyapatite, bioactive glasses were found to

have faster new bone formation and better resorption in critical bone defects in rabbit femoral condyles [32]. The makers of PerioGlas also produce several moldable grafts for use in orthopedic injuries.

Hydrogels show excellent osteoconductivity and absorptivity but lack sufficient mechanical strength [33]. Thus, they are not used as the exclusive material in a graft and are instead combined into hybrid scaffolds. Typically, bioactive glass or nanoparticles are added to give hydrogels more structural stability. In previous studies, increasing the structural stability came with a proportional decrease in the hydrogel's resorbability. Recent results with newly designed nanoparticles, however, reveal drastic improvement in mechanical strength while maintaining the hydrogel's favorable resorbability profile [34].

Metals

Currently, the metals being used in bone grafting applications, which include stainless steel, gold, titanium, and cobalt-chromium, are not resorbed but rather remain permanently in the body. This is not conducive of the most ideal outcome of a full transition to new bone. In contrast, magnesium alloys are somewhat resorbable and, since magnesium forms a divalent cation similar to that of calcium, may behave similarly in bioceramic scaffolds [35].

Incorporation of Cells into Bone Grafting Materials

Although successful incorporation of a bone graft requires the coordinated activities of matrix-secreting cells and matrix-resorbing cells [36], considerably more attention has been paid to the delivery of the cells that carry out the former activity. In particular, bone marrow-derived mesenchymal stem cells (BM-MSCs) are osteochondral progenitor cells that may be easily expanded and induced to form cartilage or bone matrix [37]. Bone marrow aspirates have shown promise due to their immunomodulatory properties and ability to secrete bioactive factors that promote growth and differentiation of MSCs [38]. Additionally, several studies have examined adipose-derived stem cells (ADSCs) [39]; however, while their yield at harvest is higher with less extraction-related comorbidity, ADSCs generally demonstrate inferior osteogenic potential than BM-MSCs. To the best of our knowledge, ADSC studies have lacked analysis of adding bioactive factors to stimulate better growth [40]. Given these properties, it is conceivable that the inclusion of MSCs or ADSCs could enhance the efficacy of bone grafting materials. Indeed, several studies have demonstrated faster rate of union and or improved healing with co-delivery of osteogenic cells as compared to scaffold material alone [41,42]. It should be noted that, in addition to MSCs or ADSCs, other cell types have shown promise in bone healing applications. For instance, a recent review comprehensively summa-

rizes the use of induced pluripotent stem cells in bone healing [43].

Incorporation of Growth Factors into Bone Grafting Materials

Numerous growth factors have been demonstrated to promote bone healing endogenously and several have been examined for the potential to augment bone graft incorporation [44]. In the section below, we highlight two medical devices approved by the US FDA and other strategies shown to improve bone engraftment.

Bone Morphogenetic Proteins (bmps) alone and in combination with other factors

Unlike ceramic and demineralized bone matrix technologies that serve as bone void fillers or autograft extenders, bone morphogenetic proteins (BMPs) are highly osteoinductive growth factors that promote robust osteogenesis [45]. BMPs belong to the transforming growth factor-beta (TGF-β) superfamily of proteins and play fundamental roles in the regulation of basic biological processes throughout the body such as growth, development, tissue homeostasis, and regulation of the immune system [30]. Two BMP proteins, BMP2 and BMP7, have received FDA approval for bone grafting applications, though BMP7 is employed only under a humanitarian device exemption [45]. While BMP advances in grafting have been promising, there remain gaps in the overall effectiveness of BMP treatment on fracture healing, in part due to the wide array of context-dependent functions that the proteins confer. Additionally, BMP signaling in osteoclasts is poorly understood and, in some circumstances, it has been suggested that BMP inhibition could be desirable for promoting bone mass accrual [31].

The fibroblast growth factor (FGF) family consists of numerous structurally similar cytokines that are implicated in a variety of processes including cellular proliferation, wound healing, and angiogenesis, among many others. FGF-2 is arguably the most established and understood member of the family and is shown to play an important role in endochondral bone formation [32]. Several studies have examined combining FGF-2 and BMPs in inducing osteogenic differentiation of MSCs *in-vitro* or in bone grafting models *in-vivo* and found that the combination of these factors induces a more potent response than either factor alone [46-48].

Vascular endothelial growth factor (VEGF) plays an endogenous role in promoting adequate angiogenesis during new bone formation. Combining this property with osteogenic growth factors such as BMPs may have significant therapeutic value [49-51].

Platelet-rich plasma

Platelet-rich plasma (PRP) has significantly grown in popularity in recent years with a very wide variety of uses. It is potentially useful as an adjunctive growth fac-

tor treatment as it is entirely autologous and relatively easy to isolate. Also, it results in an increased concentration of a cocktail of pro-osteogenic growth factors upon platelet degranulation, including but not limited to FGF, VEGF, PDGF and insulin-like growth factor. The overall benefits of PRP suggest it may emerge as an ideal choice for conferring osteoinductivity to a bone grafting scaffold. For instance, PRP has been delivered within a platelet-rich plasma poly-(lactic-co-glycolic acid) (PRP-PLGA)/calcium phosphate cement (CPC) scaffold in the treatment of radial and femoral defects in rabbits [52]. PRP was loaded into the scaffold with a unidirectional pore structure, providing directional bony in-growth. Both femoral and radial defects showed significantly improved healing at 12 weeks when PRP was used with associated improvement in angiogenesis and scaffold degradation [52]. PRP has also been delivered in the treatment of calvarial defects in rabbits via the commercially available product Skelite, which is a silicon-stabilized hydroxyapatite tricalcium phosphate scaffold. Skelite and PRP in combination demonstrated significantly increased osteoid deposition when compared to Skelite alone [53].

Conclusion

Donor site morbidity and health status have created a need for a less invasive and safer alternative to bone autografting. Allografts, though readily available, are more prone to failure. This situation results in a need for engineered bone grafting technologies, which offering promising results to address these issues. Creating osteoconductive scaffold materials; determining optimal stem cell population(s) and refining their harvesting, culturing, and concentration methods; and fine tuning cellular activity with growth factors may provide better treatment options.

Financial Support and Disclosures

Sources of Support

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