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Evolution of Bone Grafting: Bone Grafts and Tissue Engineering Strategies for Vascularized Bone Regeneration

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Abstract The regeneration of bone in segmental defects has historically been a challenge in the orthopedic field. In particular, a lack of vascular supply often leads to non-union and avascular necrosis. While the gold standard of clinical care remains the autograft, this approach is limited for large bone defects. Therefore, allograft bone is often required for defects of critical size though a high complication rate is directly attributable to their limited ability to revitalize, revascularize, and remodel resulting in necrosis and re-fracture. However, emerging insights into the mechanisms of bone healing continue to expand treatment options for bony defects to include synthetic materials, growth factors, and cells. The success of such strategies hinges on fabricating an environment that can mimic the body's natural healing process, allowing for vascularization, bridging, and remodeling of bone. Biological, chemical, and engineering techniques have been explored to determine the appropriate materials and factors for potential use. This review will serve to highlight some of the historical and present uses of allografts and autografts and current strategies in bone tissue engineering for the treatment for bony defects, with particular emphasis on vascularization.

Keywords Allograft · Autograft · Scaffold · Bone tissue engineering · Growth factors · Endothelial cells · Mesenchymal stem cells · Vascularization

Introduction

Reconstructing and regenerating significant skeletal defects have perplexed mankind for thousands of years. Grafting techniques were utilized as early as 2000 BC when Khurits utilized a piece of animal bone to repair a small skull defect, which proved successful millennia later when anthropologists discovered the remains exhibiting regrowth around the graft [1]. In the modern age, the first documented bone graft was performed in 1668 by Job van Meekeren, a Dutch surgeon. He, too, used a xenograft to repair a skull defect in an injured soldier [2]. Bone grafts and the understanding of orthopedic science were further propelled in the seventeenth century by the work of Antoni van Leeuwenhoek who is famously known for his work on microscopy. He also primitively described the microarchitecture of bone, identifying what we now refer to as Haversian canals [1, 2]. Diligent examination of bone grafting criteria and outcomes surfaced in the early 1900s with the work of Vittorio Putti who outlined the principles of grafting [1]. Putti's work established a foundation for grafting science in the field of orthopedic surgery. Since then, surgeons and researchers alike have continued to hone the science of bone grafting to allow for the most appropriate surgical intervention with the best outcomes.

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Grafted bone can come from the same individual (autograft) or from other individuals of the same species (allograft). Every year, approximately 1 million bone allografts are used [3]. Between 1992 and 2007, an estimated 1.7 million bone autografts were performed [4]. Both grafts possess unique advantages and disadvantages, but autografts began to come into favor over allograft in the early 1900s with recognition of the benefit that vascularization provides to the integrity of the graft and surrounding bone [5, 6]. Evidence continues to suggest autografts provide improved outcomes over allografts [7–14]. However, autologous and allogeneic bone grafts are now often used in combination with bioengineered scaffolds (frames upon which tissue regeneration can occur) or bone substitutes/adjuncts, which may allow for enhanced applications of allografts [15–23], so much so that allografts may be superior to autografts if combined with bone morphogenetic proteins (BMPs) and a bisphosphonate, suggested by larger and denser calluses with increased peak force in BMP + bisphosphonate graft [24].

The advancement of biomaterials' research in the past few decades has enabled the development of scaffold materials to enhance the regeneration and vascularization of bone in large segmental defects. Scaffolds have been made from many materials and have included growth factors and/or cells to specifically promote vascularization in healing bone grafts. Combinations have included vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), endothelial cells (ECs), and mesenchymal stem cells (MSCs). Addition of these compounds and/or cells to scaffolds has provided potential in improving outcomes in patients undergoing grafting procedures.

As life expectancy continues to increase, orthopedic cases continue to rise as well. In 2008, health care costs for regenerative biomaterials were estimated to exceed $240 million [25], and it is not unreasonable to assume this value will continue to rise, highlighting the importance of regenerative bone materials in orthopedic care in the near future. The most common uses for bone grafts in the USA are spinal fusion and fracture nonunion [4]. This review will address the use and characteristics of enhanced grafts, scaffolds, and bone substitutes as adjuncts in orthopedic reconstruction and bone regeneration.

Bone Grafts

Bone grafts and scaffolds are often evaluated for three characteristics: (1) osteoinduction—ability to recruit and induce MSCs to differentiate into mature bone-forming cells; (2) osteoconduction—allowing for cellular invasion of the graft; and (3) osteointegration—functional integration of the graft with the host tissue through new bone formation [26, 27]. An ideal graft harnesses adequate osteoconductive, osteoinductive, and osteointegrative characteristics; however, the necessary properties for optimal bone scaffold design remain unknown. Below, we discuss the advantages and disadvantages of allografts and autografts and touch upon adjunctive therapies that are in development to improve outcomes with use of either graft.

Allografts

Bone allografts are harvested tissue from human cadaveric donors. Cancellous allografts provide minimal to no structural strength, mild-to-moderate osteoconductive properties, and mild osteoinductive properties. Cortical allografts, on the other hand, can provide structural strength but little osteoinduction [28]. Studies have demonstrated the advantages of allografts in the setting of very significant bone defects as seen in musculoskeletal malignancies [29, 30]. When autograft use is precluded by the size of the donor site and donor site morbidity secondary to large defect, surgeons turn to allografts for reconstruction. Early research published in the New England Journal of Medicine demonstrated large allografts can prove successful in the reconstruction of bone defects following tumor removal [30]. Furthermore, functional status of patients who undergo massive allograft transplantation has been reported as satisfactory in as many as 70 % of patients [29]. Allografts may also include articular surfaces and even ligaments.

Allografts obviate many complications that arise with xenografts that were used thousands of years ago, but they also pose their own set of complexities and dangers [31–33]. Though these grafts are harvested from human cadaveric tissues, they retain the capacity to induce an immune response in recipients [34–38]. In the early use of allografts for segmental bone defects, various protocols surfaced to minimize graft–host interactions, including cryopreservation, irradiation, decalcification, and pharmacologic immunosuppression [39–43]. Cryopreservation, specifically, was demonstrated to produce shorter and more infrequent graft–host immune responses as compared to fresh, vascularized bone grafts [44]. More recently, however, protocols using nonionic detergents, hydrogen peroxide, and denatured alcohol have demonstrated an improved safety profile of allogeneic grafts [45] with union rates comparable to autologous grafts [46].

Aside from immune reactions, allografts pose a problem when concerned with union rates, structural integrity, and infections. In a large retrospective study, Hornicek et al. [47] demonstrated that of 945 patients who underwent allograft transplantation, 17.3 % of the patients experienced nonunion. Furthermore, nonunion was often

associated with infection and graft fracture [47]. Sorger et al. [48] conducted a retrospective review of graft fracture in patients who underwent allograft transplantation. In a 1046 patient sample, 17.7 % experienced structural allograft fracture at a mean time of 3.2 years after transplantation. Patients with graft fractures underwent further reconstruction, but 45.9 % of the allografts completely failed (time to complete failure not specified) [48]. Finally, infection is a large concern for allograft transplant procedures. Infection rates have been suggested to reach 12.9–13.3 % in patients who undergo allogeneic transplantation [49, 50]. Furthermore, 50 % of allograft infections were polymicrobial with poor soft tissue coverage responsible for the majority of the infections [50].

Autografts

Autografts are harvested from and implanted into the same individual. The most frequently used donor site for bone autografting is the iliac crest with other options including the proximal tibial, distal radius, and greater trochanter [51]. Autografts obviate graft–host reactions mediated by histocompatibility mismatches because the tissue is removed and transplanted in the same individual. However, autografts present their own set of complications with donor site morbidity and limited tissue availability.

Autografts are considered the standard of bone grafting, especially in craniofacial surgery, due to their significant osteoinductive and osteoconductive properties [52, 53]. Cortical autografts also provide significant structural strength to the graft [28]. Cellular viability and neovascularization are critical properties of autografts that partly account for their use over allografts and aid in the osteoinductive, osteoconductive, and osteogenic potential. Vascularization is vital to the structural integrity of bone during the healing process [54–57], and graft integration is no exception [58, 59]. As one can expect, neovascularization between any graft and recipient site during the healing phase is a complex, dynamic interplay between various cell types and growth factors, which is supported by the use of autografts [58]. Cancellous bone autografts have been demonstrated to initiate vascularization within 2 days of grafting [60]. Harnessing the neovascularization in autografts is vital to the success of grafts in recipients.

Disadvantages to the use of autologous bone include donor site pain [61–63], which can be severe and prolonged, as well as more significant complications such as fracture, pelvic instability, hematoma formation, infection, and nerve palsies [64–68]. In addition, the quantity of bone graft needed further limits the use of autografts and contributes to the likelihood of adverse events after harvest.

The limitations of both autogenous and allogeneic bone graft materials have spurred research resulting in a proliferation of natural and new synthetic biomaterials used to treat bone defects. Nanotechnology and more refined biomechanical techniques have allowed for the analysis and development of osteogenic, osteoinductive, and osteoconductive biomaterials. As the field of bioengineering continues to evolve, allografts and autografts will likely fall out of favor and be replaced by more advanced bone graft substitutes that optimize vascular and cellular potential.

Scaffold Materials

Bioengineered scaffolds have evolved dramatically over the past 40 years and provide great potential in orthopedic and maxillofacial applications without immunologic or donor site complications that arise with allografts and autografts. Potential for these materials is virtually infinite with the advancement of nanotechnology and derivation of new scaffold materials, materials that will be developed to harbor significant strength and adequate osteoconductive and osteoinductive properties. Variations in scaffold type and architecture are limitless, including material, porosity, cellular seeding capacity, and growth factor seeding capacity [69–71].

Natural—Collagen, Alginate, Hyaluronic Acid

Collagen is the most abundant protein found in bone. Thus, it has been utilized in orthopedic tissue engineering applications because of its availability and biocompatible properties [72]. It obviates many of the complications associated with the use of bone allografts and autografts, but the mechanical properties of collagen remain in question [73]. More recent developments in collagen scaffolds have provided an improved strength profile of collagen scaffolds by modifying collagen cross-linking [74–76]. Tierney et al. [77] refined the properties of collagen scaffolds, including porosity, matrix, and permeability to increase osteoblast activity. These studies point to the potential of collagen scaffolds in tissue engineering, especially in orthopedic and maxillofacial applications. In virtually, all applications of bone grafts and scaffold materials, including collagen, vascularization, remains paramount for graft success.

Alginate is an additional natural material derived from brown algae that offers potential in biomaterial engineering cell [78, 79] through its ability to form a gel in combination with water. It is a polysaccharide that is easily modified chemically and structurally to allow for enhanced application in regenerative medicine. Its viscosity and porosity allow for cellular immobilization, integration, and extended release of factors and cells from the scaffold [80].

However, it lacks intrinsic mechanical strength [81] and is often combined with other compounds (i.e., chitosan, gelatin, and hydroxyapatite) to improve osteoconductive and osteointegrative properties while providing a strong biodegradable structure [82–85]. Furthermore, alginate can be functionalized with growth factors to enhance neovascularization in and around the scaffold to improve bone growth [86, 87]. One issue within biomaterial engineering is the ability to control the release of such factors and cells to enhance their effects. Alginate has been used as a spatiotemporal delivery vehicle for BMP-2 to enhance bone regeneration in comparison with collagen sponge as a result of sustained release in vivo [88, 89] and to deliver angiogenic factors sequentially to improve scaffold vascularization and bone regeneration due to differences in binding affinity between alginate and the factors [90, 91].

Finally, hyaluronic acid (HA) is another natural compound that has been studied for use in bone tissue engineering. HA is essential to the extracellular matrix in wound healing and is well known in musculoskeletal physiology as a compound that provides lubrication to synovial membranes in joint capsules by aggregating glycosaminoglycans [92, 93]. In tissue engineering applications, HA is similar to alginate in the fact that it is often combined with other compounds [94–96] and functionalized with growth factors [97] to enhance its regenerative potential and provide functional and structural roles in constructs [98, 99]. Like alginate, as a pure compound, it lacks mechanical strength often required for weight-bearing and thus requires either sufficient fixation stability or combination with structural scaffolds.

Synthetic Materials—Polyethylene Glycol, Polycaprolactone

Polyethylene glycol (PEG) is a synthetic compound used in tissue engineering due to low toxicity and absence of an immune response. It is hydrophilic and soluble, yielding poor mechanical strength [100], but it can, like the natural compounds, be combined with other materials to improve strength and biocompatibility. PEG can be functionalized with adhesive peptides [101], growth factors, and polysaccharides, such as glycosaminoglycans [102, 103], which have improved bone growth in and around the scaffold. In addition, PEG can be used to functionalize other scaffold materials and link macromolecules to improve bone formation [104].

Polycaprolactone is a synthetic biodegradable compound used in bone tissue engineering for its mechanical profile and manufacturability. It is a porous compound manufactured via numerous processes from photopolymerization to three-dimensional printing [105, 106]. Multiple studies have demonstrated the ability to seed mesenchymal cells and growth factors to improve graft integration at the recipient site [107–110]. The opportunity to functionalize polycaprolactone scaffolds largely stems from its porous structure. For these reasons, polycaprolactone has been identified as a viable scaffold option in bone tissue engineering.

Ceramics—Bioactive Glass, Hydroxyapatite

Bioactive glass is an appealing candidate in treating bone defects due to its biocompatibility, strength, and ability to regenerate bone through release of ionic biological stimuli [111]. Pores within the glass also allow for tissue ingrowth and viability [111]. A significant drawback of bioactive glass, however, is its inherent brittleness, making it difficult to handle in implantation [112, 113]. Strategies have been developed to overcome this challenge. For example, coating or combining bioceramic materials such as bioactive glass and hydroxyapatite with a supporting matrix such as poly-L-lactide acid (PLLA) [114], polyethersulfone (PES) [115], poly D,L-lactide-co-glycolide (PLGA) [116], or p(*N*-isopropylacrylamide-co-butyl methylacrylate (PIB) [117] improves not only the mechanical properties but the osteogenic potential of such scaffolds as well [115, 118]. Furthermore, the composition of bioactive glass can be altered to a more malleable material, making it easier to manipulate [119].

Another ceramic of interest in tissue engineering is hydroxyapatite (HAp). It is biocompatible, has good osteoconductivity [120], and has been used in bone repair. Similar to bioactive glass, though, it is relatively brittle and is not ideal for bearing weight [121]. However, there are several methods in which the HAp scaffold can be produced to improve the mechanics of these constructs to improve tensile and compressive strength [122, 123]. Interestingly, 3D printing has been utilized to produce HAp scaffolds capable of sustaining cell proliferation deep inside the construct and provides an exciting prospect for the future use of HAp [124].

Growth Factors and Cells

While graft or scaffold material is important to consider, the largest hurdle to bone regeneration is arguably in the challenge of creating a vascularized structure capable of nourishing the surrounding environment and removing wastes. To enhance angiogenesis and bone regeneration, various cell and growth factor combinations have been tested in scaffolds and grafts. Such combinations have largely included VEGF, PDGF, ECs, MSCs, and BMPs. In brief, VEGF functions to regulate angiogenesis and capillary permeability, as well as EC and MSC migration and

proliferation [125, 126]. PDGF recruits fibroblasts and inflammatory cells to sites of injury, induces collagen deposition, and possesses angiogenic potential [127]. ECs are crucial because they form the lumens of blood vessels. MSCs are multipotent cells capable of differentiating into various cells such as osteoblasts, chondrocytes, adipocytes, and muscle cells, but also serve to support neovascularization by acting as mural cells [128]. BMPs function to induce bone formation through the stimulation and differentiation of osteoblasts [129].

VEGF

VEGF has been a popular candidate in tissue engineering for its angiogenic properties. It is a particularly attractive candidate in bone bioengineering for its additional effects on chondrocytes, osteoblasts, and osteoclasts [56]. VEGF has been shown to mediate chondrocyte and osteoblast survival and differentiation as well as recruit osteoclasts [130]. It has been utilized individually, paired with other growth factors, and has been infected into cells through viral vectors to promote vascularization and bone formation [131]. VEGF appears to function best when used in conjunction with other factors [132–136]. For example, VEGF combined with BMP-7 has been shown to result in earlier osteogenesis, more lamellar and trabecular bone formation, and a higher bone density than the usage of BMP-7 alone [132]. In addition, differences in vascular growth between collagen-coated PLGA scaffolds seeded with either bone marrow MSCs (bmMSCs) or VEGF were minimal, but VEGF and bmMSCs seeded together resulted in continued vascularization 14 days after implantation [133]. Combining multiple cells and growth factors in a scaffold better reflects the composition of the extracellular matrix seen in repairing bone, as the regeneration process naturally requires a multitude of factors and cell interactions.

A hurdle in the application of growth factors for bioengineering techniques is the short half-life or dissipation of growth factors after being implanted into the defect, leading to avascular necrosis or prolonged time of healing [137]. In regard to VEGF, techniques have recently been developed that allow for extended, controlled release. Scaffolds constructed of silk/calcium phosphate/PLGA have been shown capable of releasing PDGF and VEGF at a rate so that bioactivity after 28 days is maintained at 82 and 89 %, respectively [138]. Poldervaart et al. [139] demonstrated that when released from gelatin microparticles in a controlled and prolonged manner in 3D bioprinted scaffolds, VEGF promoted significantly more vascular formation than when released quickly both in vitro and in vivo. Furthermore, the gelatin microparticles allowed for the creation of heterogeneous constructs, as it was noted that the microparticles could be administered regionally. A spatiotemporal scaffold construction such as this could be of particular use when considering the potential injurious effects of prolonged action of VEGF. In a nude rat model using genetically modified bmMSCs to express VEGF, Helmrich et al. [140] examined vascular density and bone quantity on osteoconductive material. While VEGF-bmMSCs demonstrated significantly higher vascular density after 8 weeks compared to control bmMSC cells, VEGF expression induced recruitment of osteoclasts and resulted in a reduction in the amount of mature bone. Although VEGF has been supported as a critical player in induction of vascularization and bone formation, overexpression or prolonged expression can lead to deleterious consequences through activation of osteoclasts or increased vascular permeability.

PDGF

PDGF is a critical element of wound healing and has been shown to promote angiogenesis [141–144] as well as increase wound neovascularization and granulation tissue formation [145–147], early elements of the wound-healing process. PDGF and VEGF are closely related, and VEGF can signal through PDGF receptors to regulate MSC migration and proliferation [148]. In the aspect of bone bioengineering, delivering PDGF on collagen-based demineralized bone matrix scaffolds through the cross-linking of heparin enhances and prolongs its local activity, and it increases both the cellularization and vascularization of the scaffold [149]. It also has been shown to increase the amount of collagen present in bony defects [150]. PDGF's roles in angiogenesis and cellular migration and proliferation, as well as its role in conjunction with VEGF, makes it an enticing candidate in tissue engineering.

BMPs

Recombinant human BMPs (rhBMPs) 2 and 7 have been approved by the FDA for the treatment for open tibial fractures with intramedullary fixation and tibia long bone nonunion [151]. Acknowledged for their ability to induce osteoblast proliferation and differentiation, BMPs are popular choices in graft and scaffold use to increase rates to union [151]. However, usage of BMPs has been known to carry significant side effects likely due to the high dosage required, including swelling, inflammation, heterotopic bone formation, and most significantly, an increased cancer risk [152, 153].

In addition to their osteogenic potential, BMPs have been shown to increase vascularization in scaffolds as well. Zhang et al. [154] demonstrated BMP-producing bone marrow stromal cells have the potential to increase graft

incorporation and vascularization. In a cuttlefish bone scaffold soaked in BMP-2, Liu et al. [155] demonstrated that cuttlefish bone–BMP composite displays more microvasculature and bone trabeculae in rat skull defects than a scaffold of cuttlefish bone alone. The sustained release of BMP-2 seeded on 2-N,6-*O*-sulfated chitosan nanoparticles on a gelatin sponge induces bridging of segmental defects and a dose-dependent increase in angiogenesis in rabbit radius [156].

MSCs and ECs

Timing of administration of factors is important to consider when evaluating the angiogenic and osteogenic potential of a scaffold or graft, as bone regeneration is tightly regulated both temporally and spatially. MSCs can be used as a sole cell source to enhance osteogenicity in critical size bone defects [157]; however, they can also be co-transplanted with ECs. Co-transplantation of endothelial progenitor cells and MSCs increases blood vessel formation early in the healing process after 1 month and bone formation in later stages after 3 months [135]. Alternative to co-transplanting ECs and MSCs together, McFadden et al. [158] found that vascularization of a collagen-glycosaminoglycan scaffold occurs best when MSCs are added to preformed endothelial networks, as the MSCs can act as pericytes to the newly formed blood vessels. Pirraco et al. [159] also cultured ECs and subsequently added them to osteogenic cell sheets and found that this technique improves in vivo bone and vessel formation. Although MSCs and ECs cultured together provide the appropriate stimulus for vascularization and bone regeneration, MSCs are often derived from bone marrow. A challenge of utilizing bmMSCs lies in the requirement of invasive procedures to harvest the cells, as well as the limited quality of cells that are able to be obtained. It is therefore important to consider other sources. Human umbilical cord MSCs, human embryonic stem cells, and induced pluripotent stem cells have been evaluated as potential alternatives to human bmMSCs, and these alternatives have been shown capable of blood vessel and bone generation comparable to human bmMSCs [160]. These different sources of MSCs provide a potential effective and more cost-effective approach to tissue engineering.

Scaffold Vascularization Techniques

In addressing the issue of vascularization in a bony defect, one of two broad approaches can be taken. Attempts at vascularization can be done prior to placing the scaffold or graft, or the scaffold or graft can be seeded with proangiogenic factors and implanted as previously discussed. Prevascularization includes harvesting vascular bundles for the defect [161–165] or vascularizing sheets of cells prior to insertion [158, 166]. Saphenous vascular bundle constructs have shown promise in both large and small animal models, resulting in higher vascularization and osteogenesis [161, 162]. Contrary to transplanting preformed vessels, prevascularization on a smaller level with sheets of vascularized cells can be done. In an effort to construct a biomimetic periosteum prior to insertion, Kang et al. [166] created a vascularized cell-sheet-engineered periosteum by culturing human MSCs (hMSCs) and subsequently adding human umbilical vascular endothelial cells (HUVECs) to mimic the fibrous layer of the periosteum. A sheet of mineralized hMSCs designed to mimic the cambium layer was wrapped around a β-TCP scaffold followed by the vascularized HUVEC/hMSC sheet. The biomimetic scaffold resulted in enhanced angiogenesis that anastomosed with host vessels and increased bone matrix production [166]. While the use of both preformed vessels and proangiogenic factors shows promise, more research is needed to determine the efficacy among the different strategies.

Another emerging approach is stimulation of vasculogenesis through endothelial progenitor cell delivery. While typically considered important primarily during development, vasculogenesis, the process of de novo neovessel formation from progenitor cells, may also show promise as a therapeutic strategy for postnatal vascular growth. The identification of circulating endothelial progenitor cells [167], now termed endothelial colony-forming cells (ECFCs) or late-outgrowth endothelial cells (OECs) [168, 169], suggests that vasculogenesis may also be active during postnatal neovascularization. Importantly, this developmental process can be replicated postnatally by transplanted ECFCs, which participate in functional neovascular plexus formation and therefore may carry potential for therapeutic vasculogenesis. Both rat and human ECFCs have been shown to undergo vasculogenesis in bone tissue engineering constructs and enhance bone formation in vivo [170, 171].

Mechanical Regulation of Vascularized Bone Regeneration

In addition to biochemical cues, stem cell lineage specification and neovascularization are also regulated by mechanical stimuli. These mechanical cues can be characterized as either intrinsic (i.e., mechanical properties of the extracellular matrix or scaffold) or extrinsic (i.e., mechanical stimuli applied through either static or dynamic boundary conditions). Intrinsic cues such as matrix rigidity have been shown to control stem cell fate decisions

independent of biochemical signals, with soft substrates promoting adipogenic and chondrogenic differentiation of MSCs and stiff substrates driving osteogenesis [172]. Recently, Mooney and colleagues demonstrated that in addition to the elastic properties, the viscoelastic (time-dependent) properties of the extracellular matrix can also regulate stem cell fate, with stress-relaxing substrates enabling osteogenesis in spite of initially soft elastic moduli [173]. While these observations have been primarily investigated in 2D culture systems, extension of these principles to 3D hydrogels and scaffolds will provide important design constraints in the development of next-generation tissue engineering constructs.

In addition to stem cell differentiation, intrinsic matrix cues have also been shown to influence neovascularization. Early studies controlled matrix rigidity by increasing the ECM concentration [174, 175] or by mixing in additional molecules like collagen to soft matrices such as matrigel [176, 177]. However, driven by the observations that increased or different ligand presentation has the potential to influence cell behavior independent of stiffness, recent studies have developed matrices of variable rigidity that maintain constant ligand identity and density. Several different approaches have been described, including tunable cross-linking polyethylene glycol (PEG) hydrogels [178, 179] and alginate hydrogels [180], which can be modified to control ligand presentation. Other recent studies have explored matrix rigidity control through differential cross-linking of natural ECM materials such as collagen. One such approach is methacrylated gelatin, which features controllable photocross-linking by ultraviolet (UV) light [181]. Another is nonenzymatic glycation [182–184], in which reducing sugars are used to create advanced glycation end products (AGE) on collagen fibers, resulting in cross-link formation [184]. Others have exploited the natural collagen fiber cross-linking that occurs in vivo to form matrices of variable rigidity at constant collagen concentration by missing collagen monomers and oligomers, formed through native in vivo cross-links [74]. Each of these approaches has demonstrated profound effects of matrix physical properties on neovascular growth and remodeling, and may have important implications for biomaterial scaffold design.

Bone has long been known to respond to extrinsic mechanical stimuli caused by physiological mechanical loading [185], but more recent studies have demonstrated that these mechanical cues also dramatically regulate fracture healing [186] and direct tissue differentiation [187, 188]. These observations have significantly influenced clinical practices for fracture fixation and postoperative management, and emerging understanding of the mechanobiological principles that underlie these responses will further enable tissue engineers to develop viable bone graft substitutes in vitro through bioreactor culture [189, 190] or enhance large bone defect regeneration in vivo [191–194]. For example, control of in vivo mechanical loading through modulation of fixation plate stiffness can either enhance or prevent bone regeneration depending on load timing and magnitude [192–194].

Extrinsic mechanical stimuli have also been shown to regulate neovascularization [195, 196] and vascularized bone regeneration [192]. Extrinsic cues that have been shown to influence neovascular growth and remodeling include blood flow-induced luminal shear stress [196–198], luminal pressure-induced circumferential stretch [199], tensile matrix stretch [195, 200], and tissue compression [192].

Collectively, these observations may have important implications for the development of novel vascularized tissue engineering strategies. Ongoing research on the role of both intrinsic and extrinsic mechanical cues for large bone defect regeneration will continue to inform biomaterial scaffold and fixation plate design and physical rehabilitation strategies. Important questions regarding underlying molecular mechanisms and interactions with the biochemical cues described above will continue to be explored.

Conclusion

Bone grafting has served a crucial role in the repair of segmental bone defects for centuries. Pioneers in the field of bone grafting recognized the importance of allografts and autografts, including the benefits and limitations of each. Allografts were very commonly used; however, over time, there was a transition to more commonly using autografts as techniques for harvesting the graft improved. Autografts have proved quite successful but not without their own limitations, including donor site morbidity. Due to the limitations of allografts and autografts and the advancement of biomechanical research, emphasis has been placed on developing artificial scaffolds with optimum osteoinductive, osteointegrative, osteoconductive, and angiogenic properties.

Many growth factors and cells have been studied for their various properties in combination with scaffolds. These compounds and cells include: VEGF, PDGF, BMPS, ECs, and MSCs. Novel research has suggested these compounds and cells provide promising opportunities for the development of optimal materials for bone grafting that allows for vascular and regenerative potential. With the materials, growth factors, and cells available for biomechanical research, the potential for bone graft and scaffold development is endless. Taken together, these studies demonstrate opportunities that lie ahead to improve patient outcomes after a bone graft procedure.

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Compliance with Ethical Standards

Conflict of interest Kaitlyn S. Griffin, Korbin M. Davis, Jeffrey O. Anglen, Tien-Min G. Chu, Joel D. Boerckel, and Melissa A. Kacena declare they have no conflict of interest. Todd O. McKinley is a consultant for Bioventus.

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