# Engineering Principles of Clinical Cell-Based Tissue Engineering

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## Current Concepts Review

# Engineering Principles of Clinical Cell-Based Tissue Engineering

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- Tissue engineering is a rapidly evolving discipline that seeks to repair, replace, or regenerate specific tissues or organs by translating fundamental knowledge in physics, chemistry, and biology into practical and effective materials, devices, systems, and clinical strategies.
- Stem cells and progenitors that are capable of forming new tissue with one or more connective tissue phenotypes are available from many adult tissues and are defined as *connective tissue progenitors*. There are four major cell-based tissue-engineering strategies: (1) targeting local connective tissue progenitors where new tissue is desired, (2) transplanting autogenous connective tissue progenitors, (3) transplanting culture-expanded or modified connective tissue progenitors, and (4) transplanting fully formed tissue generated in vitro or in vivo.
- Stem cell function is controlled by changes in stem cell activation and self-renewal or by changes in the proliferation, migration, differentiation, or survival of the progeny of stem cell activation, the downstream progenitor cells.
- Three-dimensional porous scaffolds promote new tissue formation by providing a surface and void volume that promotes the attachment, migration, proliferation, and desired differentiation of connective tissue progenitors throughout the region where new tissue is needed. Critical variables in scaffold design and function include the bulk material or materials from which it is made, the three-dimensional architecture, the surface chemistry, the mechanical properties, the initial environment in the area of the scaffold, and the late scaffold environment, which is often determined by degradation characteristics.
- Local presentation or delivery of bioactive molecules can change the function of connective tissue progenitors (activation, proliferation, migration, differentiation, or survival) in a manner that results in new or enhanced local tissue formation.
- All cells require access to substrate molecules (oxygen, glucose, and amino acids). A balance between consumption and local delivery of these substrates is needed if cells are to survive. Transplanted cells are particularly vulnerable. Theoretical calculations can be used to explore the relationships among cell density, diffusion distance, and cell viability within a graft and to design improved strategies for transplantation of connective tissue progenitors.
- Rational strategies for tissue engineering seek to optimize new tissue formation through the logical selection of conditions that modulate the performance of connective tissue progenitors in a graft site to produce a desired tissue. This increasingly involves strategies that combine cells, matrices, inductive stimuli, and techniques that enhance the survival and performance of local or transplanted connective tissue progenitors.

The discipline of engineering translates fundamental knowledge in physics, chemistry, and biology into materials, devices, systems, and strategies to achieve practical benefits. This discipline also includes the systematic definition and assessment of each variable that may contribute to the success or failure of any engineering effort. Tissue engineering applies this conceptual framework to advance the repair, replacement, or regeneration of organs and tissues[1-3]. Current tissue-engineering strategies include transplantation of whole organs or tissues with use of pedicle flaps and microvascular techniques, transplantation of thin sections of tissues (e.g., split-thickness skin grafts), transplantation of cell suspensions (e.g., blood trans-

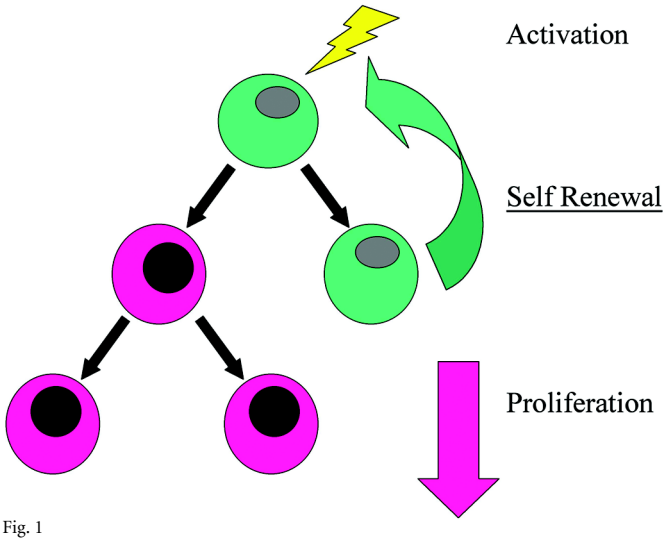


Fig. 1

Stem cell self-renewal. The defining feature of a true stem cell is the capacity for self-renewal. Self-renewal occurs when a cell that has been activated to divide does so asymmetrically. The result produces one cell that is exactly like the mother cell and one cell that takes on biological functions that are different from those of the mother cell. Without self-renewal, each activation event would result in the progressive loss of the originating stem cell population.

fusions or bone marrow transplants), and endoprosthetic replacement of tissues. In orthopaedics, bone[4], cartilage[5,6], tendon[7,8], ligament[9], meniscus, intervertebral disc[10,11], fat, muscle[12], and nerve are the primary targets.

In recent years, the options for orthopaedic tissue engineering have increased dramatically. These options include methods for harvest and transplantation of tissue-forming cells, the use of an expanding array of bioactive matrix materials as tissue scaffolds, local or systemic delivery of commercially available peptide hormones and growth factors, and other methods to control the local chemical and biophysical environment. These new options highlight a transition from the historically materials-based tissue-level approach, with which mechanically durable, bioinert, or biocompatible materials were preferred, to a focus on cell-based or bioactive materials and stimuli. This evolving approach focuses on the function of cells and the role of materials, implants, and biophysical stimuli in modulating cell function.

Cell-based tissue-engineering tools and methods create exciting new opportunities that might be useful in a broad array of potential clinical applications. These opportunities also precipitate a critical need for orthopaedic surgeons to participate actively in the design, development, critical evaluation, and informed use of these methods. Active participation requires that orthopaedic surgeons have a solid foundation in the contemporary concepts and principles of cell-based tissue engineering. This article reviews the central paradigms of contemporary tissue engineering. Specifically, it addresses stem cells and progenitor cells in musculoskeletal tissues (the cells responsible for all new tissue formation), strategies for the clinical use of these cells, barriers to cell transplantation and cell survival, and strategies and variables in the design and optimization of cell-based tissue-engineering scaffolds.

## Stem Cells and Progenitor Cells in Musculoskeletal Tissues

Stem cells and progenitor cells are present in all adult tissues and are critical to tissue health, maintenance, and response to injury or disease throughout life. Stem cells are the source of all new tissues arising from repair and remodeling and are modulated by chemical and physical signals that control their activation, proliferation, migration, differentiation, and survival. Stem cells give rise to progenitor cells and are distinguished from progenitor cells by their capacity for self-renewal, or self-regeneration, by a process of asymmetric cell division (Fig. 1). In contrast, progenitor cells (also called *transit cells*) proliferate and expand in number. Progenitor cells have a limited capacity for self-renewal and are committed to progress toward a differ-

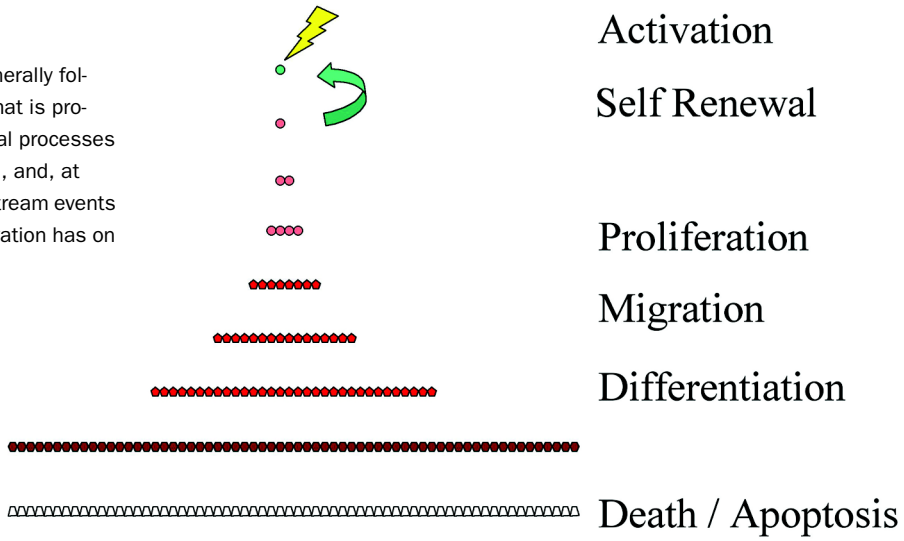


Fig. 2

The stem cell life cycle. Stem cell activation is generally followed by a clonal expansion of the daughter cell that is produced. This is associated with a series of biological processes that include proliferation, migration, differentiation, and, at some point, cell death. Regulation of these downstream events determines the net effect that each stem cell activation has on new tissue formation.

ent or more differentiated phenotype (Fig. 2)[13,14].

Stem cells and progenitor cell populations are part of continuous systems involving cell loss and regeneration in virtually all human tissues that consist of ongoing generation of new cells and the orderly transition of cells from one state to another. This turnover is most evident in the lining cells of the gastrointestinal tract (every three days) or dermis (every fourteen days). In musculoskeletal tissues, turnover is much slower and has been best characterized in bone, where the cells that give rise to and support bone tissue progress through a series of stages beginning upstream with the stem cell. Stem cells give rise to progenitor cells, which progress to become pre-osteoblasts and then osteoblasts. Osteoblasts represent yet another transit population, with a life span of only about forty days, and give rise to both the matrix of new bone tissue and the downstream cells that comprise bone tissue (i.e., osteocytes and bone-lining cells). As osteoblasts reach the end of their functional life, they have three possible fates: they may become osteocytes, they may become lining cells on the surface of mature bone, or they may die by means of apoptosis. As an osteocyte or a lining cell, the same cell may survive for a mean of twenty years or more in human cortical bone, until the region of bone in which it resides is remodeled by the progeny of yet another wave of stem cell function[14]. Bone repair and regeneration following a fracture or a bone-grafting procedure follow the same steps. The same principles apply to cells in muscle, tendon, ligament, and cartilage[15].

An intriguing feature of many tissues, including musculoskeletal tissues, is that upstream progenitors are often multipotent. Stem cells derived from bone, bone marrow, muscle, and fat have all been shown to be capable of differentiation

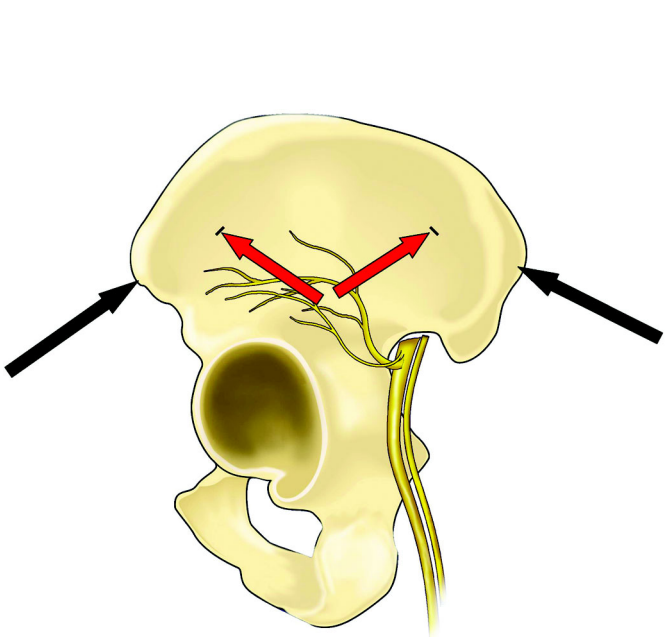

Fig. 3-A

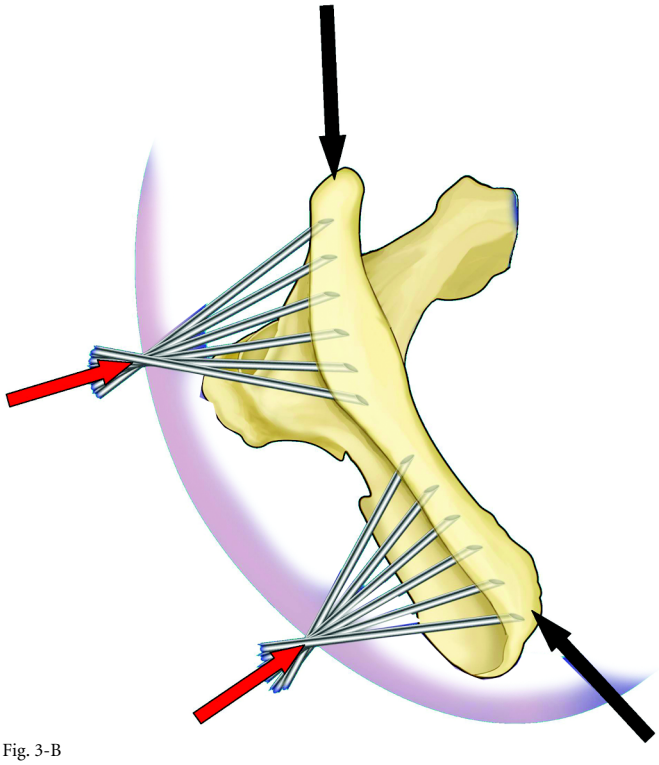

Fig. 3-B

Bone marrow harvest technique. Bone marrow can be harvested by aspiration of either the anterior or the posterior iliac crest with use of two basic approaches, as illustrated in Fig. 3-A. Prone, lateral, or supine positioning of the patient all provide one or more options. In a lateral approach, a 2-mm stab incision is made at the site or sites indicated by the red arrows. Blunt passage of the aspiration needle into the iliac surface in the direction shown (red arrows) avoids risk to the gluteal nerves or vessels. In most patients, the thickness of the iliac crest allows aspiration immediately after entry of the needle into the medullary cavity and then advancement of the needle by 5 mm once or twice to obtain two or three aspirates through the same cortical hole. In the parallel technique, the approach is made through either the anterior superior iliac spine or the posterior superior iliac spine. Only a single site of entry into the iliac crest is required with this approach. Multiple, separate aspirates can be obtained by advancing the needle between and parallel to the inner and outer tables of the iliac crest in increments of 5 to 10 mm and by redirecting the needle along various trajectories in a fan-like projection from the entry hole. In both the anterior and the posterior iliac crest, an aspiration needle can generally be advanced 6 to 8 cm and can remain within the cancellous space of the iliac wing. The needle entry sites are shown in a lateral view in Fig. 3-A. The potential paths for needle placement into the iliac bone are illustrated in a transaxial projection in Fig. 3-B. It is also possible to enter the pelvis through only one site at the anterior or posterior iliac spine and pass the needle in multiple directions through cancellous bone between and parallel to the inner and outer tables of the pelvis (black arrows).

into multiple phenotypes, including bone, cartilage, tendon, ligament, fat, muscle, and nerve[16-20]. This has important implications with regard to the design of tissue engineering strategies, in that cells derived from one tissue might be useful in forming a different tissue.

Sources of musculoskeletal stem cells and progenitor cells include bone marrow, peritrabecular tissues in cancellous bone, periosteum, cartilage, muscle, fat, and vascular pericytes[20-27]. The harvest of these tissues varies with respect to the associated host morbidity[28-34]. Aspiration of bone marrow (Figs. 3-A and 3-B) is associated with the least morbidity and provides a single cell suspension that can be readily processed intraoperatively for immediate implantation[24]. Fat has also been proposed as a low-morbidity tissue source, although it requires greater processing[20,35].

The number of stem cells and progenitor cells in various tissues can be assayed in vitro by liberating the cells from the tissues and growing them in tissue culture under conditions that promote activation and proliferation of stem cells and progenitor cells. The number of stem cells and progenitors can be estimated on the basis of the number of colony-forming units[16,19] (Fig. 4). The term *connective tissue progenitors* has been used to denote this combined heterogeneous population of stem cells and progenitor cells that is capable of both proliferation (colony formation) and differentiation into one or more connective tissue phenotypes[13].

Cells derived from connective tissue progenitors can be expanded (grown) in vitro for use in research or for tissue engineering applications. Under these conditions, isolated cells rapidly become more uniform. When cells are grown in vitro, clones of cells that divide most rapidly and those that have the greatest capacity for continued proliferation have a competitive advantage. In vitro expansion therefore produces a selective pressure favoring these traits. Culture-expanded and selected cell populations have been ascribed various names, including *bone marrow stromal cells*[16], *mesenchymal stem cells*[17], and *adult multipotential progenitor cells*[36,37]. These terms are not synonymous with regard to their precise definitions and biological capabilities[13]. However, all denote that progenitor cells can be isolated and expanded under appropriate conditions and that these cells can retain the capacity to differentiate into a variety of musculoskeletal phenotypes[16-18].

All tissues vary substantially with respect to cellularity and the prevalence of connective tissue progenitors. Aspirated bone marrow is the best characterized source of connective tissue progenitors, containing a mean of approximately forty million nucleated cells and approximately 2000 connective tissue progenitors per milliliter, or about one connective tissue progenitor per 20,000 cells, when an appropriate technique is used[24,33,38]. In contrast, fat and muscle tissues are far less cellular (approximately six million cells per cubic centimeter of tissue), but the prevalence of connective tissue progenitors in fat (as high as one per 4000 cells) may be slightly higher than that in bone marrow.

Differences between connective tissue progenitors harvested from various tissue sources and individuals are only beginning to be understood. These differences depend on the health and histological characteristics of the local tissues and the kinetics of the stem cell function at the site. These variables are in turn influenced by age, gender, and both local and systemic disease[13,24,38,39]. For example, bone marrow cellularity has been shown to decline with age. There is also an age-related decline in the prevalence of connective tissue progenitors, at least in women[38]. However, age and gender account for only a small fraction of the variation in the concentration and prevalence of connective tissue progenitors between patients[24,33,38]. As a result, osteogenic connective tissue progenitors can be harvested by bone marrow aspiration in patients of all ages.

Differences in biological potential among connective tissue progenitors derived from various tissues can have important practical implications with regard to the selection of cell sources for tissue engineering. Bone-marrow-derived con-

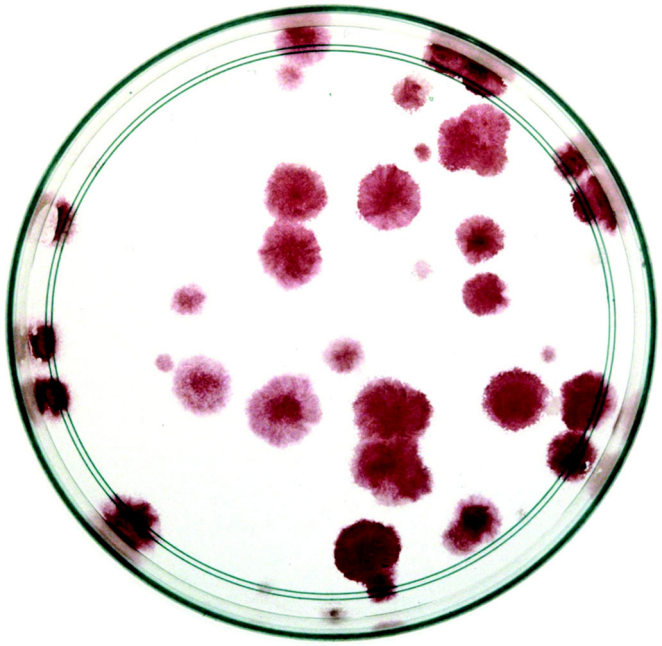

Fig. 4

Heterogeneity of connective tissue progenitors. Tissue culture of cells from bone marrow and other tissues will result in the formation of colonies of proliferating cells. The colonies shown in this image were cultured from human bone marrow for nine days and were stained for alkaline phosphatase activity, a marker of early bone formation. The image illustrates that not all connective tissue progenitors are the same. The colonies differ in size, cell density, and the extent and distribution of alkaline phosphatase activity. Some colonies do not express alkaline phosphatase and are not visible. These morphologic differences are manifestations of intrinsic differences between connective tissue progenitors at the time that they were harvested from bone marrow and placed into culture. Similar variation is present in connective tissue progenitors harvested from other tissues. These differences between colonies may represent differences between stem cells and progenitor cells in various compartments within bone.

nective tissue progenitors include cells that are capable of differentiation into a broad range of phenotypes, including bone, fibrous tissue, fat, muscle, cartilage, and perhaps even neural tissue, liver, and cardiac muscle[13,14,16,17,40-42]. In contrast, connective tissue progenitors from adult articular cartilage are only capable of forming cartilage. Connective tissue progenitors derived from muscle and fat may also have a broad intrinsic differentiation repertoire[20,27,30-32]. Some studies have suggested that fat-derived and bone-marrow-derived cells are similar[43], but others have demonstrated a decreased osteogenic potential in fat-derived cells[44] and the absence of surface markers characteristic of osteoblastic progenitors[45].

## Potential Strategies for Using Autogenous Connective Tissue Progenitors in Therapeutic Applications

There are four major types of cell-based tissue engineering: (1) local targeting of connective tissue progenitors where new tissue is needed, (2) transplanting autogenous connective tissue progenitors to augment the local population, (3) transplanting culture-expanded or modified connective tissue progenitors, and (4) transplanting fully formed tissue.

### *Targeting Connective Tissue Progenitors In Situ*

Targeting strategies are designed to promote desired tissue formation by stimulating the activation, migration, proliferation, and/or differentiation of local connective tissue progenitors. Implantation of acellular tissue scaffolds (e.g., allograft bone, ceramics, hyaluronic acid, and synthetic polymers) is an example of this strategy. The strategy relies on a sufficient local population of connective tissue progenitors. Tissue scaffolds provide a surface on which cells and connective tissue progenitors can attach and migrate as well as a protected void space in which new tissue can form and be distributed throughout the region where new tissue is desired. When these properties promote bone-healing, they are referred to as *osteoconduction*[46,47]. However, the concept of tissue conduction can be applied equally well to any desired tissue-engineered phenotype.

Locally delivered growth factors (e.g., bone morphogenetic proteins [BMPs], fibroblast growth factor-2 [FGF-2], and vascular endothelial growth factor [VEGF]) also target local cells. The capacity of some growth factors to selectively activate bone-forming connective tissue progenitors and/or enhance the probability that their progeny will differentiate into bone has been defined as *osteoinduction*. Again, the concept of induction can apply equally well to stimuli that promote activation and differentiation of connective tissue progenitors toward any desired phenotype.

Biophysical stimulation, such as mechanical loading[48-50], electromagnetic stimulation[51,52], or ultrasound[53], is also an example of cell targeting. Systemic pharmacological strategies, such as the use of parathyroid hormone for the treatment of osteoporosis[54-59] or the use of systemic growth hormones to induce an increase in muscle mass in the elderly[60,61], are types of cell-targeted tissue engineering as well.

### *Transplantation of Autogenous Connective Tissue Progenitors*

Transplantation of connective tissue progenitors was designed to compensate for a deficiency in the number or function of local connective tissue progenitors, as may occur in regions of previous trauma, infection, previous irradiation, tissue defects, scar, or compromised vascularity. Transplantation of connective tissue progenitors can improve the outcome of both conductive and inductive grafts, even in sites that are surrounded by non-diseased tissues[13,62]. This suggests that many, and perhaps all, settings of normal tissue repair may be limited by the population of connective tissue progenitors in local tissues.

Autogenous cancellous bone-grafting has long been the most prevalent and relatively effective example of cell transplantation, although only a small fraction of the transplanted cells actually survive[63-65]. In the past decade, several additional transplantation strategies have been introduced. Several uncontrolled clinical studies have suggested that transplantation of connective tissue progenitors in aspirated bone marrow has value in bone-healing applications[22,66,67]. In an uncontrolled, nonrandomized consecutive study, Connolly et al.[68,69] found bone marrow injection to be successful in the treatment of eighteen of twenty tibial nonunions. Connolly et al.[25] also showed that concentration of bone marrow cells with use of a centrifuge could increase osteogenesis further, a strategy that has been supported by other investigators[70,71].

Many surgeons now use bone marrow because of its biological value and low risk. One of us (G.F.M.) has had clinical experience (albeit without independent or detailed prospective documentation) with aspiration of bone marrow in more than 900 patients undergoing elective orthopaedic procedures over the past fifteen years. The aspiration volumes in the patients ranged from 16 to 200 mL. Patient cohorts representing a subset of this experience have been reported on in two publications[24,33]. There were only two reported bruises, no hematomas, no infections, and no chronic pain at the aspiration site. On direct questioning, most patients reported no pain at the aspiration site during their hospitalization, and in no case was the bone marrow aspiration site the reason for the patient taking pain medication, a factor limiting rehabilitation, or the cause for a delay in discharge from the hospital.

The aspiration technique is important. One of us (G.F.M.) and colleagues[24] found that limiting the volume of the aspirate to ≤2 mL per site reduces dilution with peripheral blood and significantly increases the concentration of marrow-derived connective tissue progenitors ($p < 0.001$). Recent data have shown that the efficacy of a bone marrow graft can be significantly enhanced by the use of the surface of some porous implantable materials to selectively concentrate and select marrow-derived connective tissue progenitors from bone marrow ($p < 0.001$)[62]. Selective retention of connective tissue progenitors can be used to rapidly enrich the population of marrow-derived connective tissue progenitors, by removing red blood cells, serum, and most other cells in marrow and contaminating blood. Grafts enriched in this way have signifi-

cantly improved the results of bone-grafting in a canine spinal fusion model ($p < 0.05$)[62] and have been approved by the United States Food and Drug Administration for clinical bone-grafting in spinal fusion and in treatment of bone fractures and defects, although to date no clinical studies on this strategy have been published, to our knowledge.

### *Transplantation of Culture-Expanded Autogenous Cells*

Culture-expanded cells can also contribute to new tissue formation[42,72-74]. Preliminary studies have suggested that culture-expanded cells from muscle, fat, and bone marrow may be useful in regeneration of bone, cartilage, muscle, and tendon tissue[73,75-79]. In vitro expansion offers the potential to generate a large number of progenitor cells. However, culture expansion also adds substantial cost and some risks, such as contamination with bacteria or viruses or depletion of the proliferative capacity of the connective tissue progenitors prior to implantation[80-82]. In vitro selection of the most rapidly proliferating cells may also select cells with mutations or epigenetic changes that might confer a tumor-forming potential. However, we are not aware of any reports of human tumors being formed by culture-expanded cells, and the risk of tumor formation appears to be very low. At present, the only Food and Drug Administration-approved clinical use of culture-expanded cells in orthopaedics is for the repair of cartilage defects, where expanded autogenous chondrocytes are transplanted under a periosteal flap[6,83].

### *Transplantation of Genetically Modified Cells*

The intrinsic biological potential and performance of connective tissue progenitors can be genetically modified with a variety of means that either transiently or permanently alter the genes that a cell expresses[84]. Both connective tissue progenitors and nonconnective tissue progenitors have been engineered to secrete factors (e.g., BMP-2) that will influence the behavior of that cell or the cells nearby. The introduction of new or modified genes is usually accomplished with use of *vectors* that are created by modifying naturally occurring viruses, such as a retrovirus, lentivirus, adenovirus, or adeno-associated virus[85,86]. Nonconnective tissue progenitors have also been transfected to express genes that control other genes. LMP-1 (LIM mineralizing protein-1) is one example. LMP-1 is a nuclear transcription factor, a protein that, when expressed, functions within the nucleus to activate or inhibit the expression of a number of other genes. LMP-1 is therefore not secreted but functions only within the nucleus of a cell that is transfected to express LMP-1. However, LMP-1 activity within the cell induces the secretion of a variety of pro-osteogenic factors that can target osteogenic connective tissue progenitors[87,88].

The biological risk associated with genetic manipulation is greater than that associated with the alternatives. As a result, demonstration of safety is currently as great a challenge as is demonstration of biological efficacy[89]. While transplantation of genetically modified cells may not play a role in elective clinical tissue engineering in the near future, it has substantial potential value, particularly in the setting of inherited genetic defects (e.g., osteogenesis imperfecta)[90] and in tissues, such as cartilage, that consist of relatively homogeneous long-lived cells and in which phenotypic expression may be induced and durably maintained by expression of a single gene[86,91].

### *Ex Vivo Tissue Generation and Transplantation*

Creation of fully organized and mature tissues outside of the body (ex vivo) followed by functional transplantation and integration is the common public vision of tissue engineering. However, this strategy involves three great challenges: (1) generation of functional tissues, (2) transplantation in a manner that preserves the viability and function of cells, and (3) biological and mechanical fixation and integration with surrounding tissue.

In some areas, transplantation of thin tissue grafts (e.g., cartilage, corneal, and skin grafts) is possible without an immediate connection to a developed vascular system. However, in most tissues, cell survival requires a functioning vascular system. Some reports describe ex vivo generation of vascular transport systems[92,93], but these approaches are not currently clinically practical.

## Mass Transport Processes and Metabolic Demand

In all settings in which cells are transplanted, access to substrate molecules (oxygen, glucose, and amino acids) and clearance of products of metabolism ($CO_2$, lactate, and urea) are critical to cell survival. The movement of these molecules in and out of the graft site is collectively referred to as *mass transport.* Mass transport can be mediated by fluid flow (convection), both in the circulatory system and in the extracellular space, between the vessel lumen and the cell membrane. The pressure gradients driving this fluid flow can be induced by tissue deformation (movement), mechanical loading, muscle contraction, gravitational pooling, Starling flow, and arterial pulsation. Convection can be particularly important when cells are embedded in a dense extracellular matrix, such as bone and cartilage, where it has been shown to play a major role in enhancing the transport of large molecules (e.g., proteins and growth factors)[94-97]. However, in most tissues, passive diffusion along concentration gradients is the principal mechanism for mass transport, particularly for small molecules.

In metabolically active tissues such as trabecular bone and bone marrow, the distance that oxygen must diffuse between a capillary lumen and a cell membrane is almost never more than 40 to 200 μm[98,99]. This diffusion distance is critical to maintaining the balance between oxygen delivery to a site and consumption of oxygen by cells, both in native tissues and in tissue engineering strategies involving cell transplantation. When cells are transplanted clinically, the vessels that deliver oxygen are initially confined to the outer surface of the graft site. As one moves deeper into the graft site, each transplanted cell competes for oxygen and other nutrients with other transplanted cells. Transplanted cells also compete with other cells

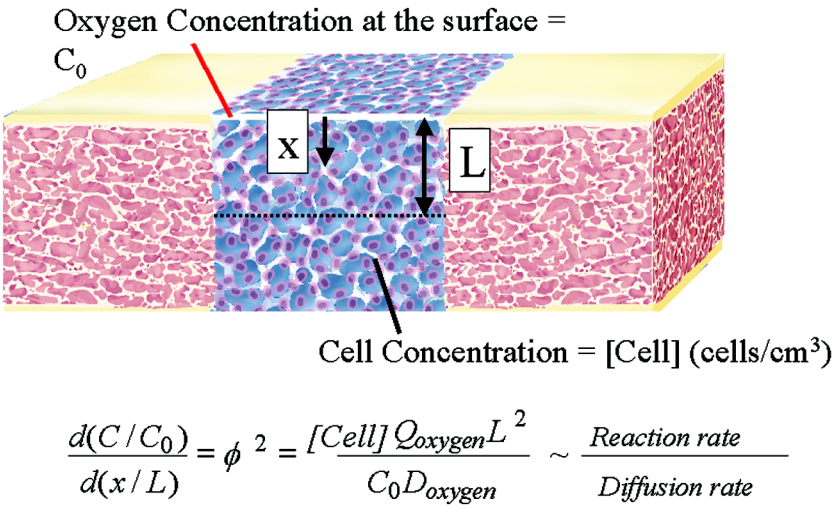


Fig. 5-A

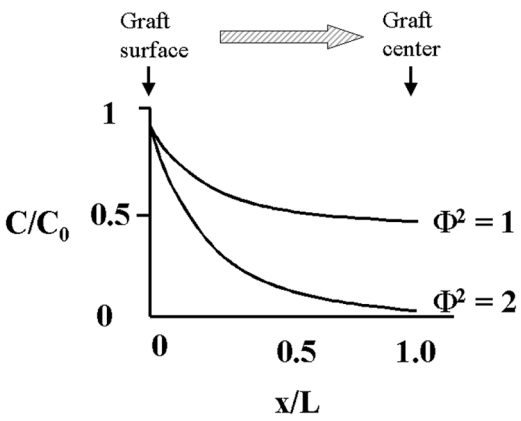


Fig. 5-B

Theoretical calculations of oxygen diffusion and reaction kinetics in a cellular implant. An idealized scaffold matrix is illustrated after implantation with cells. The diffusion distance to the center of the scaffold is defined as L. The concentration of cells in the scaffold is represented as [Cell]. The oxygen concentration at the surface of the device is defined as $C_o$ (the oxygen concentration in the tissues at the site of implantation). Oxygen diffuses into the graft site and is consumed by cells at a volumetric rate of $Q_{o2} = Q_{cell}x[Cell]$, where $Q_{cell}$ is the oxygen consumption rate per cell per second and x is the depth within the matrix. The equation governing the balance between diffusion and consumption is $D_{o2}d^2C/dx^2 = Q_{o2}$, where $D_{o2}$ is the diffusion coefficient of oxygen and C is the local oxygen concentration within the device. The solution to the equation provides the oxygen concentration profile in the device: $C/C_o = 1 - Q_{o2}L^2/(D_{o2}C_o)[x/L - 0.5\ (x/L)^2]$. The minimum oxygen concentration will occur at the center of the device (i.e., x = L). In order for the oxygen concentration at the center of the device to be greater than zero, the parameter grouping $Q_{o2}L^2/(D_{o2}C_o)$ must be less than two. Therefore, the maximum cell concentration that can be supported by oxygen diffusion without central necrosis can be represented by rearrangement as: $[Cell] < 2D_{o2}C_o/(Q_{cell}L^2)$.

that are recruited as part of the local inflammatory response following implantation. In most clinical grafts, the diffusion distance for oxygen and other metabolites from the edge of the graft to the center of the graft is a minimum of 5 mm, or approximately fifty times the normal diffusion distance. In this setting, diffusion is able to support only a limited number of transplanted cells before the balance between metabolic demand and diffusion creates a zone in the center of the graft where oxygen tension is too low to support viable cells, resulting in central necrosis. The size of the necrotic region and the number, distribution, and type of cells that do survive in the deeper regions of the graft site are a function of many variables, which can be analyzed with use of basic engineering principles. The principal variables are the concentration of oxygen at the surface of the graft site ($C_O$), the concentration and distribution of cells in the site (including inflammatory cells), the rate of oxygen consumption by cells within the site, fluid flow within the site, the diffusion constant for oxygen, and the biological response of cells (survival, proliferation, migration, and differentiation) to hypoxia. Relatively little is known about the balance between diffusion and consumption of proteins, peptides, or the signaling molecules. However, because diffusion of oxygen is relatively slow and oxygen consumption is high, the transport of other nutrients (e.g., glucose and amino acids) is generally more favorable than that of oxygen. Oxygen is therefore the limiting factor in cell survival in most grafts. As a result, few cells tolerate diffusion distances of >0.2 mm. For example, rat osteoblasts seeded on porous scaffolds in vitro form a viable tissue that is no greater than 0.2 mm thick[100]. Islet cells show necrosis when the diffusion distance exceeds approximately 0.1 mm[101-103]. Cartilage is exceptional, maintaining viability in avascular regions >1 mm thick[104], although oxygen transport in cartilage in vivo may be enhanced by convective flow[95,105].

Theoretical modeling can be used to explore the relationships among cell density, diffusion distance, and cell viability within a graft. Figures 5-A and 5-B illustrate a theoretical cellular implant, where diffusion of oxygen in the x-direction is balanced by cellular consumption. When the differential equation describing the balance between diffusion and reaction is made nondimensional, a parameter, denoted $\phi^2$, emerges as the relative rate of reaction to the relative rate of diffusion. When $\phi^2 = 1$, the oxygen concentration in the center of the graft is 50% of the concentration at the surface, and when $\phi^2 = 2$, the oxygen concentration in the center of the graft is zero. A rough estimate of the limit of cell density and diffusion distance can be made by setting $\phi^2 = 1$. Assembling these variables, oxygen consumption averages about $4 \times 10^{-17}$ mol/cell-sec but varies with cell type (hematopoietic stem cells, $0.47 \sim 3.3 \times 10^{-17}$ mol/cell-sec[106]; fibroblasts, $4 \sim 7 \times 10^{-17}$ mol/cell-sec[107]; and granulocytes and monocytes, $0.6 \sim 18 \times 10^{-17}$ mol/cell-sec[98,99,107]). The diffusion coefficient of oxygen in tissue is $\sim 2 \times 10^{-5}$ cm²/sec at 37°C[98,99,107], and oxygen concentration in normal tissues ($C_O$) is about 0.07 mM, slightly greater

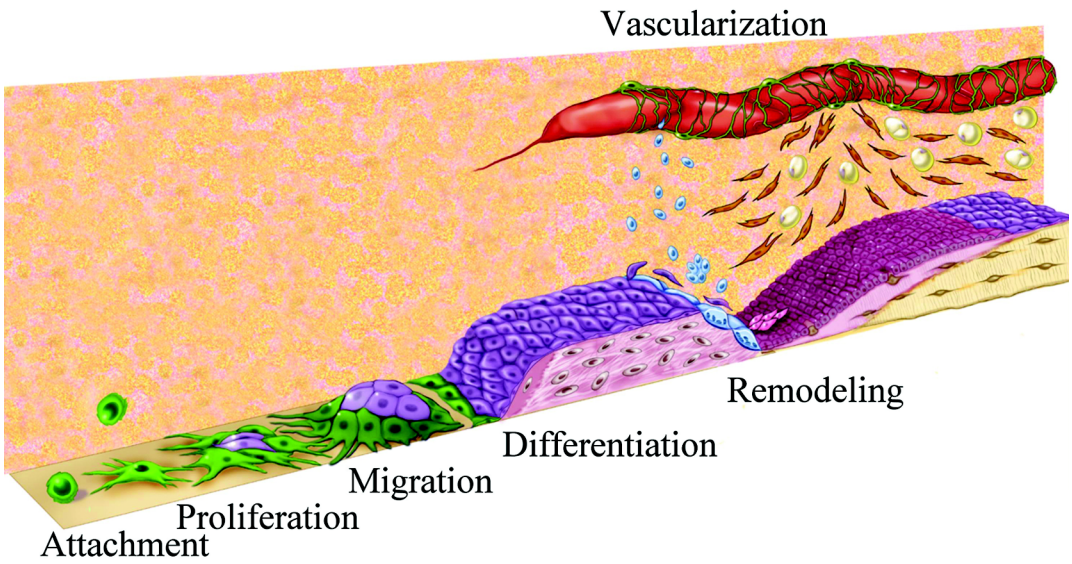


Fig. 6

Stages of bone tissue ingrowth. The sequential stages in the formation of new bone tissue are illustrated. Attachment and/or activation of a stem cell (green) is followed by continued proliferation and migration of the resulting progeny, forming a clone or colony of new cells. Less mature and more stem-cell-like progenitors continue to proliferate and migrate at the periphery of the colony (lighter green). Differentiation is characterized by the elaboration of an appropriate tissue matrix, beginning in the center of the colony. In this case, the tissue formed first is woven bone, although cells may also follow a pathway that results in cartilage formation or direct apposition of new lamellar bone. Elaboration of a mature bone phenotype does not occur in the absence of a new or existing local blood supply (i.e., a sufficient local oxygen tension). Remodeling involves the coupled process of osteoclastic bone resorption followed by recruiting and activation of additional stem cells and progenitors from upstream osteoblastic cells in bone marrow.

than that in venous plasma. Using these values for a graft of a given thickness, one can estimate the maximum concentration of cells that can be delivered without central necrosis ($[Cell]_{max}$). For example, in a graft that is 2 cm thick, $[Cell]_{max}$ is roughly 70,000 cells/cm$^3$. In contrast, a 1-cm-thick graft could support four times more cells, or 280,000 cells/cm$^3$. This is about 1000-fold lower than the concentration of cells in native autogenous cancellous bone (~$5 \times 10^8$ cells/cm$^3$) and 100-fold lower than the mean concentration of cells in a marrow aspirate (~$4 \times 10^7$ cells/cm$^3$)[24]. These estimates predict hypoxia and central necrosis in almost any graft site with a diffusion distance of more than 500 to 1000 μm. They also define what is approximately an inverse square relationship between $[Cell]_{max}$ and diffusion distance. For example, increasing a graft dimension by a factor of 5 (e.g., transitioning from a rat to a dog) will decrease $[Cell]_{max}$ by a factor of 25. This is one reason why many cell transplantation methods work very well in small animals but fail in larger animals and humans.

Several factors may modify these calculated estimates. First, because cell delivery systems are generally prepared in room air, the initial implant is usually saturated with oxygen. This dissolved oxygen will support cell respiration for at least several hours after implantation, blunting the abrupt decrease in regional oxygen concentration. Second, not all implanted cells continue to respire at basal rates. Cells that are very sensitive to the trauma of transplantation may die, promptly reducing the initial metabolic demand within the implant. However, cell death also results in the local release of products of cell lysis and adds debris to the site. This may increase the intensity of local inflammation and the metabolic demand from inflammatory cells at a later time.

The survival of transplanted connective tissue progenitors also depends on the response of these cells to the transplantation environment. Observation has long supported the concept that at least some connective tissue progenitors in bone and bone marrow have a high capacity to survive in hypoxic conditions[63,64,108]. Experimental data also have shown that many stem and progenitor cells, including connective tissue progenitors in bone, exhibit a remarkable tolerance to, and are even stimulated by[109-113], hypoxia, not unlike endothelial cells[114,115]. The capacity to convert to glycolysis transiently in response to hypoxia is one adaptive mechanism[116]. The rate and extent of revascularization are also critical. Prompt revascularization favors osteoblastic differentiation, whereas prolonged hypoxia favors formation of cartilage or fibrous tissue[17,117-119].

These concepts can be converted into several practical strategies to optimize cell survival in clinical grafts. One method is to reduce the concentration of transplanted cells. Another is to limit the transplanted cells to only those cells that contribute to the formation of the desired tissue (i.e., connective tissue progenitors and perhaps endothelial cells), while excluding red blood cells and the vast majority of other nucleated cells. Both concentration[25] and selection strategies[62]

have been shown to enhance graft performance. Finally, in the future, culture-expanded cells might be preadapted to hypoxic conditions prior to transplantation and/or selected to enrich for those most likely to survive. Other options to improve the local matrix environment and to enhance mass transport are discussed below.

## Design and Selection of Scaffolds or Tissue Engineering

Three-dimensional porous scaffolds play a critical role in both cell targeting and cell transplantation strategies. Scaffold matrices serve as space-holders to prevent encroachment of surrounding tissues into the graft site. They provide surfaces that facilitate the attachment, survival, migration, proliferation, and differentiation of stem cells and progenitors. They also provide a void volume in which vascularization, new tissue formation, and remodeling can occur (Fig. 6). In addition, scaffolds can provide a vehicle for delivery of cells into a graft site, facilitating their retention and distribution throughout the region where new tissue is desired[1,46,120-122].

A broad range of scaffolds is already available for clinical use, and many new scaffolds are under development. Differences between scaffolds can generally be categorized into one or more of six domains: bulk material, three-dimensional architecture and porosity, surface chemistry, mechanical properties, initial scaffold environment (osmolarity and pH), and late scaffold environment (degradation characteristics). Each domain has important implications with respect to the biological response to a scaffold and its utility in transplanting or supporting local stem cells and progenitors.

### *Bulk Materials*

Current clinical scaffolds are made from a broad range of bulk materials. These include tissue-derived materials (e.g., allograft bone matrix, skin, and intestinal submucosa), biological polymers (e.g., collagen, hyaluronan, fibrin, and alginate), ceramics or mineral-based matrices (e.g., tricalcium phosphate, hydroxyapatite, and calcium sulfate), metals (e.g., titanium, tantalum, and other alloys), and composites of two or more materials. A variety of synthetic polymers are also being adapted or developed. These new materials include water-insoluble polymers (e.g., poly[lactide], polytyrosine carbonates, poly[caprolactone], varying copolymers, and synthetic gel-like polymers [polyethylene oxide-based])[123].

### *Three-Dimensional Architecture and Porosity*

*Matrix architecture* refers to the way in which a bulk material is distributed in space, at the nanoscale, microscale, and macroscale (i.e., molecular, cellular, and tissue-length scales, respectively). Matrix architecture defines the mechanical structure of the scaffold, but it also defines the initial void space that is available for connective tissue progenitors to form new tissue, including new blood vessels, as well as the pathways for mass transport (convection and diffusion). Most scaffolds are designed to have an internal porous structure of void spaces that are interconnected through pores or channels on the scale of 50 to 1000 μm. The pore size used for most bone ingrowth settings is between 150 and 500 μm, which is just large enough to support ingrowth of vascular tissues, depending on the depth of penetration required. Figures 7-A, 7-B, and 7-C illustrate the process of bone ingrowth into a surface with a pore size of ~150 μm. Larger pores generally support deeper penetration of new tissues, but the optimal pore size for ingrowth deeper than 3 to 4 mm into tissue scaffolds has not been studied systematically, to our knowledge. This is relevant to the current clinical practice of filling large voids with particulate or granular materials, since the void spaces between packed particles are generally an order of magnitude larger than the stated microstructure or pore size of most granules themselves. This may provide the larger macrostructure needed for deeper revascularization.

Options for the structural design of tissue scaffolds are almost infinite. The macrostructures include regular geometric shapes (e.g., blocks, pellets, and dowels), amorphous structures (e.g., randomly packed chips, granules, or fibers), randomly integrated structures (e.g., foams or freeze-dried materials), and formally designed regular structures (e.g., machined, printed, woven, or assembled structures). Gel or putty preparations can also be made from powders or fibers, by mixing them with plasticizing agents (e.g., glycerol, cellulose, and hyaluronan) or by conducting in situ polymerization with use of chemical, photochemical, or enzymatic methods[120]. In some cases, a desirable structure has been borrowed from nature, such as the highly interconnected porous structure of cancellous bone or some corals[124]. Selective processing (machining, size and density selection, washing, and demineralization) now provides a variety of relatively optimized materials for use in special clinical settings.

Most methods for fabricating porous scaffolds—i.e., particulate leaching[100], freeze-drying[125], gas infusion[126], and phase separation[120]—create isotropically distributed voids and connecting pores (such as in a sponge) by using particles or bubbles when the scaffold is solidified. In the past decade, substantial advances have been made in the methods for producing more precise hierarchical microstructures from a variety of materials. These are now being applied to create strategically oriented channels and pores and defined macroscopic shapes. The most notable innovations involve solid free-form fabrication methods (e.g., three-dimensional printing process and stereolithography)[1,120], which provide a feature resolution of approximately 200 μm. Creation of more defined porous structures offers the potential for greater control over the distribution of bulk material within a graft site as well as control over patterns of cell migration, fluid flow, and diffusion throughout the device[127-129].

Nanostructural features (<100 nm) may also play an important role in scaffold function. Nanopores are too small to influence where cells can or cannot migrate, but they may still have important effects on cell behavior by changing surface texture or diffusion of soluble materials. All other features

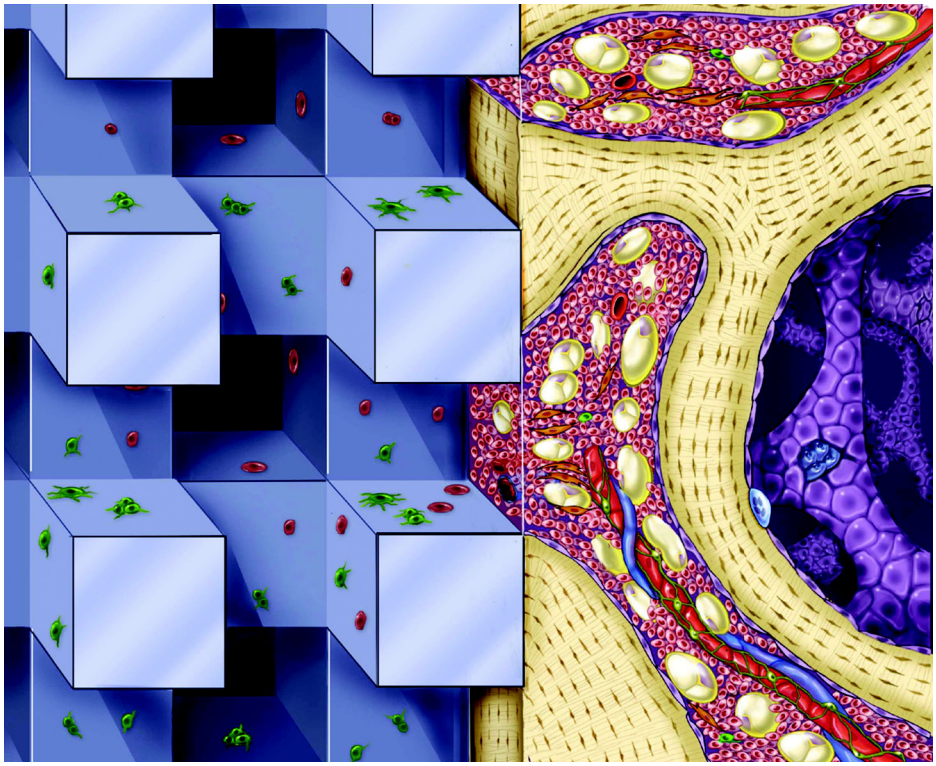

Fig. 7-A

**Figs. 7-A, 7-B, and 7-C** Conceptual illustration of the biological processes of bone formation within and around a porous implantable scaffold matrix that delivers osteogenic cells into a graft site. **Fig. 7-A** Osteogenic stem cells or progenitors are shown as adherent to the matrix (green) and uniformly distributed in the matrix, which is being used as a delivery system for the attached cells. Other, nonosteogenic cells (pink) may also adhere to the matrix. The matrix shown has 150-$\mu m^2$ pores and 150-μm-thick walls. Following implantation, the osteogenic cells near the surface of the implant experience an environment in which the concentration of oxygen, glucose, and other nutrients is nearly normal. Cells deeper in the matrix and farther from the vascular tissue bed experience progressively lower concentrations of oxygen and other nutrients because they must compete with all of the other cells within the matrix. Mild-to-moderate hypoxia may actually represent a stimulus for these transplanted cells to proliferate, migrate, and secrete cytokines that induce other cells to migrate into the matrix.

being equal, the presence of interconnected nanoporosity within the walls of a porous structure can open up a much wider path for mass transport, thereby improving cell survival in the scaffold.

*Mechanical Properties*

Sometimes, graft sites must bear loads at, or close to, physiological levels very soon after implantation. Internal fixation often provides the necessary early stability. However, in some bone or soft-tissue settings, the scaffold must bear or share substantial load immediately, and then high-strength materials and structures such as cortical bone, metals, ceramics, or carbon-fiber-based polymers are required.

A scaffold's mechanical properties (strength, modulus, toughness, and ductility) are determined both by the material properties of the bulk material and by its structure (macrostructure, microstructure, and nanostructure). Matching the mechanical properties of a scaffold to the graft environment is critically important so that progression of tissue healing is not limited by mechanical failure of the scaffold prior to successful tissue regeneration. Similarly, because mechanical signals are important mediators of the differentiation of connective tissue progenitors, a scaffold must create an appropriate stress environment throughout the site where new tissue is desired.

One of the greatest challenges in scaffold design is the control of the mechanical properties of the scaffold over time. Scaffolds that do not degrade (metals and ceramics) simplify this problem and can provide excellent and durable function in some settings. However, these materials can also compromise tissue repair and function. It is obvious that persistence of a scaffold or implant precludes the formation of new tissue in the space that it occupies. In addition, following integration of a rigid nondegradable implant, adjacent tissue is often mechanically protected (stress-shielded), changing local mechanical signals and resulting in loss of desired local tissue. Stress concentration at the interface between a high-stiffness implant and native tissue can increase the risk of mechanical failure (e.g., fracture) and pain. Finally, if subsequent procedures require removal of the im-

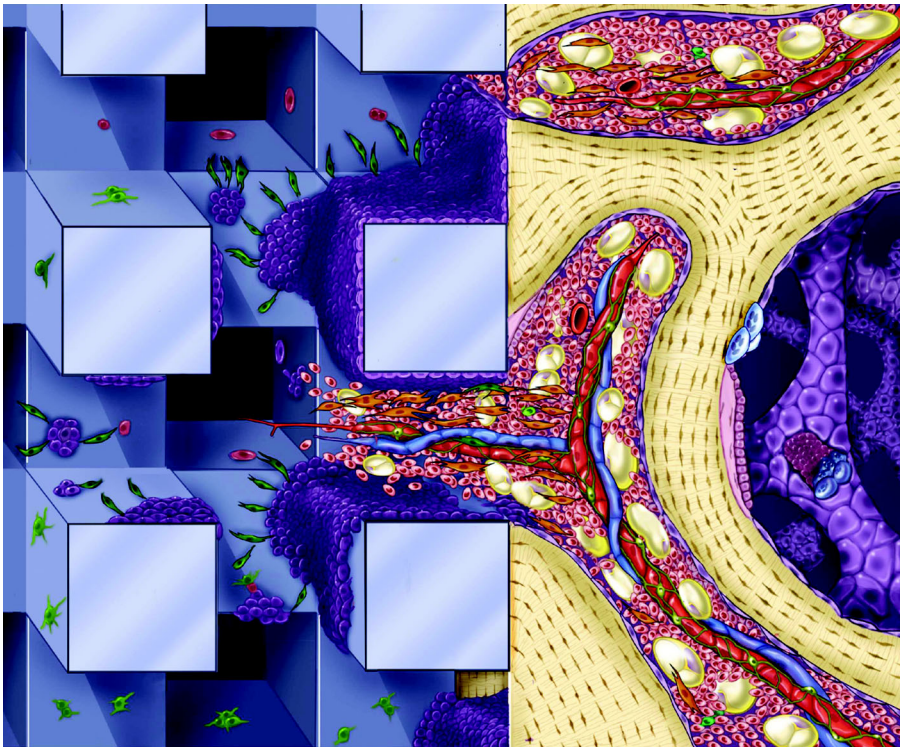

Fig. 7-B

Several days later, the cells migrating into the matrix may include inflammatory cells, additional progenitor cells derived from local stem cell activation (orange), and also vascular endothelial cells supporting an angiogenic response. These additional cells also compete with cells deeper in the scaffold. The most effective way to limit the loss of transplanted stem cells and progenitors is to limit the number of cells that arrive at the site prior to revascularization. This can be accomplished by limiting the transplanted cells to stem cells and progenitors and other cells that directly contribute to the bone-healing response. Selection of noninflammatory materials and limiting debris (e.g., degrading red blood cells and other material) in the matrix and surrounding tissue reduces the competition for oxygen and other nutrients from inflammatory cells. The surviving and invading osteoblastic stem cells and progenitors proliferate, migrate, and differentiate to form a network of new woven bone on and within the implanted material.

plant scaffold (e.g., because of infection or migration), all of the new tissue within the implant may also be lost, eliminating the value of the initial procedure.

Problems arising from retained implants have increased the desire to use resorbable scaffolds whenever feasible. One example of that strategy is the use of impaction grafting for reconstruction of contained periprosthetic defects. Another is the recent shift from the use of very slowly degradable ceramics (e.g., hydroxyapatite) for bone-void fillers to the use of more rapidly resorbed materials (e.g., tricalcium phosphate)[130]. This

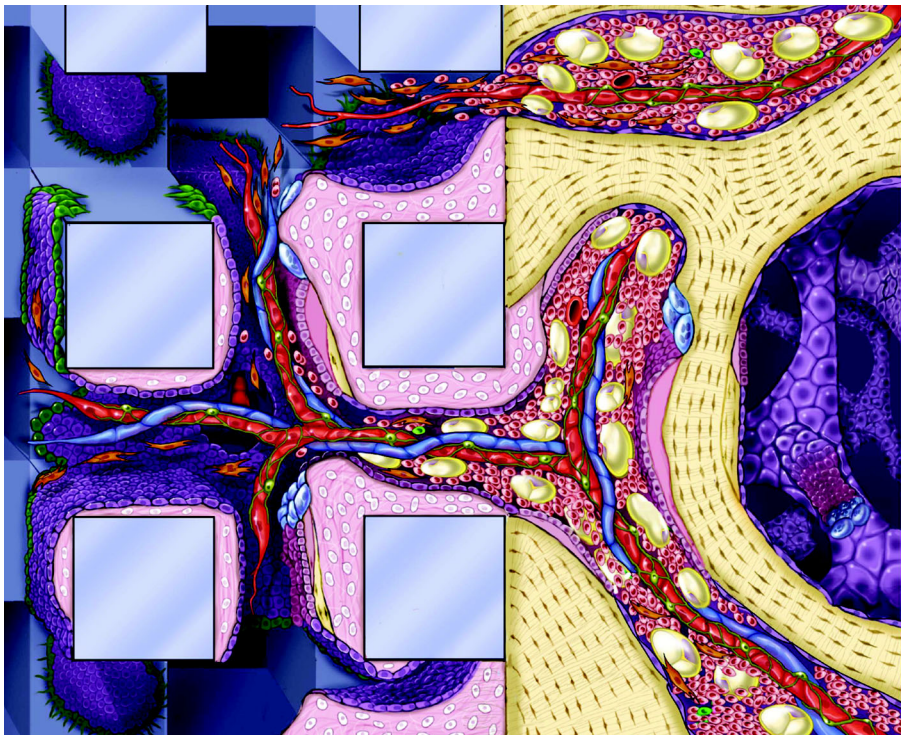

Fig. 7-C

An increase in bone remodeling is seen both within the implant site and in the bone adjacent to the implant. Initial woven bone is replaced with lamellar bone, forming an interconnected network extending deeper within the implant. Vascular remodeling also occurs. Void spaces within the scaffold are remodeled into normal marrow elements. Deeper regions of the implant, in which transplanted cells may not have initially survived, are progressively colonized by migration of additional stem cells and progenitors.

demand for resorbable scaffolds continues to fuel the development of resorbable inorganic polymers[120].

Almost all new materials being developed for tissue engineering ultimately resorb, and three key features of the degradation or resorption process appear to influence performance: the rate at which the matrix loses its mechanical properties, the rate at which the matrix is removed from the site, and the nature and concentration of the soluble products that are released into the site as the material is broken down.

Controlled degradation of mechanical properties alone is a major challenge. Mechanical properties can be lost as a result of internal degradation of the bulk material (e.g., hydrolysis) or the accumulation of fatigue damage. In recent years, it has become more evident that, even in controlled settings, the mechanical and chemical degradation of the same polymer can vary substantially between species, individuals, anatomic locations, and clinical settings. As a result, it has been very difficult to define an optimal degradation rate for materials to be used in general clinical practice. In general, most design strategies tend to extend degradation time over months, in order to minimize the risk of early failure in preference to minimizing the risks associated with delayed resorption[131].

*Surface Chemistry*

Interactions between cells and scaffolds occur at the surface and are the direct result of the unique chemical environment that is created. The surface chemistry depends on the properties of the bulk material but is not defined by the bulk material. This is due to the fact that almost all implanted materials rapidly become coated with proteins and lipids, and these adsorbed biomolecules are the principal mediators of the cellular response to most materials. The net effect involves an interaction between a given surface and available biomolecules that adsorb to the surface. Furthermore, when a protein adsorbs, it usually undergoes a change in conformation, which may include denaturation or unfolding. This, in turn, may either hide or expose sites within the protein that interact with cell surface receptors. For example, fibronectin is a more active adhesion molecule on hydrophilic surfaces (e.g., glass) than on hydrophobic surfaces (e.g., Teflon or polyethylene)[132-135].

Biological fluids contain a vast diversity of proteins, and cells have hundreds of different types of cell surface receptors. There are twenty-four distinct cell-matrix receptors in the integrin family alone[136]. As a result, it is not surprising that scaffold materials have been discovered and selected empirically. However, powerful surface analytical techniques are being used to illuminate the protein adsorption properties on various surfaces in an effort to find out why some materials are so favorable for bone cell adhesion and bone formation[137]. For example, it has been speculated that hydroxyapatite and some other ceramics may preferentially sequester growth factors, growth factor-binding proteins, or adhesion molecules that are important for bone regeneration. Indeed, hydroxyapatite and tricalcium phosphate materials perform successfully as depot delivery vehicles for BMPs, in both animals[138] and humans[139,140].

Like other implanted materials, allograft bone matrices (both mineralized and demineralized) rapidly accumulate biomolecules on their surface, which have biological effects on local cells. However, allograft bone already contains many embedded adhesion molecules and growth factors[141]. These include the BMPs, although the concentration is far lower than that delivered with use of purified recombinant BMP products and the release is much slower, requiring matrix degradation by local cells. Furthermore, the concentration and presentation of bioactive molecules in allograft bone may vary widely depending on the age, gender, and genetics of the donor; the tissue site of origin; and the tissue processing procedures[142].

The attachment, survival, proliferation, and differentiation of stem cells and progenitor cells can be modulated in vitro if implants are precoated with selected bioactive proteins[143,144]. Furthermore, proteins can be selectively concentrated and presented on surfaces with use of nonspecific surface interactions (e.g., dip-coating or lyophilization). This is the strategy that is used to deliver BMP-2 (Infuse; Medtronic Sofamor Danek, Memphis, Tennessee) and OP-1 or BMP-7 (OP-1 Device; Stryker Biotech, Hopkinton, Massachusetts) in two clinical products that are currently available for improving bone-healing[145-147]. In the case of the BMP-2 product (Infuse), BMP-2 is provided in solution and is dripped into an absorbable collagen sponge. BMP-2 binds to the collagen and is then released. In the case of the OP-1 (BMP-7) product (OP-1 Device), 3500 μg of OP-1 is lyophilized onto 1 g of bovine type-I collagen powder. OP-1 is then released by solubilization from the collagen surface.

The pharmacokinetics of delivery of BMPs have been shown to be an important clinical variable in a variety of materials, including degradable polymers[148-155], type-I collagen[156-158], and calcium phosphate ceramics[159,160]. The retention time of implanted BMPs have been shown to correlate with biological efficacy, presumably because the longer a BMP is retained, the higher the likelihood that it will act on an appropriate target cell. Protein residence time can be estimated in vivo by measuring the rate of clearance following the implantation of radioactive protein, with the assumption that the protein remains active as long as residual radiation can be measured. Retention has been shown to be related to solubility[161] and protein isoelectric point[138]. The release kinetics of BMP-2 from a degradable collagen sponge in rabbits was described by Bouxsein et al.[162]. Approximately 25% of the delivered BMP-2 was released rapidly from the implantation site, but as much as 37% remained at the site one week after implantation.

The advantage of the current clinical strategies for protein delivery is that they are technically simple. However, they require that the protein be delivered in a high concentration. This delivery strategy presumably allows the protein to diffuse into tissues adjacent to the implant site to act on the local connective tissue progenitor target population. This release pattern may also establish a concentration gradient around the graft site that may be important for chemotactive factors. However, the disadvantage of current strategies is that they provide relatively little control over the rate of delivery, conformation, presentation, clearance, or degradation of the delivered protein. While current strategies for delivery of BMPs can be effective, there is rea-

son to believe, given the very large supraphysiological doses of BMPs that are needed, that the vast majority of the protein that is delivered is wasted and that only a small fraction actually comes into contact with target cells to elicit a receptor-mediated signal that enhances new bone formation. These methods therefore leave substantial room for improvement in the delivery kinetics (rate and duration) and distribution of bioactive proteins to optimize efficiency.

Proteins and small bioactive peptides can also be selectively concentrated and presented by covalently linking them to a surface[1,120,140]. This provides more control over conformation, a slower rate of release from the surface, and longer retention. While this method may not be appropriate for many soluble proteins, particularly signaling molecules that need to be internalized for function (e.g., steroid hormones), there is growing evidence suggesting that presenting growth factors in a matrix-bound fashion not only may be suitable for clinical use, but also may better mimic the native physiology and improve outcomes[163-166]. This strategy may also be particularly well suited for the design of matrices with selective affinity for specific cells or sets of cells (e.g., connective tissue progenitors, endothelial cells, and platelets).

### *Initial Scaffold Environment: Osmolarity and pH*

A scaffold that is used as a delivery system for viable cells must provide and maintain an environment with physiological pH and osmolarity. For most scaffolds, simple hydration with normal saline solution prior to exposing them to cells avoids cell injury. However, some matrices do not allow an isotonic condition to be created for cell delivery. Examples include many bone matrix materials that are prepared with use of solutions containing high concentrations of low-molecular-weight materials to improve handling (e.g., glycerol) and materials that dissolve rapidly in water, releasing hyperosmolar concentrations of local ions (e.g., calcium sulfate). If such materials are mixed with cells, they can be expected to induce osmotic injury, reducing or precluding cell viability. Matrices containing high-molecular-weight carriers (e.g., cellulose, starch, and hyaluronan) may be acceptable. There is much less osmotic pressure (proportional to the molar concentration of the solute) with these high-molecular-weight carriers.

### *Late Scaffold Environment: Degradation Products*

All degradable matrices release degradation products into the graft site environment that must be further degraded or cleared. The effect that these degradation products have on the cells within the graft site depends on their concentration, their effect on local pH, and their relative biological toxicity. Concentration, in turn, is a function of the rate of release of these products and their rate of clearance from the graft site.

Polyesters, such as polylactides and polyglycolides, are currently the so-called workhorses of synthetic degradable surgical materials. They have been used for decades as sutures, surgical meshes, and more recently as fixation hardware (e.g., suture anchors and screws)[120]. The degradation of these materials can be controlled over a range of weeks to years. Polymer hydrophobicity and crystallinity both influence the rate at which water penetrates and hydrolyzes the solid polymer and thus the rate of device breakdown[140]. However, the degradation products of these materials (lactic acid and glycolic acid) are not ideal for tissue regeneration. Furthermore, they tend to be released as a bolus after a long period of residence. This is due to the fact that they are degraded by hydrolysis in a process that first randomly degrades the bulk polymer, progressively reducing the molecular weight and the mechanical properties of the material but leaving the total mass of polymer essentially the same until the molecular weight of the fragments that are created is small enough to make them soluble. When this occurs, soluble material is generated rapidly, liberating the bulk polymer into solution but creating a profound local decrease in pH. Regression of local bone formation and sterile cysts in bone and soft tissue have been commonly observed[15,167]. Furthermore, in some settings, the polymer can crystallize as it degrades, creating particles that persist for years. In highly porous devices, the effects of degradation products may be less pronounced because the volume fraction of the bulk polymer in the graft site is smaller and the degradation products that are released are cleared more readily through a broader surface of contact with local extracellular fluids and vascular perfusion. Regardless, the use of polylactides and polyglycolides for bone regeneration remains controversial, and devices based on these materials are not widely used in clinical practice.

The limitations of polyesters are being addressed by the development of new classes of degradable materials that possess reasonable mechanical strength and do not release acidic degradation products. One material consisting of a copolymer of polyethylene oxide and polybutylene terephthalate showed promising osteoconductive results in animals but failed to induce bone formation in the iliac crests of humans[168]. A different class of materials, pseudo-polyamino acids, have been synthesized with a range of degradation properties and have been shown to offer improved behavior in bone sites in animals[169,170]. An innovative approach to matching degradation rate to tissue ingrowth is being developed by Hubbell et al., using polyethylene oxide-based gels that contain both cell adhesion molecules and other peptides, which are selected to provide specific biological activity and are released as the gel is degraded[171,172]. The gels can be formed in situ, and they function essentially at a synthetic extracellular matrix designed as a true surface eroding polymer. The gels are degraded by specific proteases that are elaborated by cells as they invade the structure. As a result, the scaffold is removed from the implantation site gradually, in concert with cell invasion, and it maintains its mechanical properties until it is degraded. This avoids the late burst release of material that is characteristic of bulk eroding polymers.

## Opportunities for Rational Design of Future Materials, Devices, and Strategies

Rapidly advancing knowledge and capabilities in many fields

are driving the next wave of tissue engineering strategies and products. Tissue engineering strategies that are particularly rich in opportunity include (1) improved methods for intraoperative selection and concentration of stem cells and progenitor cells; (2) cell delivery systems that enhance the survival of transplanted cells by managing the balance of mass transfer and metabolic demand at the graft site; (3) three-dimensional scaffolds with architectural and mechanical features that are customized for specific clinical applications; (4) chemically defined surfaces that present covalently tethered biologically active molecules (adhesion sites, growth factors, and synthetic peptides) creating local concentrations and gradients to elicit desired cell attachment, migration, differentiation, and survival; (5) defined microtextured surfaces that elicit the desired cell attachment, migration, differentiation, and survival; (6) scaffold materials that degrade in a manner that delivers biologically inert or even biologically active molecules, rather than molecules that may be harmful in the graft site; and (7) delivery systems for soluble molecules (e.g., BMPs and other protein growth factors) that ensure both a biologically active conformation and a local concentration or concentration gradient that is appropriate for the target cell population, minimizing the total dose of bioactive agent that is required and the risk of unwanted collateral effects.

Optimizing combinations of cells, matrices, and locally and systemically active stimuli will remain a complex process characterized by a highly interdependent set of variables with an almost infinite range of possible combinations. As a result, these developments must also be informed by a combination of clinical experience, knowledge of basic biological principles, medical necessity, and commercial practicality. The responsibility for rational development is shared by the entire orthopaedic community (developers, vendors, and physicians). The need for objective and systematic assessment and reporting is made particularly urgent by the recent rapid addition of many new options for clinical use. Prospective, randomized preclinical and clinical trials will play a critical role in the initial evaluation of new materials for specific indications. Prospective cohort studies will also be valuable in several settings, including testing whether the results of controlled studies can be generalized to the broader orthopaedic community, defining settings where current practice may fall short of reported or desired outcomes, and assessing settings where randomization is impractical or unethical because of the absence of equipoise (i.e., the absence of an alternative method for comparison that is perceived as being comparable in effectiveness or morbidity).

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