



## New frontiers in bone tissue engineering

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Bone tissue engineering is an interdisciplinary field composed of teams of clinicians, engineers, and scientists whose goal is to create a limitless and readily available supply of off-the-shelf bone substitutes [1–3]. Current bone tissue engineered products have largely failed to meet clinical expectations. Surgical procedures to repair defects resulting from congenital craniofacial anomalies, trauma, tumor resection, and degenerative disease continue to require autogenous bone grafting. Until the reliability of bone substitutes matches that of traditional bone grafting, autogenous bone will remain the gold standard despite the problems associated with harvesting of bone grafts from the patient. Bone tissue engineering remains the focus of numerous universities and corporations around the world because of the strategic importance of bone. Recent advances suggest that this global effort will eventually lead to the development of effective and reliable substitutes. This article reviews the need for engineered bone and provides a historical perspective of bone engineering research, current research efforts, and the future direction of this work.

### Clinical need

Plastic surgeons are constantly faced with the difficult task of reconstruction of bony defects [4]. Each different anatomic area offers difficult challenges in terms of the specific needs for bone reconstruction in terms of form and function. Although there have been multiple ingenious solutions to reconstruct these

defects, each of these operations incurs the costs of hospitalization and possible donor and recipient site morbidity.

In the head and neck region, vascularized and nonvascularized bone grafts are used for the reconstruction of a variety of congenital, traumatic, and post-oncologic resection defects. Nonvascularized split calvarium has been used for cranioplasty, immediate bone grafting of traumatic defects, and onlays to the maxillofacial skeleton. The calvarium has also been used for bone grafting of alveolar clefts, in addition to the more common source—the iliac crest. Ribs have been used in a vascularized and nonvascularized fashion for cranioplasty, mandible reconstruction, and contour restoration [5]. Even more complex defects are faced for post-oncologic reconstruction where aesthetics must be considered in addition to primary wound healing and function. In view of the hostile environment where these grafts must survive, with previously irradiated wound beds and exposure to saliva, vascularized bone grafts are more commonly used [6]. Frequent sites for these bone grafts include the fibula, scapula, radius, and iliac crest.

Similar problems are faced in the reconstruction of extremities where bone grafts are used in the treatment of a variety of traumatic and oncologic defects. Vascularized and nonvascularized bone grafts have been used. For smaller bone defects and a favorable wound bed, nonvascularized cancellous bone grafts from the iliac crest, tibia, and radius have been used. For larger bone gaps or a hostile wound bed, a vascularized bone graft such as the fibula is used.

For each of these donor sites, there are several potential difficulties that patients face. Among these are pain, deformity, injury to adjacent structures, length of the operations, scarring, cost of the operation

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as well and prolonged hospitalization, and possible graft loss or resorption.

### Historical perspective

Urist first popularized the concept of a bone-generating protein in 1965 when he made the discovery of bone morphogenetic protein (BMP) [7]. He observed that there must be some substance within the bone that is responsible for inducing and maintaining bone formation. He hypothesized that if this substance could be isolated, it would have the potential to generate bone formation on its own. This concept came to bare the isolation of the bone morphogenetic proteins and osteogenic proteins in later years [8].

However, it was not until the late 1980s that Wozney was able to clone bone morphogenetic protein [9]. This spawned an era of research that held the promise of solving the problem of generating bone *in vivo*. It was thought at that time that within 3 to 5 years after the cloning of this gene that there would be no need to ever take another bone graft. However, this has not been the case. Many studies involving delivery of the BMP-2 for healing of defects in animals have shown initial benefit [10,11], but the results in general practice are still being evaluated.

Since the cloning of BMP-2, multiple BMPs and osteogenic proteins have been isolated and cloned, and recombinant products have been generated for experimental and clinical use [7,9-18]. Other growth factors have been found to be valuable in the bone generation arena. Factors such as vascular endothelial growth factor, insulin-like growth factor 1 and 2, acidic fibroblast growth factor, and basic fibroblast growth factor have been investigated as potential bone-enhancing substances for bone tissue engineering [5,7-41]. These factors have had mixed results depending on the method of use and the model of experimentation. More investigation and evaluation is necessary before clinical use is widely accepted.

Most of the research involving these osteogenic proteins being performed around the world is linked to using them in conjunction with some type of carrier substance and possibly a cell population thought to be able to facilitate the process of bone formation. The materials research associated with the quest for engineered bone has centered on a few key substances. These include tricalcium phosphate (TCP), hydroxyapatite (HA), polylactic acid co-glycolic acid, and polylactic acid. Novel materials are on the horizon and are discussed later in this article.

After Urist used the demineralized bone matrix for the creation of neobone, it was thought that the mineral

component could be used effectively as a bone substitute. Researchers turned their attention to the use of TCP and HA for the creation of new bone. Great progress was made through the ensuing decade as many investigators attempted to form bone using various combinations of demineralized bone matrix, TCP, and HA [3,5,42-44].

Holmes [43,45] made great strides in being able to evaluate the bone formation within engineered bone implants such that the histomorphometric analysis techniques were solidified. This advance allowed others to perform bone genesis research and advance the research through standardized evaluation techniques.

Hollinger [10,11] created the critical size defect model, which remains the gold standard for bone tissue engineering testing. A critical size defect model is an experimental bone defect that will not heal on its own. Hollinger et al worked out the critical size defect in many locations across many species such that any researcher could have a reliable model for testing bone tissue engineering strategies *in vivo*. This work has facilitated the evaluation of the many bone tissue engineering strategies.

Weiland [46,47] began to approach bone formation in HA with the use of autogenous bone marrow cells. The goal of his group was to develop a synthetic vascularized bone graft using ceramic and bone marrow cells. The initial success of Weiland's strategies was tempered by the inability to seed the cells into the central portion of the block of ceramic. Later, Weiss and Calvert [48] patented a solid freeform fabrication method to begin to address this problem (see the section on solid freeform fabrication below).

### Current approaches

The current approach to bone tissue engineering involves the integration of a scaffold material, some type of cell, and growth factors [1,3,49]. This is considered to be the classic tissue engineering paradigm whereby the scaffold provides a base substance for the cells to lay down the matrix of the tissue and the growth factors help direct the cells to the phenotypically desirable type. The construct is implanted into the patient where the normal wound healing and tissue regeneration mechanisms take over the construct and cause the formation of the desired tissue.

This paradigm has certain limitations. It implies that the host bed is healthy and capable of providing all of the normal physiologic functions necessary to have the tissue regenerate. A wound bed that has been radiated or infected might not be able to provide these

"services" to the engineered construct, and the engineered tissue may ultimately fail. Furthermore, if the blood supply of the local wound bed does not integrate into the construct, the cells will die, the scaffold will be nonvascularized and become encased in scar tissue or become infected, and the construct will fail. Thus, as the current components to bone tissue engineering are fairly entrenched, there are many hurdles yet to be cleared on the way to the ultimate bone tissue engineering methodology.

## Materials

The materials used in bone tissue engineering are common surgical materials. These include metals, ceramics, and polymers [1,50-57]. The materials that are used are designed to provide strength, shape, and a functional space in which the new bone can form. There is also a desired functional component to the materials used in these bone tissue engineering strategies. The most commonly used materials are TCP, HA, poly-lactic acid, and poly-lactic co-glycolic acid.

The basic concept with the use of a scaffold material is that the scaffold guides the growth of new bone. In some cases, it is thought that the scaffold itself induces cells to make bone. These properties are known as osteoconduction and osteoinduction. The ideal scaffold material is osteoinductive and osteoconductive. Furthermore, some scaffold materials are absorbable, and others are nonabsorbable. For example, certain forms of HA are not absorbable, whereas others are absorbable. Depending on the application of the materials, these properties may or may not be desirable. Thus, the variable properties of certain scaffold materials must be optimized and matched to the clinical scenario for which they are intended. No single scaffold material is a panacea.

The problems confronting bone materials engineers today are rooted in the limits of nonviable materials. Many of the materials available for bone tissue engineering are brittle. Polymers and ceramics can fracture easily and have poor handling characteristics. They are not easy to place in the body and can be unfavorable environments for cells to occupy. Furthermore, polymers tend to induce a significant foreign body inflammatory reaction, complete with lymphocytic infiltrates and giant cells [2,11]. This is not conducive to formation of a new tissue or to mineralization of bone matrix.

To address these problems, novel approaches to scaffold design must be used. Marra et al have patented a material now known as Caprotite [58]. This material was designed to meet the clinical needs of a

surgeon requiring a bone substitute for the healing of a bone defect. The materials used to create this mixed combination include poly(caprolactone), poly(D,L-lactic-co-glycolic acid), and HA. Each component of this mixture imparts a specific property on the composite.

## Cells

There has been a great deal of focus on the cells under investigation for bone tissue engineering. Stem cells have been highlighted for decades as the answer to the cellular need. Adult stem cells and embryonic stem cells have the potential to answer the need [42,46,47]. However, it is also the method of use that may make the most impact on the field. Corporate production of adult stem cells derived from bone marrow has shown promise in experimental therapy for cancer. Further testing for tissue engineering applications is needed. There is also potential for stem cells derived from adipose tissue to serve this need. In experimental studies, liposuction-derived adiposites have demonstrated the potential to mineralize matrix *in vitro* [59]. *In vivo* testing is pending at this time.

## Growth factors

Growth factors will most likely be a major part of any successful strategy to create synthetic bone. The results of many studies have demonstrated the efficacy of a myriad of growth factors in creating new bone in experimental models and clinical examples. The morphogenetic proteins from the TGF- $\beta$  family and insulin like growth factor family show the most promise [5,7-41,60]. However, the current techniques of delivery are crude and unpredictable. Milligram doses of growth factors are being used in situations such as spine fusion, where the normal concentration of growth factor in the internal milieu is 100,000 fold less. It is important to consider the endless combinations of growth factors that might be used and the limitless methods of delivery. It is this expanding number of combinations that continue to make bone tissue engineering a field popular with cross-specialty collaboration.

## Current limitations

Despite some success in the arena of bone tissue engineering, the limitations of these techniques, materials, and strategies are evident in the clinical

arena. The central issue is how to generate a blood supply within any bone construct such that the new bone functions as normal bone. Blood supply allows the bone to fight infection and to receive circulating factors and nutrition that aid it in long-term incorporation and remodeling at the site of implantation. Without a method of generating an adequate blood supply, bone regeneration cannot occur to the level necessary for the major bony clinical problems.

An issue that has been omitted from many strategies for bone tissue engineering is the concept of regeneration. What is truly desired is to regenerate bone in a way that the new bone is equivalent to normal bone. The closest bone tissue engineers have to come to this is with bone distraction. Bone distraction creates bone that is vascularized, mineralized, and fully integrated into the site where it is required. Because distraction cannot be performed at all wounds, bone regeneration must be mimicked with materials, cells, growth factors, and technical strategies for bone replacement. However, regeneration is poorly understood. There is a complex interaction of timed events and spatial relationships that occur in a dynamic, three-dimensional tissue such as bone. However, present strategies of scaffold, cells, and growth factors mixed together do not approximate what is anticipated as necessary for true bone regeneration. Thus, novel strategies must be examined for their potential to address some of these issues.

#### **Novel concepts: solid freeform fabrication of engineered bone**

The scaffold's external shape and material composition are important; in addition, its internal architecture is critical for proper functioning and optimal performance. The design of the scaffold's microstructure (ie, the size, shape, orientation, and spatial distribution of its internal voids) affects cell seeding efficacy, cell attachment, mechanical properties, nutrient transport, and vascular in-growth. Tissue engineers therefore need manufacturing processes that can precisely build scaffolds according to specified designs.

Solid freeform fabrication (SFF) processes can address this need. SFF refers to computer-aided-design/computer-aided-manufacturing (CAD/CAM) methods that can fabricate, automatically, complex shapes directly from CAD models [61]. SFF processes are based on a layered manufacturing paradigm that builds shapes by incremental material deposition and fusion of thin cross-sectional layers. Although SFF processes are used predominantly for industrial appli-

cations, SFF is also used to manufacture scaffolds with controlled microstructures for numerous tissue engineering applications [3,4,51,62-86].

Beyond the capability to manufacture scaffolds with custom shapes and complex internal microstructures, it is important for next-generation scaffold designs to have complete spatial control over the internal distribution of cells, signaling molecules, and materials. For example, cells migrate along concentration gradients of growth factors; therefore, the capability to directly build in directional gradients would be beneficial (eg, gradients of vascular endothelial growth factors for guiding angiogenesis from surrounding vascularized tissue into the interior of the scaffold). For another example, heterogeneous scaffold designs, such as bone/cartilage/vasculature constructs, would require different cell types and material compositions in different regions.

SFF has the potential to enable complete spatial control scaffold composition by incorporating selective material deposition processes and by simultaneously and selectively adding cells and growth factors to the layers as the scaffolds are being built up [4,49,64,70,71,75,78-80,87-92].

Calvert and Weiss are developing a new SFF process for tissue engineering based on a patented assembly method [4]. Relatively thin, prefabricated cross-sectional layers and layer segments of scaffolding are stacked up to form three-dimensional structures by mating layers together with miniature biodegradable fasteners. With this assembly approach, each prefabricated section can be seeded with cells or growth factors before final assembly. Then, normal tissue growth across the layers, *in vitro* or *in vivo*, fuses the assembly together as the scaffold degrades. The assembly approach permits the manufacture of heterogeneous scaffolds that are composed of various materials with different microstructures, cell types, and growth factors used in different sections of the assembly. This method is useful for working with scaffold materials that require heat or toxic chemicals to form the materials because these processes precede cell seeding operations in the assembly approach. Another advantage is the ability to form heterogeneous scaffolds with integral fixation plates for selected layers for load-bearing applications.

Another SFF process being developed by Weiss and Campbell (unpublished data) manufactures fibrin-based scaffolds with concentration gradients of growth factors. Briefly, it uses focused ink-jet print heads to co-deposit fibrinogen, thrombin, growth factors, and cross-linking factors to produce, layer-by-layer (by mixing of the droplets at the printed surface) three-dimensional patterned fibrin structures. The process is

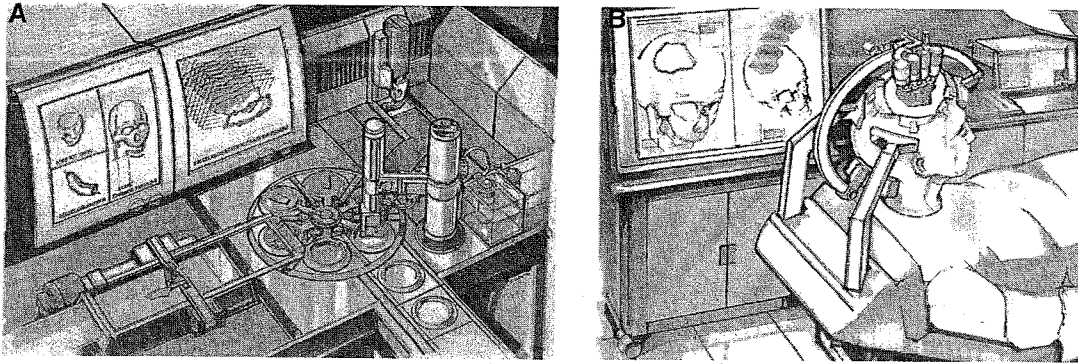


Fig. 1 (A,B) Envisioned CAD/CAM scaffold fabrication systems. (See also Color Plate 20).

compatible with in situ printing to address surgical handling issues (Fig. 1).

#### Future directions

Bone tissue engineering encompasses all of the challenges of creating any engineered three-dimensional tissue that is vascularized. Bone is a dynamic connective tissue that serves many functions throughout the body and has great variability depending on the location. Bone provides strength to the skeleton, protection of structures, barrier functions, stability, and houses the marrow. It is a key component of the appearance of the body and has aesthetic value. It is capable of remodeling, healing, and responding to changes in demand. It has a blood supply and can fight infection when it is well vascularized.

The ability to create a synthetic form of bone that can function as well as autograft depends on the ability to generate new materials, new methods of cell and growth factor management, and novel strategies of integration of the multiple components and technologies that are capable of contributing to the clinical answers to these problems. No single scientific field can generate the ideal method of engineering bone. However, through collaboration and expansion of programs in bone tissue engineering, the right combination of materials, cells, growth factors, and methodology will come together for each clinical situation such that harvesting bone grafts will become obsolete.

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