A Review of Materials, Fabrication Methods, and Strategies Used to Enhance Bone Regeneration in Engineered Bone Tissues

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Abstract: Over the last decade, bone engineered tissues have been developed as alternatives to autografts and allografts to repair and reconstruct bone defects. This article provides a review of the current technologies in bone tissue engineering. Factors used for fabrication of three-dimensional bone scaffolds such as materials, cells, and biomolecular signals, as well as required properties for ideal bone scaffolds, are reviewed. In addition, current fabrication techniques including rapid prototyping are elaborated upon. Finally, this review article further discusses some effective strategies to enhance cell ingrowth in bone engineered tissues; for example, nanotopography, biomimetic materials, embedded growth factors, mineralization, and bioreactors. In doing so, it suggests that there is a possibility to develop bone substitutes that can repair bone defects and promote new bone formation for orthopedic applications. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 85B: 573–582, 2008

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INTRODUCTION

According to the AAOS (American Academy of Orthopedic Surgeons), there are ~6.3 million fractures each year in the United States. In addition, the costs associated with these fractures are extremely expensive. In fact, in 2005 there were more than 500,000 bone graft procedures performed in the U.S. costing ~$2.5 billion.1 About 90% of these procedures were involved with the use of either autograft or allograft bone tissues. The current standard tissue used is autograft tissue, which is usually harvested from the iliac crest of the patient. Although autografting has been a major treatment, it has several limitations including patient pain, cost, and limited supply. As an alternative, allografting has been studied due to its abundant source. However, its drawbacks, including the uncertainty of compatibility and disease transmission, have limited its use. As society finds many more baby boomers on the list of fracture patients, we need to search for better replacements for use in bone grafting procedures.

To overcome limitations of autografting and allografting, bone scaffolds or bone engineered tissues made from biocompatible materials and bone cells have been developed. Several bone graft substitutes have been designed over the last decade, and these bone grafts can be clarified to different groups by a base material as suggested by Laurencin et al.1 These include factor-, cell-, ceramic-, and polymer-based bone graft substitutes. For example, factor-based bone grafts consist of natural and/or recombinant growth factors used alone or combined with other materials, whereas polymer-based bone grafts are involved with degradable and nondegradable polymers used alone or combined with other materials. Many advances, new materials, and novel approaches to produce bone graft substitutes continue to be discovered and investigated. The reader is referred to several recent review articles on allograft bone graft substitutes,1,2 allowing this review article to focus primarily on a promising bioengineering approach, bone tissue engineering (BTE), to develop bone graft substitutes for the treatment of bone diseases. In particular, factors used in bone engineering tissues, techniques employed to form bone scaffolds, and strategies to enhance
bone growth in these scaffolds will be discussed in this review article.

MATERIALS, CELLS, AND SIGNALS USED IN ENGINEERED BONE TISSUES

Several criteria have been followed to develop an ideal engineered bone tissue that could attain success in bone graft procedures. A scaffold needs to be integrated with the surrounding bone tissue and provide the initial three-dimensional (3D) framework upon which cells may adhere, proliferate, and eventually produce extracellular matrix (ECM) proteins. For the scaffold to integrate with surrounding tissue, it should mimic the structure and morphology of the natural bone tissue. The bone structure is composed of inorganic hydroxyapatite (HA) and the organic matrix containing mostly (~95%) collagen type I. The morphology of the bone has also been described as a porous (50–90% porosity) tissue depending on the bone type.3 In addition, in order to provide a proper 3D structure, several important requirements for scaffolds are as follows: (i) biocompatibility, (ii) a design that closely resembles the natural extracellular structure, (iii) a highly porous microstructure with interconnected pore networks to allow cell in-growth and reorganization, (iv) appropriate surface chemistry to promote cellular attachment, differentiation, and proliferation, (v) sufficient mechanical strength to withstand in vivo stresses and physiological loading, and (vi) controlled degradation consistent with sufficient structural integrity until the newly grown tissue has replaced the scaffold’s supporting function. The pore size, distribution, and structure should also be tailored to produce specific application requirements as they strongly influence cellular adhesion, proliferation, and matrix deposition as well as the formation of blood vessels within the scaffolds to help tissue ingrowth.4,5 To produce ideal bone scaffolds according to these requirements, three major factors: materials, cells, and signals, used in BTE will be discussed in detail in the following section.

Materials

Several biomaterials, including bioceramics, biopolymers, metals, and composites, have been used in BTE to form bone scaffolds. For each material used, there are properties of resorption, surface reactivity, and biocompatibility that play into their effectiveness to house cells and enhance cellular adhesion and proliferation. Of those materials, bioceramics have been well investigated due to their effectiveness. Bioceramics including hydroxyapatite (HA), Bioglass, A-W glass ceramic, and β-tricalcium phosphate closely mimic bone tissues. These materials also provide an environment where bone ECM proteins are absorbed, resulting in osteoblasts adhering and proliferating more rapidly than other materials including titanium.6-8 An in-depth review of various mechanisms that are responsible for increased cell attachment, growth, and bone/implant integration on bioceramics has been discussed by Ducheyne and Qiu.7

In addition to bioceramics, several biopolymers have also been utilized. One of the most widely used materials from the past decade is a group of synthetic polymers and their copolymers known as polyesters. These polymers include polylactic acid (PLA), polyglycolic acid (PGA), and the copolymer poly (lactic-co-glycolic) acid (PLGA). These materials have played a large part in BTE due to their biocompatibility and ease of manufacture into different shapes.9 The remarkable property of these polymers is their ability to support mechanical needs for a wide variety of applications such as screws and fixation devices in orthopedics. Degradation is another important property of these materials. In an ideal case (i.e. an ideal biodegradable material property), as the growth of bone into the scaffold promulgates and the bone cells naturally build an infrastructure, the initial supporting scaffold is degraded and a handoff of mechanical stress and strain is passed onto the neo-tissue structure.10 In addition to polyesters, other synthetic polymers also have significance in BTE, including poly(methyl methacrylate), poly(ε-caprolactone), polyhydroxybutyrate, polyethylene, polypropylene, polypeptide, polyurethane, poly(ethylene terephthalate), polyetherketone, and polysulfone.

Besides bioceramics and polymers, metallic alloys have also been used in orthopedic implants for their strength >100 GPa for an elastic modulus), especially in load-bearing areas.11 The metal alloys used in BTE include cobalt-chromium, stainless steel, aluminum, and titanium alloys. The advantages of metallic alloys include a light-weight nature and strength and biocompatibility. Although metallic alloys have been used to produce bone prostheses, these materials have several limitations. These drawbacks are permanence, cracking, and the potential of releasing metallic ions and introducing corrosion products into the body from these materials, raising concerns for their use.12 Unlike biodegradable materials, metals cannot be used to produce a complete tissue replacement for bone defects because of their permanent property. In addition, the released (wear) metal particles have been found to affect the release of inflammatory factors, inhibit bone formation markers, and stimulate bone loss or resorption. For example, studies have shown that titanium and its alloy particles inhibit bone-cell proliferation and reduce bone formation markers.13 An increase in the release of inflammatory factors such as interleukin-6 and transforming growth factor (TGF-β1) has also been noted.14,15 Moreover, metal implants have less integration with the tissues surrounding the implant site because there is a significant difference in stiffness between metal implants and bone tissue. These metal implants are often stiffer than the natural bone, leading to a stress shielding effect that causes poor osseointegration.

Bioactive composite materials have recently been developed to incorporate the strengths of two or more distinct
Most composite materials are used to produce specific strength, stiffness, or toughness properties that match natural bone. The cortical bone has a wide range of mechanical properties: 7–30 GPa for Young’s modulus, 50–150 MPa for tensile strength, and 1–3% for elongation at fracture. Biocompatibility is also provided by at least one of the constituents making the composite materials bioactive. For instance, hydroxyapatite reinforced high density polyethylene (HA/HDPE) composite and PLGA/HA composite materials have been developed to mimic the structure and match the properties of the bone, suggesting these materials as good matrices for bone cell differentiation and mineralization. In vitro and in vivo studies of these composites found that osteoblast cells adhered to HA preferentially, leading to subsequent cell proliferation, and the composite implants displayed good bone integration and osteoconductivity in rabbit models. The rationale for designing composite materials is to provide many attractive properties that each individual material cannot have. For example, in the case of metal-matrix composites, the metal will provide the strength to bear large loads, whereas polymeric materials produce the environment for the incorporation of bioactive molecules and integration of surrounding bone cells at the implant sites. In general, several materials and bioactive composites have been investigated for bone graft substitutes over the last decade. Each of these materials has its distinctive characteristics and advantages, yet it remains a great challenge to produce a material that is similar to nature and that bone cells like to grown on.

Cells

In addition to materials, cells have been used in BTE for seeding in bone scaffolds before implantation. Numerous cell sources, such as embryonic stem cells, bone marrow stromal cells, and muscle-derived stem cells, have been investigated in BTE. One of the most used cell sources is bone marrow stromal cells. Adult stem cell sources such as human bone marrow are used in BTE to produce autologous bone tissues and to avoid ethical issues and the immune responses generated by the human body against cells isolated from different sources. Bone marrow consists of hematopoietic stem cells and mesenchymal stem cells, which can differentiate into various cell types including osteoblasts. Bone marrow has also become a large source of osteoblasts because of the availability and ease of harvesting bone marrow from animals for research purposes in BTE.

To detect the success of bone cell proliferation and differentiation within scaffolds, several assays and biomarkers have been studied. To assess cell proliferation, DNA assays are done fluorimetrically using various dyes (e.g., Hoescht, PicoGreen) bound to the cell DNA, which is proportional to the cell density. H-thymidine is also used to label cells and can be detected radioactively when cells proliferate. To study bone cell differentiation, several biomarkers exhibiting the osteoblastic phenotype have been used. For example, assays for osteocalcin, an ECM as a bone formation marker, are performed using Iodine-labeled osteocalcin, which can eventually be detected in a gamma counter. Alkaline phosphatase (ALP), an enzyme found in bone tissue, is also studied as a bone formation marker using ELISA. Furthermore, stainings for phosphate (Von Kossa method), calcium (Alizarin Red S), and ALP (immuno-staining) have been used to reveal these biomarkers in cell cultures and sectioned tissues.

Signals

Besides materials and cells, several signals such as media additives and chemical cues have been investigated to guide bone cell differentiation and proliferation in BTE. To differentiate bone marrow stem cells into osteoblasts, the medium used for cell cultures and bone scaffolds often includes chemical differentiation additives. The most prominently used agent is dexamethasone (Dex). Dex has been shown to promote marrow cell differentiation and express bone marking proteins such as ALP, osteopontin, and osteocalcin. Other additions to bone specific media include β-glycerolphosphate and L-ascorbic acid.

Chemical cues such as growth factors have also been used to enhance bone growth within the scaffold. The commonly used growth factors include TGFs, bone morphogenic proteins (BMPs), pleiotrophin, platelet-derived growth factors, insulin-like growth factors, and fibroblast growth factors (FGFs). TGFs with their most common isoform, the β-isoform, have been useful in augmenting the growth of bone tissue. For example, TGF/βs (types 1, 2, 3, and 5) stimulate osteoblast proliferation, mineralization, and matrix structures. They also play a key role in bone resorption and bone formation. In addition to TGFs, BMPs including BMPs 2–7 also induce the formation of bone, cartilage, and connective tissues. For critically sized bone defects, BMP 2 shows the most promise, while both BMP 7 and 2 are successful in large defects. Furthermore, pleiotropin, a cysteine-rich peptide known as a signaling molecule for bone formation, and hyaluron demonstrate the ability to promote adhesion, migration, and differentiation of human osteoprogenitor cells.

FABRICATION METHODS

Several fabrication processes have been utilized to manufacture bone scaffolds that meet the earlier requirements. Although various traditional fabrication techniques such as solvent casting are available; the production of scaffolds using these methods lacks consistency and reproducibility. Recently, with the use of modern techniques like rapid prototyping (RP), it is possible to mimic naturally-occurring scaffolds, even with their complex structures. The purpose of this section is to provide a quick overview...
of some traditional procedures, like material injections and solvent casting, and to expand upon new fabrication techniques, such as RP, to produce bone scaffolds for BTE.

**Material Injections**

Injectable pastes have been developed for use as a fixation material or to fabricate bone constructs for cell ingrowth or preseeding. Injectables have an advantage over preshaped scaffolds in that they are minimally invasive for surgical procedures and can conform to any shape that they are pressed into. In this method, polymerization can be done *in situ*. For example, a poly (propylene fumarate) solution can be injected and polymerized at the site of a bone defect to fill in any defect shapes. Studies have also shown that the chitosan-calcium phosphate composite is a good material for an injectable, resorbable scaffold. The chitosan tends to be in a paste form at a pH value of 6.5, and when injected into the body it undergoes a phase transition and becomes a solid ceramic at the defect site. Furthermore, an injection of hyaluronic mixed with FGF at the site of a fracture in a rabbit fibula showed increased bone formation and good mechanical strength in a short period of time. Generally, properties of the injectable materials, such as viscosity, setting time, and initial mechanical strength, play a large part in their success. The major advantages of this method are as follows: (a) the material can fill the gaps in the defect irrespective of the shape of the defect, (b) it is possible to incorporate bioactive and therapeutic agents by mixing them together with the injectable materials, and (c) there is no need for a surgical procedure to replace the material.

**Solvent Casting**

Solvent casting (i.e. particulate leaching) has shown promise for its ability to produce scaffolds at room temperature. In this technique, a scaffold is produced by adding a polymer solution (e.g. PLA or PLGA dissolved in an organic solvent such as chloroform and methylene chloride) into a mold containing a solid porogen (e.g. salt crystals). These salt crystals are generally insoluble in the organic solvent. During subsequent processing the porogen is leached out by immersing the scaffold in water to form a pore structure within the scaffold. In this method, the pore size and porosity of scaffolds can be varied by changing the size and morphology of the salt crystals. Scaffold properties, such as polymer degradation time and mechanical strength, can also be altered by changing polymer concentration and the amount of salt crystals. For instance, increasing polymer concentration has been found to result in induced scaffold compression modulus, whereas scaffolds fabricated with higher amount of salt crystals with smaller sizes were more brittle than others. Although solvent casting has been effective to produce scaffolds sufficiently strong for bone implantation, it lacks reproducibility and the ability to provide designed pore geometries and morphologies. In addition, the thickness of scaffolds made by this technique should be less than 4 mm to have a uniform pore structure, making it more difficult to fabricate a large 3D scaffold. To overcome this, lamination of several highly porous membranes has been used. A successful 3D scaffold made of PLLA and PLGA was prepared using this laminating technique, and the pores were interconnected forming a continuous pore structure.

**Rapid Prototyping**

To overcome the limitations of conventional fabrication methods, rapid prototyping (RP) technologies (a.k.a. solid freeform fabrication or additive manufacturing technologies) have been developed and increasingly applied in BTE. The process of producing an RP part begins when an object is scanned using a computed tomography (CT) scanner or modeled in a Computer-Aided Design (CAD) software package. The CAD file is typically converted to an STL file, the standard RP file format, which represents the model as a collection of surface triangular patches. The STL file can be scaled to compensate for shrinkage and/or rotated and translated along any geometric axis to orient the part for optimum manufacturing. This file is then numerically sliced into two-dimensional (2D) cross-sections, which are then fabricated and stacked in such a manner that the 3D object is formed and the product is finished. The accuracy of the 3D object relative to the digital data is a function of the accuracy of the process which forms and stacks the 2D cross-sections as well as thickness of the cross-sections.

RP technologies offer several advantages over conventional scaffold fabrication techniques for producing scaffolds, due to the fact that both the geometry and material composition can be controlled. Using the RP manufacturing approach, it is possible to create scaffold geometries, which are reproducible, reliable, geometrically complex, and highly-customizable. Because of the additive nature of RP, tissue engineers are also able to custom design a desired level of complexity into the scaffold, which is a capability not available using traditional manufacturing methods. In addition to the geometric flexibility, some RP machines have material flexibility such that multiple materials, including living cells in some cases, can be deposited to form a tissue engineered structure. These types of tailored multi-material structures are not possible using traditional methods, which produce only single material structures.

RP technologies have been used in various applications including the manufacturing of medical devices, controlled drug delivery systems, and engineered tissues. Of these, engineered tissues is a growing application area for RP technologies. Various processes have been successfully employed to fabricate 3D scaffold structures using biodegradable materials. In addition, a number of investigators have successfully produced various artificial
TABLE I. Advantages and Disadvantages of Common Rapid Prototyping Techniques. 39,50,54,55,67

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Stereolithography</td>
<td>Hydrogel materials, high-resolution and accuracy, liquid build material can easily be removed from within complex scaffolding</td>
<td>Limited choice of materials, may require furnace postprocessing (e.g. bioceramics), high material cost, complex and expensive equipment</td>
</tr>
<tr>
<td>Laser sintering</td>
<td>Wide range of material choices, good mechanical properties, lower material cost, good accuracy</td>
<td>Materials may thermally degrade during the process, undesired porosity, hard to remove trapped powder, complex and expensive equipment</td>
</tr>
<tr>
<td>3D printing</td>
<td>Wide range of material choices, low cost, quick process, multimaterial capabilities through multi print-heads</td>
<td>Hard to remove trapped materials, low to Medium resolution, powder particles may not bind well, binders are always necessary to bind powders</td>
</tr>
<tr>
<td>Fusion deposition modeling</td>
<td>No trapped materials, minimal material waste, low cost</td>
<td>Materials may thermally degrade during the process, lower range of material choices, medium resolution</td>
</tr>
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Various methods for RP have been developed over the past 20 years starting with the advent of stereolithography in 1986. Several common RP processes, which have been used for tissue engineering, are overviewed below to illustrate the variety of processes and techniques in RP. The advantages and disadvantages of common RP techniques are shown in Table I.

**Stereolithography.** Stereolithography (SLA) was the original RP process introduced in the late 1980’s by 3D Systems Incorporated. SLA utilizes the ability of photopolymerizable liquid polymers to solidify when ultraviolet light is focused on the surface of the liquid. A platform supporting the developing model is brought near the surface of a liquid vat, and a UV laser is used to solidify the first cross-sectional layer of the model to the platform. By lowering the platform one cross-sectional layer thickness, recoating the liquid on top of the first layer, and then passing the UV light over the new liquid layer, a second layer is deposited. The process is repeated layer by layer until completion, after which the prototype is typically cleaned and completely cured by placing it in an ultra-violet oven. 50,55,68 Several biopolymers have been utilized in SLA. For example, an aqueous poly(ethylene glycol) (PEG) hydrogel solution has been processed and research results have been shown that the hydrogel is capable of preventing human dermal fibroblast cells encapsulated in the solution from damage during the SLA process. 69 Thus, by combining the SLA process and biopolymers such as PEG, it is possible to fabricate complex 3D structures with bioactive agents embedded within the structure. Although biocompatible materials used in SLA are typically biopolymers, considerable research has been conducted on the use of bioceramics. A bioceramic suspension is first prepared by adding bioceramic powder into a photopolymer before SLA processing. The photopolymer acts as a binder to maintain the geometry of part during fabrication. The suspension is cured during the SLA process, and a “green part” is produced. The “green part” is postprocessed by heating in a furnace to remove the photopolymer binder and sinter the bioceramic into a porous network.70

**Selective Laser Sintering.** In selective laser sintering (SLS), parts are made by passing a laser over a thin layer of polymer powder. The laser will raise the temperature of the powders, which will cause neighboring particles to fuse together both laterally and to the preceding layer below. Once a layer of polymer is fused, an elevator platform supporting the part is lowered by the height of the next layer and a roller applies new powder over the previously processed layer. Post processing of the final part can include dissolving out unsintered particles (for instance, using a calcium phosphate solution) to leave microporosity within the structure. HA and calcium phosphate particulates with a polymer binder have been used in SLS as possible implant materials. 51,71 SLS has also been used to manufacture bone scaffolds from a HA/PLLA composite with PLLA selected as a binder due to its higher degradation time and lower melting temperature. Results showed that the elastic modulus of HA/PLLA parts range between 140.47 and 257.27 MPa, whereas the bending strength ranges from 1.57 to 4.05 MPa, which is comparable to the modulus and bending strength of cancellous bone. 72 Other SLS research has employed HA/HDPE composites. 73 The disadvantages of SLS compared to SLA are a rough surface finish, lower dimensional accuracy, and porosity. 74 However, the ability to directly sinter biocompatible materials without a high-temperature post-processing step gives SLS inherent material advantages over SLA.

**3D Printing.** 3D Printing uses ink-jet printing technology to precisely place a “binder” solution on a bed of powder, thus gluing the powder together in a cross-sectional layer, much as the lasers in SLS use heat to bind powder layers. Conceptually, any powdered material, including polymers, metals, or ceramics can be fused using an ink-jet head...
which passes over and applies droplets of a binder solution to the powder. A platform is lowered and more powder is deposited and processed in a layer by layer fashion. The unbound powder in the void spaces of the prototype can then be removed by compressed air or by manually brushing it away. A number of studies have investigated the application of 3D printing technologies for direct and indirect manufacturing of scaffolds from various materials.65,75–77 By varying the composition of the “glue” which is printed (much like colors can be varied in a traditional color ink-jet printer), the composition of the resulting scaffold can be varied as well in three dimensions.

**Fused Deposition Modeling.** Fused deposition modeling, another RP technology used for tissue engineering, uses a small temperature-controlled orifice to extrude filament material and deposit semimolten polymer onto a platform, which immediately solidifies. At the end of the deposition of each layer, the platform is lowered so that the next layer can be deposited. By changing the direction of each layer and the spacing between materials, scaffolds with highly uniform internal structures are obtained.64,78 Using multiple heads or other deposition processes simultaneously, a multi-material scaffold can be created with complex internal geometry.

Several other variations of RP processes have been developed and are being used for scaffold fabrication. Most of these processes are based either on material printing, extrusion, or spraying techniques, which involve multiple deposition heads or material feeders so that multimaterial scaffolds can be formed, which are capable of optimizing both geometric and material composition simultaneously.67,79–86

**STRATEGIES TO ENHANCE BONE GROWTH**

To enhance bone growth in scaffolds, several strategies have been developed. These include generation of nanotopography on the scaffold surface, development of biomimetic materials, formation of mineralized layers on bone scaffolds, and utilization of bioreactors for cell seeding and feeding. The principle of these approaches is to mimic the natural environment in which bone cells grow. Studies on nanotopography, biomimetic materials, embedded growth factors, mineralization, and growth environment of bone scaffolds are discussed in the next section.

**Nanotopography**

Several studies have shown that bone grafts with nanotopographic surfaces (or nanotopography) similar to native tissues have better implant-tissue integration.87 In native bone tissues, bone cells are exposed to substrates and structures with nanoscale features, such as ECM proteins, minerals, and pores in membranes and tissues.87 By mimicking this nanotopography, researchers hope to enhance bone cell growth and tissue integration. In fact, nanotopographic titanium surfaces obtained by chemical treatment with H2SO4/H2O2 increased both bone tissue growth and ALP activity.88 Similarly, TiO2-coatings that contained substantial “surface pores” at dimensions of 15–50 nm promoted the formation of bone mineral-like calcium phosphate as well as induced tissue attachment.89,90 Furthermore, calcium and phosphorus mineral contents are induced more in nanophase Ti6Al4V compared to microphase Ti6Al4V.91 One interesting study investigated the osteoblastic cell response to nanostructured islands (11, 38, and 85 nm produced by a polymer-demixing (polystyrene/polybromostyrene) technique) and found that the smaller nanoisland produced an increase in cell adhesion and proliferation as well as in ALP activity.92 Nanotopography was also a significant factor for differentiation of mesenchymal stem cells (or osteoprogenitor cells) to osteoblastic phenotype.93 Results from these studies suggest that nanotopography of bone scaffolds will stimulate bone formation and enhance bone-implant integration, leading to better tissue repair and regeneration at the bone implant-biomaterial interface.

**Biomimetic Materials**

Besides nanotopography, biomimetic materials have been investigated to increase cell adhesion and proliferation into a scaffold.94 In nature, osteoblasts often attach or adhere (spread) onto a surface based on their cell membrane proteins. The main family of these membrane proteins is integrins, which are composed of two chains, an α-chain and β-chain. Osteoblasts have been shown to express α1, α2, α3, α4, α5, α6, αv, β1, β3, and β5 integrin subunits. These integrins mediate cell attachment and regulate cell migration, growth, differentiation, and apoptosis. Several attempts have been made to develop materials that mimic integrin-binding in various biological systems. For example, a three peptide sequence of arginine-glycine-aspartic acid (RGD) bound to several of these cell membrane integrins has been incorporated into a scaffold to enhance bone cell adhesion and growth.95 In addition, ECM proteins such as collagen I, laminin, fibronectin, vitronectin, and fibrinogen as well as peptides designed from these proteins have been applied onto scaffolds to induce cell adhesion and proliferation.94,95 Of these, fibronectin and vitronectin have been viewed as the optimum adhesion proteins for osteoblastic cells.94 Advances in biomimetic materials have been discussed in detail by Shin et al.96

**Embedded Growth Factors**

In addition to nanotopography and biomimetic materials, growth factors may also be used in conjunction with scaffold materials to enhance bone ingrowth in scaffolds. In the case of nonunion fractures, growth factors, most notably BMPs, are needed for enhanced healing.97,98 In the past, delivery of these growth factors using carrier matrices to the site of concern was needed for successful bone recon-
Mineralization of Bone Scaffolds

Another strategy to enhance bone ingrowth is generating mineralized layers on bone scaffolds. Since inorganic minerals have been found in biological bone structures, researchers have manipulated implant surfaces to induce a bone-like structure (mineralization) in which new bone may form after implantation or reconstruction procedures.\textsuperscript{101,102} For instance, implant surfaces modified with an apatite layer are favorable to induce osteogenesis.\textsuperscript{102–105} Materials containing the functional groups Si—OH, Ti—OH, Zr—OH, Nb—OH, and Ta—OH have been shown to form apatite layers in the presence of an acellular protein-free simulated body fluid.\textsuperscript{102,104} As apatite particulates are generated on synthetic materials, new bone may form on this mineralized layer, and implants may be stabilized by increasing material-bone interface strength.

Growth Environments (Bioreactors)

A novel addition to the field of BTE is the augmentation of bone ingrowth by generating mineralized layers on bone scaffolds. Since inorganic minerals have been found in biological bone structures, researchers have used bioreactors to enhance cell proliferation in bone scaffolds in various studies.\textsuperscript{110} Recently, researchers have used bioreactors for cell seeding\textsuperscript{111} and circulation of media through 3D scaffolds for waste removal and nutrient diffusion.\textsuperscript{112–115} Oscillating perfusion of cell suspensions produced higher seeding efficiency, better cell distribution, and stronger cell-matrix interactions.\textsuperscript{111} Bioreactors have also been shown to increase the proliferation of bone cells.\textsuperscript{112} The same group elucidated that increasing shear forces leads to the enhancement of osteoblastic phenotype expression, which increases mineralized matrix production.\textsuperscript{112–114}

**CONCLUSION**

Engineered bone scaffolds have been developed to overcome the limitations of autografting and allografting. The development of successful scaffolds requires appropriate materials, cell sources, bioactive molecules, and fabrication techniques. Challenges in BTE are being overcome by applying novel strategies to enhance bone formation, such as mimicking natural nanostructures. As interdisciplinary fields donate their expertise, new methods for BTE will lead to further successes. This can be seen with the application of RP technologies to BTE.


