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"user friendly" for the critical care nursing and medical staffs, who are under inordinate stress when dealing with acutely ill patients and when decision times are short. These are the challenges faced collectively by bioengineers, cell biologists, and clinicians alike as we look to the future of the BAL bioreactor in clinical medicine.

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VIEWPOINT

# Tissue Engineering—Current Challenges and Expanding Opportunities

**Linda G. Griffith[1]* and Gail Naughton[2]**

**Tissue engineering can be used to restore, maintain, or enhance tissues and organs. The potential impact of this field, however, is far broader—in the future, engineered tissues could reduce the need for organ replacement, and could greatly accelerate the development of new drugs that may cure patients, eliminating the need for organ transplants altogether.**

## Introduction

The field of tissue engineering exploits living cells in a variety of ways to restore, maintain, or enhance tissues and organs (*1*, *2*). Tissue engineering conjures up visions of organs built from scratch in the laboratory, ready to be transplanted into desperately ill patients. The potential impact of this field, however, is far broader—in the future, engineered tissues could reduce the need for organ replacement, and could greatly accelerate the development of new drugs that may cure patients, eliminating the need for organ transplants altogether.

To engineer living tissues in vitro, cultured cells are coaxed to grow on bioactive degradable scaffolds that provide the physical and chemical cues to guide their differentiation and assembly into three-dimensional (3D) tissues (*3*). The assembly of cells into tissues is a highly orchestrated set of events that requires time scales ranging from seconds to weeks and dimensions ranging from 0.0001 to 10 cm. Coaxing cells to form tissues in a reliable manner is the quintessential engineering design problem that must be accomplished under the classical engineering constraints of reliability, cost, government regulation, and societal acceptance.

Even though fewer than five engineered tissues have been approved by the Food and Drug Administration (FDA), more than 70 companies are spending a total of $600 million per year to develop new products (*2*). There are still many technical challenges to overcome before we create "off-the-shelf" tissues that represent the translation of scientific discoveries into treatments for millions of patients. The successful large-scale production of engineered tissues requires an adequate source of healthy expandable cells, the optimization of scaffolds, and the creation of bioreactors, which mimic the environment of the body and that are amenable to scale-up. Additional challenges include the preservation of the product so that it has a long shelf-life and the successful use of various approaches to prevent tissue rejection.

## Biological Challenges: Cells and Their Sources

There are three principal therapeutic strategies for treating diseased or injured tissues in patients: (i) implantation of freshly isolated or cultured cells; (ii) implantation of tissues assembled in vitro from cells and scaffolds; and (iii) in situ tissue regeneration. For cellular implantation, individual cells or small cellular aggregates from the patient or a donor are either injected into the damaged tissue directly or are combined with a degradable scaffold in vitro and then implanted. For tissue implantation, a complete 3D tissue is grown in vitro using patient or donor cells and a scaffold, and then is implanted once it has reached "maturity." For in situ regeneration, a scaffold implanted directly into the injured tissue stimulates the body's own cells to promote local tissue repair.

Sources of cells for implantation include autologous cells from the patient, allogeneic cells from a human donor who is not immunologically identical to the patient, and xenogeneic cells from a different species. Each category may be further delineated in terms of whether the cells are adult or embryonic stem cells (capable of both self renewal and differentiation into a variety of cell lineages), or a mixture of differentiated cells at different stages of maturation (including rare stem and progenitor cells). Some approaches use cell mixtures, whereas others rely on separation or enrichment of stem cells.

Although the prospect of using xenogeneic cells for tissue repair remains controversial because of the potential for transmitting animal pathogens to humans, xenogeneic cells could perhaps temporarily support an ailing tissue until either a human donor organ becomes available for transplant, or the tissue repairs itself. For example, pig liver cells (hepatocytes) grown in extracorporeal biore-

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actors are being tested clinically to see whether they can support patients with liver failure until a liver transplant can be performed [see Viewpoint by Strain on page 1005 (*4*)].

Allogeneic cells have been used successfully to treat skin ulcers (*5*), diabetes (*6*), and liver disease (*7*). Patients with diabetic or venous skin ulcers have been treated with two FDA-approved living skin products engineered in the lab. One product is composed of neonatal dermal fibroblasts obtained from human foreskins. The neonatal fibroblasts are expanded in culture and seeded onto a thin scaffold composed of the polymer polylactide coglycolide (originally developed for use in surgical sutures), which breaks down gradually in the presence of water (*8*). The cells on their scaffold are cultured in custom-designed bioreactors for several weeks until they form a tissue similar to the inner dermal layer of skin. This neo-dermis is then frozen for shipment to physicians. The second skin product has both dermal and epidermal layers. It is composed of dermal fibroblasts in a collagen solution that forms a gel when heated to body temperature; the gel is coated with several layers of human epidermal cells (keratinocytes). After transfer to the patient, this skin product is at least partially replaced by host skin cells as healing progresses. The dermal fibroblasts in the skin products naturally secrete extracellular matrix proteins and are able to respond to growth-regulatory molecules secreted by the host (*9*). These skin products can persist for up to 6 months after implantation.

The success of engineered dermal implants for treating skin injuries and burns has not been as easy to replicate for organs such as the liver and pancreas, partly because expanding hepatocytes or pancreatic islet cells in culture is much more difficult than expanding dermal fibroblasts or keratinocytes. There is an FDA-approved autologous cell product for the repair of articular cartilage (*10*). A small piece of cartilage is removed from the healthy section of a patient's injured knee. Cartilage cells (chondrocytes) are isolated, expanded in culture, and are then implanted at the injury site. In a variation on this approach, mesenchymal stem cells have been harvested from patient bone marrow, expanded in culture, and then induced to differentiate into cells that can help to repair damaged bone, cartilage, tendon (see the News story by Pennisi on page 1011), or ligament (*11*). Given that donor and patient cells are already being exploited therapeutically, why is there such intense interest in adult and embryonic stem cells? Stem cells hold great promise for treating damaged tissue where the source of cells for repair is extremely limited or not readily accessible. Embryonic stem (ES) cells are attractive because they can be expanded in an undifferentiated state in vitro and can be induced to form many different cell types. Although ES cells can be coaxed to assemble into tissues as diverse as insulin-secreting pancreatic islets (*12*) and blood (*13*), they have not, as yet, been able to cure an animal model of disease. We still need better markers to identify stem cells and their progeny, better ways to expand them in culture, and more research to see whether there is an immunological barrier to implanting stem cells derived from allogeneic donors (*14*).

Adult bone marrow stem cells can be collected from the circulation (after mobilization with cytokines) and used clinically to treat a range of blood disorders. Recent reports that marrow-derived stem cells can give rise to hepatocytes, cardiac muscle cells, and lung tissue suggest that efficient recruitment of bone marrow stem cells to sites of injury or their injection into these sites may provide a source of cells for tissue repair. At least one model of animal liver disease has been cured by a bone marrow transplant (*15*). The addition of bone marrow to engineered bone grafts improves healing of bone defects; concentrating and selecting for the marrow stem cells that form bone improves healing still further (*16*).

## Engineering Challenges

*Blood vessels of the microcirculation.* One of the principal constraints on the size of tissues engineered in vitro that do not have their own blood supply is the short distance over which oxygen can diffuse before being consumed (a few hundred micrometers at most). Once implanted in the patient, cells in the engineered tissue will consume the available oxygen within a few hours, but it will take several days for the growth of new blood vessels (angiogenesis) that will deliver oxygen and nutrients to the implants. How can this problem be overcome? Implanting cultured cells directly into the existing vascular beds of the patient's liver and spleen looks like one promising strategy. Hepatocytes injected directly into human liver show engraftment and sufficient biochemical activity to ameliorate the symptoms of liver disease, although this is clearly not a cure (*7*). Some diabetic patients with pancreatic islet cells implanted into the liver (a very vascular organ) exhibited normal glucose tolerance for several months after the procedure (*6*).

Unfortunately, cells implanted for the repair of bone or tendon, for example, cannot exploit existing vascular beds. Inducing or speeding up angiogenesis by engineering a scaffold to slowly release growth factors, such as vascular endothelial cell growth factor (VEGF) or fibroblast growth factor (FGF), may be the answer. For example, controlled release of both VEGF and platelet-derived growth factor (PDGF) from the same scaffold implanted into rats resulted in blood vessel induction, maturation, and stabilization (*17*). However, blood vessel formation may still be too slow and the ultimate quality and stability of vessels suboptimal with this approach. Interestingly, angiogenesis also can be induced using engineered skin products because the dermal fibroblasts that they contain produce angiogenic growth factors (*8*, *18*). The need for preformed vascular beds or rapid angiogenesis could be avoided altogether by exploiting what may be a common property of many stem and progenitor cells—their resistance to low-oxygen conditions (*19*–*23*).

When small pieces of bone tissue are implanted at the site of bone injury, existing microvessels in the implant connect with blood vessels at the injury site. This has prompted the inclusion of endothelial cells (which form blood vessels) in cultures of the cells to be expanded, so that rudimentary tubelike vessels form within the assembling tissue (*24*). Even more ambitious is the goal to form fully vascularized tissues for implantation that contain blood vessels of sufficient size that they can be fused with the patient's own blood vessels during surgery. The complexities associated with organizing millions of cells into 3D structures such as blood vessels can be simplified using computer modeling, which translates the tissue's 3D structure into a 2D template. Composed of a degradable polymer, the 2D template precisely guides cells to their correct positions, the engineered tissue finally being folded up to form the 3D structure (*25*).

*Scaffolds.* Scaffolds are porous, degradable structures fabricated from either natural materials (collagen, fibrin) or synthetic polymers (polyglycolide, polylactide, polylactide coglycolide). They can be sponge-like sheets, gels, or highly complex structures with intricate pores and channels fabricated using new materials-processing technologies. Virtually all scaffolds used in tissue engineering are intended to degrade slowly after implantation in the patient and be replaced by new tissue.

Many epithelial and connective tissues have a simple macroscopic architecture consisting of a number of thin layers. Bladder, intestine, and blood vessels are composed of a layer of smooth muscle sandwiched between a layer of collagenous vascularized support matrix and an epithelial lining. Such structures can be built by seeding the different cell types for each layer sequentially ondegradable scaffolds made from synthetic fibers of polyglycolide or its derivatives that are 10 to 20 micrometers in diameter. Polyglycolide, unlike polylactide, does not dissolve in solvents such as chloroform. Thus, 3D polyglycolide scaffolds can be sculpted by dipping them in a solution of polylactide dissolved in chloroform and shaping the wet fabric on a mold. When the chloroform evaporates, the polylactide serves as a solid glue to hold the fabric in the desired shape (*26*, *27*). A scaffold

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made in this way in the shape of a bladder and seeded with urinary epithelial cells and smooth muscle cells has been implanted into dogs. This artificial bladder acquired near-normal function (*27*). New methods are being developed to process fabrics for more demanding scaffolding applications. For example, using solid free-form fabrication techniques, complex 3D polymer structures have been built from a series of very thin 2D layers, starting with a computer model of the desired shape derived from an MRI (magnetic resonance imaging) or computerized tomography image of the patient's original tissue (*28*) (Fig. 1).

Scaffolds can also be designed to release growth factors that induce cellular differentiation and tissue growth in vitro, or cell migration into the wound site in vivo. For example, a dual-release scaffold composed of PDGF encapsulated in polylactide coglycolide microspheres together with a powder of the same polymer linked to VEGF promoted angiogenesis by releasing VEGF quickly and PDGF slowly, thus mimicking the physiological production of these growth factors (*26*). Scaffolds containing small, degradable polymer beads that release nerve growth factor improve the viability of fetal neural cells transplanted into rat brain (*29*).

The fragile nature of proteins has motivated design of scaffolds that release naked plasmid DNA containing genes that encode growth factors (*30*). When a collagen scaffold fabricated to release the gene for parathyroid hormone (a protein that regulates bone growth) was implanted at a bone injury site in the dog, new bone was formed according to the "dose" of the gene (*30*). Controlling the diffusion rates of genes and proteins from scaffolds so that they are in the physiological range is the next challenge. New bioactive materials, such as those that covalently incorporate growth factors and other molecules that regulate cell behavior, offer alternatives for enhancing scaffold performance.

*Biomaterials.* A crucial mainstay of tissue engineering is the biomaterial from which scaffolds are fashioned (*3*) [see Viewpoint by Hench and Polak on page 1014 (*31*)]. Many biomaterials direct the growth of cells in culture. However, tissue regeneration in vivo involving the guided growth of nerve, bone, blood vessels, or corneal epithelia across critical injury sites requires that cells receive more specific instructions. In vivo, the cells that repair and regenerate damaged tissues are bombarded with molecular signals, both from the "hostile" wound site and from healthy surrounding tissues. The ideal biomaterial for a scaffold would selectively interact with the specific adhesion and growth factor receptors expressed by target cells in surrounding tissues required for repair of damaged tissue. The scaffold could guide migration of these target cells into the injury site and stimulate their growth and differentiation, finally degrading in response to matrix remodeling enzymes released by the cells as tissue repair progresses (*32*).

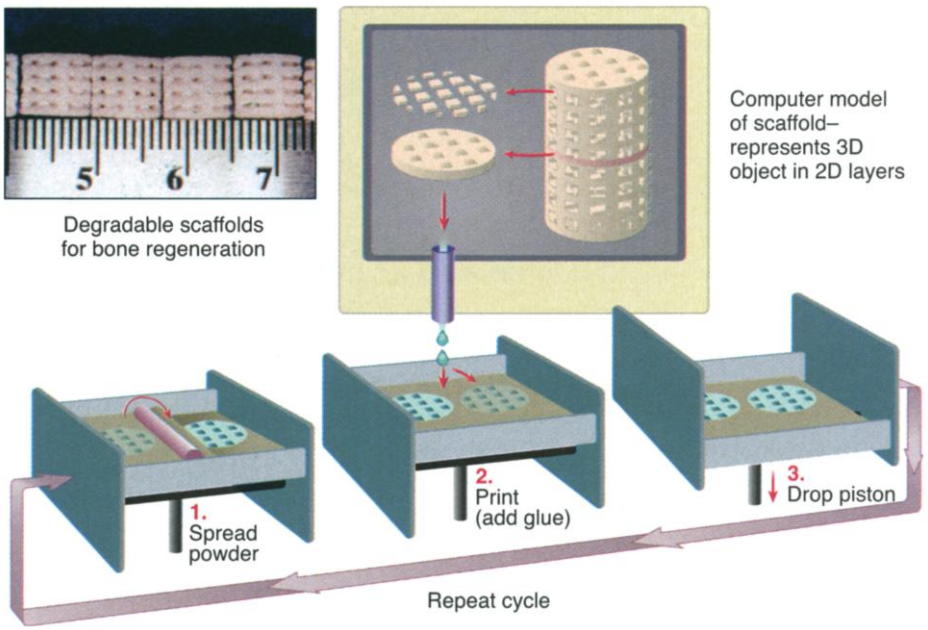


**Fig. 1.** The 3DP printing process is a solid free-form fabrication method that builds complex 3D objects from a series of 2D layers (*61*). First, a fine powder is spread in a thin layer on a piston. A binder or "glue" (green drops) is then "printed" into specific regions of the powder according to a computer program that has parsed the 3D object into thin 2D layers. The glue binds the particles together in precise regions, and the 2D layer starts to take shape. The piston is then lowered, the cycle repeated, and the next layer printed. This layer-by-layer process continues until the entire 3D object is fabricated, allowing the generation of gradients in composition, surface chemistry, and porosity. This provides 3D scaffolds that are suitable for growing composite tissue structures such as bone. [Illustration: Preston Morrighan]

The discovery of adhesion domains in fibronectin and other extracellular matrix glycoproteins containing the amino acid sequence Arg-Gly-Asp (RGD) has enabled the design of synthetic materials that can modulate cell adhesion (*33*). Yet the process is not as simple as identifying an adhesion peptide and incorporating it into a biodegradable material. Cell motility is an adhesion-dependent process required for cell migration, angiogenesis, and regrowth of severed nerve ends, among many other physiological events. An engineering model that incorporates the biophysics of how cells bind to extracellular matrix adhesion molecules and how they contract predicts that increasing the number of adhesion contacts between cells and the extracellular matrix may not always be advantageous (*34*). If too few adhesive ligands (such as RGD) are available, cells cannot get a strong enough grip to enable them to move, but if there are too many ligands, cells adhere so firmly that they remain stuck in place. Thus, intermediate adhesion is required for optimal cell migration (*34*). In vivo, bone scaffolds coated with adhesion proteins containing the RGD motif promote maximal tissue ingrowth only at intermediate values of ligand surface density (*35*); likewise, only at an intermediate density do adhesion proteins on scaffolds induce neural progenitor cells to extend neurites, a prerequisite for nerve regeneration (*36*). Cells are also responsive to the nanoscale spatial organization of RGD peptides—such peptides more effectively induce cell adhesion and migration when they are clustered rather than random (*37*, *38*). The ability of fibrin scaffolds modified with the peptide ligand L1Ig6, which binds to the cell surface adhesion receptor integrin $\alpha_v\beta_3$, to promote angiogenesis is influenced by the supramolecular organization of the fibrin (*39*).

Design principles are emerging for modulating the interactions of cells with growth factors. Apart from hematopoietic cytokines, successful use of growth factors for human tissue regeneration has been notoriously difficult. Many growth factors, including the angiogenic factors VEGF and FGF, are bound tightly to the extracellular matrix of normal tissues. A ligand for the epidermal growth factor receptor (EGFR) is immobilized within tenascin, a large extracellular matrix molecule (*40*). Presenting growth factors as part of an extracellular matrix, rather than just releasing them into the liquid medium, has improved nerve regeneration and growth of smooth muscle cells during engineering of artificial arteries (*41*, *42*). Using gel scaffolds that incorporate a complete compendium of growth factors and their correctly presented adhesion sites may be the next step (*32*).

*Tissue architecture.* The correct molecular and macroscopic architecture of cartilage, blood vessels, bone, and other tissue is essential for proper tissue function. Connective tissue cells grown on 3D scaffolds in vitro secrete biochemically appropriate extracellular matrix molecules

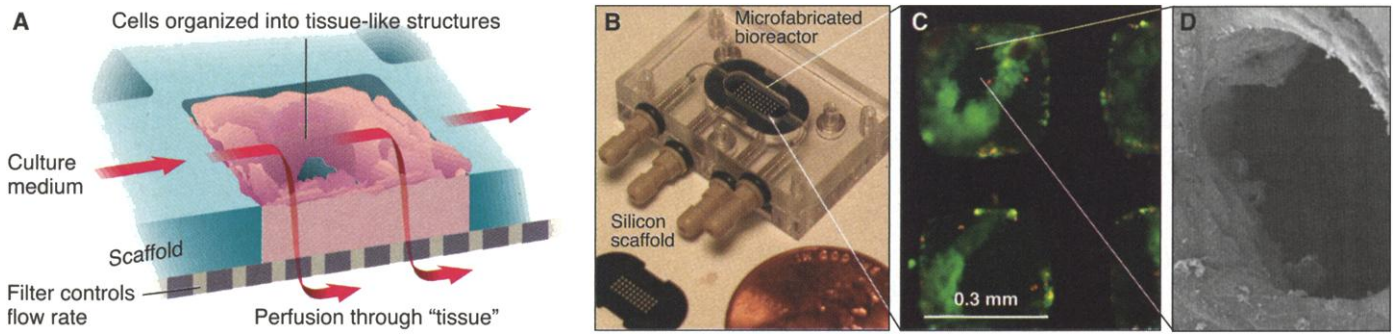


**Fig. 2.** A microfabricated bioreactor for perfusing 3D liver tissue engineered in vitro (*54*, *55*). **(A)** A cross section showing tissue aggregates growing attached to the inside walls of the narrow channels of the silicon-chip scaffold. Culture medium flows across the top of the scaffold as well as through the narrow channels, enabling tissue aggregates to extract oxygen and nutrients. The design of the scaffold promotes self-assembly of the cells into tissues. **(B)** A bioreactor containing a 0.2-mm-thick silicon-chip scaffold etched with 0.3-mm-diameter channels. **(C)** Hepatocytes seeded onto the scaffold of the bioreactor attach to the walls of the channels (four channels are shown) and reorganize to form 3D structures that are reminiscent of liver cords. Bile canaliculi and tight junctions can be seen with high-power microscopy (*54*, *55*). Live cells are green and dead cells are red as visualized with the calcein AM/ethidium homodimer stain. **(D)** Scanning electron micrograph showing vessel-like structures assembled from endothelial cells at the fluid-tissue interface in the bioreactor channels. [Illustration: Preston Morrighan]

yet fail to acquire the appropriate tissue architecture. The answer may lie in providing appropriate physiological stresses during engineering of the tissue in vitro. The first generation of bioreactors for culturing cells were designed simply to pump nutrient liquid (culture medium) through the assembling tissue. The next wave of bioreactors designed for growing blood vessels and cartilage subjected the nascent tissue to compression, shear stresses, and even pulsatile flow of culture medium. Such stresses profoundly improve the mechanical properties of engineered vessels, cartilage, and cardiac muscle (*43*). In a promising result for the ~500,000 patients who need heart bypass surgery, an artificial artery engineered in the laboratory under pulsatile conditions using endothelial cells seeded on a polymer scaffold developed a burst strength of 2000 mmHg compared with 300 mmHg for unstressed tissues (although physiological responses to vasoactive factors were not completely normal) (*44*). A typical saphenous vein graft used surgically has a burst pressure of ~1000 mmHg; normal systolic pressure exerted on blood vessels in vivo is about ~120 mmHg.

A critical issue for engineering 3D tissues in vitro is scale-up for clinical use. Hundreds or thousands of tissues must be grown and cryopreserved under sterile conditions. This has certainly been feasible for the allogeneic dermal fibroblasts that comprise the FDA-approved engineered skin products. These cells can be expanded quickly in culture without the need for external stresses, and are readily amenable to cryopreservation (*45*). If cells to be engineered into tissues for implantation must first be derived from the patient, then a separate culture system will be required for each patient, posing formidable regulatory challenges and high costs. Thus, there is much interest in the in situ growth of tissue from injected cells where mechanical stresses are applied naturally. Blood vessels with superior mechanical properties have been grown in situ in an animal model using a natural scaffold (the small-intestine submucosa stripped of cells but with an intact extracellular matrix) that recruited endothelial cells (*46*). However, translating vascular successes in animals to humans is notoriously difficult because human and animal endothelia behave very differently (*47*).

### Tissue-Engineered Model Systems

Tissue engineering can be applied to the development of drugs to treat many diseases that could be prevented or even cured if such drugs were available today. For example, the hepatitis C virus has infected more than 170 million people worldwide and is currently the leading cause of liver failure in the United States (*48*–*50*). Infecting cultured liver cells with the virus is extremely difficult, and the lack of small-animal models has hampered the development of drugs to combat this pathogen. Unfortunately, human hepatocytes quickly lose many of their liver-specific functions, including susceptibility to viral infection and ability to metabolize drugs, when they are cultured. Chimeric animal models made by implanting human hepatocytes into mice (*51*–*53*) may provide an alternative system for studying hepatitis C virus and other hepatitis viruses. In the future, engineered liver tissue may provide a cheap in vitro system with greater control of variables for studying viral infection.

We speculate that the greatest impact of tissue engineering in the coming decade will be for designing in vitro physiological models to study disease pathogenesis and for developing molecular therapeutics. For example, engineered 3D capillary beds could be used to study the effects of a variety of insults in a high-throughput manner that is reasonably physiological (*54*, *55*). The creation of tissues containing hierarchical cell-cell interactions under appropriate mechanical stresses (including perfusion shear as found in the microcirculation) will take in vitro systems even closer to living tissues (see Fig. 2). In Europe, human skin engineered in vitro (*56*) has replaced animals in the regulatory approval process for testing drugs and other agents for their skin corrosiveness (*57*, *58*). New microfabrication and microfluidics technologies are beginning to transform the diagnostics field, allowing multiple chemical reactions to take place on a small plastic chip (*59*, *60*). Tissues engineered in vitro that can be easily manipulated have the potential to expand tissue engineering into areas such as cancer drug development. For example, a good in vitro model

## Clinical Endpoints

How are engineered tissues ultimately judged to be good enough for clinical application? With tissues such as kidney or bone marrow, analysis of urine or blood provides valuable information about the success or failure of the tissue in both human patients and animal models. But different methods are needed to quantitatively assess the long-term outcome of engineered connective tissues, such as cartilage, tendon, and blood vessels. The ultimate failure of these tissues (or their engineered counterparts) is defined as a failure to perform mechanically, resulting in pain and disability. Metrics used in current clinical trials of engineered cartilage, for example, are a reduction in pain, appearance of new tissue, and qualitative mechanical probing. Because connective tissues are relatively avascular, they take a long time to be remodeled and repaired, so that metrics must be obtained over the long term. In the absence of mechanistic models of long-term tissue repair, gene-expression changes obtained by microarray analysis, or imaging of 3D tissue structure with MRI, may be valuable alternative strategies.

of cancer metastasis that mimics the lodging of a single tumor cell in a capillary bed would facilitate the development of antimetastatic drugs.

The increasingly intimate combination of engineering and biology offers the prospect of sophisticated physiological in vitro models of many different human tissues. These physiological surrogates will ultimately allow major advances in prevention, diagnosis, and molecular treatment of diseases that are currently considered potential targets for tissue engineering. Ultimately, this may result in a greater emphasis on treating different target diseases, such as trauma and congenital defects, with engineered tissue.

With a scientific foundation firmly established, we now need a robust infusion of biology-based engineering analysis and design to move the tissue-engineering field from an era of phenomenological observation and serendipity to one of commercially viable products that will improve the lives of millions of patients.

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VIEWPOINT

# Third-Generation Biomedical Materials

**Larry L. Hench* and Julia M. Polak***

**Whereas second-generation biomaterials were designed to be either resorbable or bioactive, the next generation of biomaterials is combining these two properties, with the aim of developing materials that, once implanted, will help the body heal itself.**

Initially, the choice of biomedical materials for use in the body was dependent on those already available off the shelf. Until an understanding of the immune system developed, many of the materials selected proved to be either pathogenic or toxic. During the 1960s and 1970s a first generation of materials was developed for use inside the human body. The goal of all early biomaterials was to "achieve a suitable combination of physical properties to match those of the replaced tissue with a minimal toxic response in the host" (*1*). In 1980 there were more than 50 implanted devices (prostheses) in clinical use made from 40 different materials (*1*), and some 2 to 3 million prosthetic parts were implanted in patients in the United States annually. A common feature of most of the materials was their biological "inertness." The principle underlying the bulk of biomaterials development was to reduce to a minimum the immune response to the foreign body, and this is still valid 21 years later. Tens of millions of individuals have had their quality of life enhanced for 5 to 25 years by use of implants made from such "inert" biomaterials.

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## Second-Generation Biomaterials

The field of biomaterials began to shift in emphasis from achieving exclusively a bioin-

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