Published in final edited form as:
J Long Term Eff Med Implants. 2010 ; 20(4): 303–315.

Novel Approaches to Bone Grafting: Porosity, Bone Morphogenetic Proteins, Stem Cells, and the Periosteum

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Abstract

The disadvantages involving the use of a patient's own bone as graft material have led surgeons to search for alternative materials. In this review, several characteristics of a successful bone graft material are discussed. In addition, novel synthetic materials and natural bone graft materials are being considered. Various factors can determine the success of a bone graft substitute. For example, design considerations such as porosity, pore shape, and interconnection play significant roles in determining graft performance. The effective delivery of bone morphogenetic proteins and the ability to restore vascularization also play significant roles in determining the success of a bone graft material. Among current approaches, shorter bone morphogenetic protein sequences, more efficient delivery methods, and periosteal graft supplements have shown significant promise for use in autograft substitutes or autograft extenders.

Keywords

bone graft; allograft; permeability; periosteum; tissue engineering

I. INTRODUCTION

Critical size bone defects result from one or more pathological events (e.g., tumor, trauma, inflammation, or radiotherapy)[1] and can lead to a delayed union or a nonunion of fracture. The lost bone mass can be replaced using a number of techniques and materials. In some cases, a patient's own bone may not be available or may not be obtainable in sufficient volume to repair a given defect. In these cases, natural, synthetic, and artificial materials can be used to replace autograft materials, and a variety of clinical outcomes have been achieved with these bone graft materials.

Autogenous iliac crest bone graft is considered the gold standard for bone grafting procedures due to its natural osteoinductivity; however, the disadvantages of autografting procedures such as donor site pain, increased operative time, and the limited amount of obtainable material have led surgeons to search for alternative materials. Acceptable bone substitutes should offer one or more of three characteristics that are provided by autograft materials: osteoinductivity, osteoconductivity, and osteogenicity. For example, allograft materials eliminate the need for donor site tissue removal and provide good osteoconductivity; however, these materials are associated with poor osteoinductivity.[2] Unlike patients receiving autograft materials, patients receiving allograft materials undergo

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shorter operative times as well as receive satisfactory outcomes; however, allograft recipients have been shown to experience longer postoperative fevers.[3] In addition, allograft transplantation is relatively safe from infection transmission. Current sterilization practices used in hospital bone banks virtually eliminate infection transmission from frozen cadaver bone.[4]

Synthetic materials exhibit several beneficial properties, including unlimited supply, straightforward sterilization, and simple storage.[5] These materials can be engineered to exhibit osteoinductive properties through the incorporation of bone morphogenic proteins (BMPs). BMPs are important supplements to synthetic materials since they can induce bone and cartilage formation as well as other biological activities, such as cell proliferation, migration, and apoptosis.[6] Their increased application in clinical fracture healing, however, raises efficacy and dose concerns.[7] In addition, there is room for possible improvement through the use of shorter BMP-2–related peptide sequences.[8] Ensuring good vascularity is also a problem that any bone graft material faces. Use of periosteal grafts, growth factors, and porous surfaces in combination may provide an approach for increasing the success of graft vascularization. Advances in cell harvesting, three-dimensional (3-D) matrices, and recombinant signaling molecules may also provide graft material improvements. This review will consider advances in the synthetic materials, tissue engineering approaches, and allograft enhancements that are used in bone grafting procedures. It will also outline the necessary general evaluation criteria that must be considered for any given bone graft material. The optimal bone graft material should possess mechanical properties similar to bone, provide controllable BMP delivery, and exhibit sufficient porosity as well as microscale and nanoscale features for bone regeneration. In addition, the material should be nontoxic, simple to sterilize, and dissolvable at a controlled rate.

II. POROSITY

II. A. Pore Size and Shape

Porosity is an important factor in determining tissue-implant material integration. Porosity can be incorporated within natural and synthetic scaffold materials in order to impart desirable properties to these materials. For example, polymers such as poly(lactic-*co*-glycolic acid) (PLGA) and polycaprolactone (PCL) can be prepared with porous structures to obtain improved protein loading and controllable degradation rates.[9] Porous ceramics and composites such as beta tricalcium phosphate (β-TCP) and hydroxyapatite (HA) can be prepared with strength equaling or exceeding that of human cancellous bone.[10,11] In addition, these materials can be prepared with interconnected porosity, including both macroporous and microporous features.[12] These features facilitate BMP loading and delivery.[12] The properties of natural materials may be improved through the incorporation of porous structures. For example, a recent study involving collagen scaffolds showed that cell spreading and migration in collagen were more dependent on porosity and pore size than on matrix stiffness.[13] It should be noted that an increase is porosity is associated with several drawbacks. Collagen and scaffold materials exhibit increases in absorption rates and decreases in mechanical properties with decreases in density (i.e., increases in porosity).[14] Scaffold materials are commonly engineered to have interconnected porosity and controlled pore dimensions, which may be used to modulate cell growth, seeding, and differentiation.[15] Novel advancements in scaffold fabrication, such as computer-assisted solid freeform fabrication, facilitate the preparation of structures with precise shapes and uniform pore characteristics.[16] In addition to scaffolds with large pores, porous scaffolds with multiscale porosity more closely resembling that of natural bone have been created. Sponge-like iliac crest bone, which is commonly used as an autograft material, exhibits a natural supporting structure that is made up of a network of smaller trabeculae (micropores). These trabeculae enclose larger voids (macropores), providing 55–70% interconnected porosity.[17] Pore size

and pore shape in scaffolds must be similar to those of natural bone. In addition, the pore characteristics must facilitate diffusion of oxygen and other nutrients. Although there has been significant discussion regarding optimal pore size, most studies focus on scaffolds with pore sizes above 100 μm.[18] One concern regarding pore size involves optimizing the surface area that is available for cell attachment. If the pores are too small, they may inhibit cellular migration and produce necrosis.[19] If the pores are too large, they may not provide sufficient surface area for cell attachment[20,21] and they may compromise structural integrity.[10,14] A recent study of interconnected porous PCL scaffolds found that 350-μm and 800-μm pores play a limited role in bone regeneration, indicating that pore features other than size may play important roles.[22] Another study showed that although initial cell adhesion (48 h postseeding) reaches a maximum value for a mean pore size of 120 μm, overall cell migration is greatest for pore sizes larger than 300 μm.[23] This study suggests that it is important to consider parameters other than size (e.g., pore shape, interconnection, and overall permeability). In addition, cone-shaped (diameter gradient) pores were associated with significantly higher oxygen concentrations and cell proliferation than uniform-diameter pores.[24] An increase in porosity, particularly with interconnected pores, may be associated with a decrease in mechanical stability. This relationship is dependent on the material. For example, scaffolds based on pure HA have much better mechanical properties than biphasic composites based on HA/β-TCP.[25]

II. B. Oxygen Diffusion and Permeability

The role of oxygen diffusion through pores has been studied extensively by means of in vitro studies, which indicate that oxygen diffusion plays an important role in angiogenesis. A problem for bone graft materials has been the inability to induce angiogenesis at the center, as well as at the periphery, of the scaffold.[26] In addition, there are difficulties associated with maintaining a nutrient supply to newly formed tissue.[27,28] The lack of oxygen can reduce cellular respiration as well as pore invasion; alternatively, the lack of oxygen (when reduced to a degree) promotes angiogenesis through the hypoxia-inducible factor-1 pathway.[29] When cells adhere to a given surface, they need additional space in the form of interconnected pores for acquisition of nutrients, removal of wastes, and transport of proteins.[30,31] An alternative approach to looking at pore size and structure alone is to determine the overall permeability of a scaffold material. One study used Forchheimer's equation and Darcy's Law to find values for overall permeability that are consistent with literature data for porous HA scaffolds.[30] Forchheimer's equation is an empirical relationship that describes a parabolic dependence of the pressure drop through a scaffold with the resulting superficial velocity. [31–34] Darcy's Law is a linear relationship between fluid velocity and pressure drop.[31] This relation accurately determines flow permeability and takes porosity, pore size, and tortuosity (amount of curvature in the network) into account. Permeability should be obtained in a simulated environment that resembles in vivo fluid, pressure, temperature, and velocity conditions as closely as possible.

II. C. Roughness and Microporosity on the Nanoscale

Scaffold surface properties have a significant influence on cell-material interactions, and these properties should be examined at the nanometer and micrometer scale. The extracellular matrix of a cell is composed of nanometer-sized features, such as pores, fibers, and ridges, and these features have influence on cell migration and orientation.[35,36] It is a goal of scaffold processing to fabricate a scaffold that exhibits the appropriate surface topography for a desired cell response. The relationship between titanium implant surface roughness and osteoblast behavior or *Staphylococcus* behavior has been examined, and osteoblasts and Staphylococci had dissimilar preferences for surface roughness types.[37] The study suggests that lateral lengths of topographical features and vertical roughness parameters on a titanium surface can be optimized to simultaneously promote osteoblast

adhesion and minimize bacterial interaction. These effects of surface topography are independent of scaffold chemistry. Surface topography-dependent properties provide specific contribution to toxicity,[38] immune response,[39] cell motility,[40] and other factors. Recent advances have provided more precise control over the surface features of nanoscale materials. Cell proliferation studies have been used to examine various surface features, including the incorporation of microporous features within bone scaffold materials. In a study comparing TiO_2 nanotubes to moderately rough blasted surfaces that are used in bone implants, nanotube surfaces were shown to provide significantly increased osseointegration and new bone formation. In addition, more cell contact at the bone-implant interface was noted. The diameters and heights of the nanotubes were modulated by altering reaction times; nanotubes with diameters of around 90 and 108 nm were obtained. These dimensions are orders of magnitude smaller than those of macroporous features; macroporous features are typically one hundred micrometers or more in diameter.[18] Studies have also shown the positive effects of incorporating microporosity within macroporous scaffolds in animals.[41–43] For example, significant increases in capillary penetration, bone volume, and mineral apposition rates have been observed with ceramic constructs, including HA.[41] In addition, the amount of the increase varied with different levels of microporosity.[24] Ceramic composites made from calcium phosphate and HA are attractive scaffolds because one can obtain structures with microporous features, such as pores and rods.[18,44–47] Macroporous biphasic calcium phosphate (MBCP) is a ceramic material that is similar in composition to the mineral phase of bone, and it forms a very strong attachment to host tissue.[48–51] In addition, it can be custom prepared for bone grafting procedures[52] and it can be modified with microporous features.[53] For example, one study used microrobotic deposition to create microporosity within MBCP rods. It was found that recombinant human (rh)BMP-2 was associated with a microscale positive effect; however, no positive macroscale effect was observed. BMP was not necessary for bone formation within the micropores.[18] Microporosity also theoretically eliminated “dead space” in the scaffold, which theoretically improves load transfer between the tissue and the material and overall toughness. In addition, it facilitates use of the material for the establishment of a continuous mechanosensory network.[9–18] In addition to ceramics, polymers may also be fabricated with microporous features.[54,55] For example, resorbable polyurethane materials have been fabricated into 3-D microporous scaffolds, which may be used for cartilage tissue reconstruction[54] with an autogenous periosteal flap[56,57] (discussed later in this review). Biodegradable polyurethane scaffolds have been used to support the attachment and proliferation of chondrocytes and osteogenic cells.[58–60] These materials, which contain open interconnections, can be used as microporous templates.[61] Phase-inversion techniques have been used to prepare such microporous polymeric membranes with well-controlled and well-defined pore sizes and geometries.[54] Satisfactory mechanical properties make these materials an attractive choice for microporous tissue engineering scaffolds, providing the ability to supplement or replace periosteal flaps.[54]

III. BONE MORPHOGENIC PROTEINS

III. A. Collagen Carriers

At the present time, two BMPs are clinically available, bone morphogenetic protein 7 (BMP-7) and recombinant human bone morphogenetic protein 2. BMP-7 (osteogenic protein-1), which is distributed by Olympus Biotech (Hopkinton, MA), uses a bovine collagen carrier in granular form. Recombinant human bone morphogenetic protein 2, which is distributed by Medtronic (Minneapolis, MN), uses a collagen sponge carrier. The carrier materials slow the release of BMP during administration.[4–7] Collagen sponges and similar carrier materials exhibit excellent biocompatibility and have good cell and macromolecule interactions.[62] Collagen is also an attractive material for use as a delivery vehicle and as a material coating due to its favorable influence on cellular infiltration and wound healing.

Collagen can also be processed in an aqueous base, which minimizes potential contact with toxic chemicals. It is a well-studied material, as aqueous injectable collagen dispersions, powders, sutures, wound dressings, shields, sealants, and spongy implants have been utilized in a clinical setting.[63] Collagen is also a well-known hemostatic agent. Furthermore, it is suitable for carrying pharmacologic agents, including protein-based agents and antibiotics. It should be noted that collagen carriers do have several drawbacks. Bone grafting substitutes require a large amount of BMP to be delivered to the fracture site. rhBMPs are known to become soluble and can escape the delivery site, particularly when administered in large doses.[64] Due to these factors, rhBMP collagen carriers are not ideal materials for sustained rate-controlled delivery of BMPs. The high market price and the need for large doses of BMPs have driven the development of highly controlled delivery vehicles. When used in large doses, BMPs have been shown to cause side effects, including local inflammation during spinal fusion as well as unwanted ectopic bone formation.[65]

III. B. Gelatin Carriers

1. Gelatin as a BMP Carrier—Gelatin is an attractive alternative to collagen as a BMP carrier. It is a natural biodegradable polymer that is used in various medical applications, including skin regeneration,[66] bone grafting,[67] and controlled drug release.[68] Gelatin contains denatured collagen; it also exhibits lower antigenicity than collagen. The isoelectric point of gelatin can be selected to be acidic or basic.[69] This property allows growth factors with several isoelectric point values to be loaded into gelatin, while maintaining the biological activity of these factors. The degradation rate and growth factor release can be varied by controlling the amount of cross-linking in the gelatin, which influences the in vivo rate of enzymatic decay. Thermal stability, resistance to water dissolution, and collagenase digestion are all dependent on the amount of cross-linking that is present.[70] One study addressed the problem of early diffusion and absorption of BMPs through the use of a slow-release gelatin hydrogel layered on top of a biodegradable poly-L-lactide/ecaprolactone copolymer. The results showed that BMP was released over a period of several days, and the formation of new healthy bone was noted in a canine model.[71] Gelatin is also suitable for cell delivery, and this material may be used for tissue engineering of bone and other tissues.[72] The delivery and slow-release properties of gelatin can be combined with other materials to create protein-loaded gelatin microspheres. A recent study examined the effect of incorporating gelatin microspheres within calcium phosphate bone cement (CPC).[73] In this study, the CPC/gelatin composite healed defects more quickly and had a higher bone mineralization rate than CPC loaded with rhBMP-2 without gelatin.[73]

2. Gelatin-Based Absorbable Polymer Matrices—Gelatin can be cross-linked with chitosan to create porous scaffolds for bone tissue engineering. These scaffolds are able to support adhesion and osteogenic differentiation of bone marrow mesenchymal stem cells (MSCs) in a rat model.[74] Gelatin/beta-chitosan porous scaffolds can be used to immobilize the amine groups of rhBMP-2 on the carboxylic groups of the scaffold surface, and this material may be used for dental applications.[75] Gelatin scaffolds can also be used to carry lyophilized adenovirus encoding BMP-2 (AdBMP-2). A recent study points to the possibility of using lyophilized viral BMP and gelatin to prepare premade constructs for the treatment of bone defects.[76]

Another notable gelatin scaffold is an injectable product called E-Matrix (Pioneer Surgical, Marquette, MI), which is derived from porcine collagen. E-matrix contains gelatin that is copolymerized with a high-molecular-weight branched glucose polysaccharide (dextran).[77] The large carbohydrate molecules stabilize unwound collagen strands, which typically exhibit helical structures. The scaffold attempts to mimic the open extracellular matrix structure of embryonic mesenchymal tissue to encourage increased interactions between

cells and the scaffold surface. Ionic and molecular interactions hold the copolymer matrix together. Polar amino acids (e.g., cysteine, glutamic acid, arginine, and lysine) also provide structural stability. The exposed polar amino acid binding sites are thought to interact with host cells, which participate in the growth and repair of bone as well as other tissues. Polar amino acid sequences are typically obscured from host cells in the extracellular matrix by the tightly wound triple-stranded helix of collagen.[77] The modified open gelatin scaffold has the potential to promote tissue-specific responses depending on growth factor loading and enhanced cellular responses (e.g., the osteoinductive response to BMPs). In a recent study, E-matrix was used as a rhBMP-2 carrier in a rat spinal fusion model, and the results showed that it enhanced spinal fusion.[78] Overall, gelatin scaffolds and sponges have promising futures for use as BMP carriers. They have been shown to be effective in several animal models, including rabbits[79] and mice.[80] In addition, they are an attractive alternative to collagen BMP carriers for clinical applications.

III. C. P24 Oligopeptide

Even if delivery can be provided in a controlled manner, natural BMPs have several shortcomings that limit their clinical use. For example, BMPs are susceptible to rapid degradation, and they exhibit complex structures, limited availability, and the potential to contribute to unwanted reactions.[81] BMP-2 is known to have the strongest ability to induce formation of new bone.[82] It has two subunits, which are antigenic determinant epitopes that bind to either receptor type I or type II.[83] These epitopes are known as the “wrist epitope” and the “knuckle epitope.” The knuckle epitope of BMP-2 binds to BMP receptor type II.[84] The BMP-2 receptor is thought to merge at the 73–92 peptide of the knuckle epitope of BMP-2.[82] An alternative to using costly natural BMPs involves creating a synthetic version of this functional region. The most well-known approach uses solid-state synthesis and chromatography to achieve high yields of oligopeptide P24, which is designated the number 24 because it contains a 24-amino acid sequence from the BMP-2 functional region. Several studies investigated the feasibility of coupling the P24 oligopeptide to alginate, and these materials have been evaluated in in vivo studies involving small animals. Synthetic P24 has been shown to significantly increase osteoinduction on mineralized recombinant collagen, nano-hydroxyapatite/recombinant human-like collagen/poly(lactic acid) (nHA/RHLC/PLA) porous scaffold,[85] and PLGA materials[86] when implanted into rat and rabbit models.[87] Another study suggested that P24 exhibits the same biological activity as natural BMP-2, and the results indicated that P24 and natural bone morphogenetic protein 2 showed equal potential to induce ectopic bone formation.[88]

IV. ALLOGRAFTS

IV. A. Vascularity and the Periosteum

Allograft material (i.e., cadaveric bone) is commonly used for bone replacement. Allograft material is utilized in a wide variety of structures, including whole bone segments, demineralized matrix, and bone chips. One benefit of allograft material over autograft material is that a donor site is not required. In a follow-up study that involved the use of autogenous iliac crest bone graft and banked allograft bone in scoliosis surgery, patients receiving allograft bone demonstrated significantly better postoperative results. Three months after surgery, half of the patients receiving autograft material had physically limiting donor site pain at the wound site. On the other hand, patients receiving allograft material had successful results and returned to their preoperative level of function soon after surgery.[89] There are theoretical concerns regarding donor harvesting, donor screening,[90] and allograft material storage. There are also theoretical concerns regarding infection transmission if allograft material is not properly processed. Allograft material is an effective alternative to autograft material for anterior cruciate ligament reconstruction.[91] One study looked at

postoperative surgical site infection 1 year following spinal fusion surgery.[92] The authors reported that no significant difference in the rate of infection was noted among irradiated allograft, nonirradiated allograft, and autograft materials.[92] This result may in part be attributed to the compatibility of cadaver bone with sterilization methods. Another study evaluated the effect of sterilization processes on the mechanical strength of cortical bone allograft material prior to implantation.[93] Cylindrical cortical bone cadaveric materials were sterilized by chemical sterilization; chemical and gamma irradiation; as well as chemical treatment, lyophilization, and terminal sterilization with rehydration. Untreated materials were examined for comparison purposes. The cadaveric materials were subsequently tested to failure by means of axial compression, diametral compression, shear, and bending studies. No significant differences in ultimate stress, strain, or fracture energy data among the groups were noted.[93] Allograft materials are associated with some shortcomings. For example, these materials exhibit limited resorption and new bone replacement, which tends to occur at the periphery of the allograft material. The challenge for allograft materials is to achieve well-vascularized new bone by means of a process known as allograft revitalization. Retrieval studies involving human subjects have shown only 15–20% replacement with new bone after 5 years.[94] Implanted allografts demonstrate good soft tissue attachment, and 80% coverage of graft surface area after 2 years has been noted. In addition, they become enveloped by well-vascularized muscle. On the other hand, allograft failure is often associated with insufficient vascularity. A study involving the use of allograft materials in rabbits showed no vascularization in these materials despite the fact that the allograft materials were combined with autograft adipose-derived stem cells.[95] Additional studies have examined a combined approach and have obtained different outcomes. A more successful study involving a combination of adipose-derived stem cells, rh-BMP-2, periosteum, and structural bony porcine mandibular allograft constructs suggested that periosteum plays a significant role in determining allograft vascularization. The allograft constructs were implanted within a periosteal envelope after rib extraction (thoracic) or wrapped within rectus abdominis muscle. The rectus abdominis implants showed little vasculature and were encased within scar tissue. On the other hand, the periosteal envelope implants resembled normal healthy bone and were superior in many ways. All of the periosteal envelope implants had bony processes in development between them and the native ribs. In addition, these materials were more firmly affixed and had predominately smoother surfaces.[95] Although the individual effects of rhBMP-2, stem cells, and periosteum could not be determined, the study underscored the importance of periosteum. In addition, the rectus abdominis implants showed limited new bone growth, which occurred in locations with sufficient vascularity. On the other hand, the periosteal envelope implants were highly vascular throughout, suggesting that the periosteum was a critical factor in establishing a good vascular supply.[95] It is important to note that the origin of the periosteal graft material plays an important contribution. The use of periosteum has been studied extensively for use in chondrogenesis. The chondrogenic potential of periosteum is known to significantly vary based on the donor site, and iliac grafts show the highest potential. On the other hand, skull grafts show almost no chondrogenesis.[96] This variation is likely caused by differences in MSC amounts for various locations and by differences in the structure of periosteum found in various locations. In vivo studies involving animal models have indicated the possibility of optimizing periosteal graft performance by pretreatment with transforming growth factor-beta 1 (TGF-β1)[97] or insulin-like growth factor-1 (IGF-1)[98] via subperiosteal injection. Although the studies focused exclusively on osteochondral defects, they suggest possible use in fracture treatment. For example, TGF-β injection resulted in increased extracellular matrix production in the region surrounding a bone graft.[99]

Periosteal cells have been shown to be biocompatible with synthetic and natural scaffolds in vivo.[100] Furthermore, these cells have been shown to induce new vascularized bone formation on scaffolds in animal studies. The periosteum is composed of an osteogenic layer

that is known to contain MSCs, a fibrovascular intermediate that contains fibroblasts and endothelial cells, and a collagenous outer layer. These layers contain cellular components that promote osteogenesis and vascularization. In addition, these layers contain collagenous matrix, which is vital to the success of a bone graft. One study proposed a technique that gently inverts the periosteum in a manner that allows the collagenous layer to contact the bone and allows the osteogenic layer to contact the graft material and the surrounding tissue.[101] Although inverted periosteum has less stem cell potential than bone marrow aspirate, it is considered to be a viable supplement to bone graft material.[101] A human case study using a combination of adipose-derived stem cells, BMP-2, allograft material, and periosteum resulted in healthy lamellar craniofacial bone, obviating the need for osteocutaneous free flaps or additional allograft material.[102] Another study stressed the importance of harvesting the periosteum with the cortical components, which contain the cambium layer that is thought to be responsible for osteogenesis. This study further confirmed the efficacy of periosteal flaps, and a75% success rate in treating recalcitrant nonunions that were resistant to conventional therapies, including cancellous autografts and cadaveric allografts, was demonstrated.[103] Such findings suggest the possibility of preparing periosteal grafts for both allograft enhancement and synthetic graft enhancement in situ. Periosteum supplements are a promising solution to poor vasculature for both synthetic materials and allograft materials, and these materials may be used in combination with BMPs and stem cells. If available, periosteum should be utilized as a graft or preserved at the site of implantation for improved graft vascularity and osteogenesis.

V. CONCLUSIONS

The ideal alternative to autogenous bone grafts is a combination of existing materials that impart the desirable characteristics of the component materials. The surface should exhibit optimal permeability, cellular ingrowth, and cellular differentiation characteristics. Porosity should also be optimized in terms of size, shape, distribution, and interconnection. Delivery of BMPs should be through the use of slow-release carriers as well as shorter, more precise peptide sequences, which facilitate controlled delivery and decrease the unwanted effects of natural BMPs, respectively. In addition, allografts and synthetic materials stand to benefit from periosteal supplements for improved vascularity.

Acknowledgments

Roger J. Narayan acknowledges financial support from the National Institutes of Health (Project Title: Autonomous Treatment of Opioid Abuse, Project Number: R21DA026980).

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