Tissue Engineering
Many Challenges Ahead

by Nilesh V. Patil

Tissue engineering is evolving as an alternative strategy to autografts and allografts, which have inherent limitations such as donor site morbidity and risks of disease transmission and immune rejection. Tissue engineering strategies generally make use of highly porous scaffolds, which may serve as delivery vehicles for cells and/or growth factors, with the ultimate goal being to replace injured or diseased organs and tissues. During the past decade, many highly porous biodegradable scaffolds have been created and used for a variety of such strategies (1–3).

Tissue engineering is the persuasion of the body to heal itself achieved by delivering to the appropriate site cells, biomolecules, and supporting structures. The technology specifically involves regeneration of new tissue to replace that which has become diseased or injured, an ability that adult humans do not normally possess. We may repair ourselves under some very limited circumstances (e.g., bone fractures and injured skin), but even when that does occur, such healing often involves nonspecific reparative tissue (scar tissue) rather than the specific functional tissue that was affected (4).

The essence of tissue engineering is that those cells capable of initiating and sustaining the regeneration process are “switched on,” perhaps through growth factors or genes, so that they generate new functional tissue of the required variety. This may be achieved with the help of a scaffold or matrix to guide the geometrical/architectural shape of the new tissue and may be either customized at the site of the injury in an individual patient or on a more industrial scale in an ex vivo bioreactor, with the resulting tissue construct reimplanted into a patient (5).

The field of tissue engineering exploits living cells in a variety of ways to restore, maintain, or enhance tissues and organs (6, 7). The phrase conjures up visions of organs built from scratch in the laboratory, ready to be transplanted into desperately ill patients. The potential is far broader: In the future, engineered tissues could reduce the need for organ replacement and greatly accelerate the development of new drugs that may eliminate the need for organ transplants altogether.

To engineer living tissues in vitro, cultured cells are coaxied to grow on bioactive degradable scaffolds that provide physical and chemical cues to guide their differentiation and assembly into three-dimensional (3D) tissues (8). 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 cm to 10 cm.

Many technical challenges must be overcome before we can create “off-the-shelf” tissues. Successful large-scale production of engineered tissues requires an adequate source of healthy, expandable cells, as well as the optimization of scaffolds and development of scalable bioreactors that mimic the environment of a human body. Additional challenges include product preservation and stability for long shelf-lives and prevention of tissue rejection.

CURRENT CHALLENGES

Microcirculation: One principal constraint on the size of tissues engineered in vitro without their own blood supply is the short distance over which oxygen can diffuse through tissue before being consumed (a few hundred micrometers at most). Once implanted in a patient, cells in engineered tissue will consume available oxygen within a few hours — but it takes several days for the growth of new blood vessels (angiogenesis) that will deliver fresh oxygen and nutrients to the implants. How can this problem be overcome? Implanting cultured cells directly into existing vascular beds of a patient’s liver and/or 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 does not represent a cure (10). Some diabetic patients with...
pancreatic islet cells implanted into their livers (very vascular organs) have exhibited normal glucose tolerance for several months afterward (9). Unfortunately, cells implanted for repair of bone or tendon, for example, cannot exploit existing vascular beds. The answer may be to induce or speed up angiogenesis by engineering a scaffold to slowly release growth factors such as vascular endothelial cell growth factor (VEGF) or fibroblast growth factor (FGF). For example, controlled release of both VEGF and platelet-derived growth factor (PDGF) from the same scaffold implanted into rats caused blood vessel induction, maturation, and stabilization (12). However, blood vessel formation may yet be too slow with this approach, and the ultimate quality and stability of vessels could be suboptimal. Interestingly, angiogenesis also can be induced using engineered skin products because their dermal fibroblasts produce angiogenic growth factors (11, 13). 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 (14–18).

When small pieces of bone tissue are implanted at the site of a 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 cells to be expanded so that rudimentary, tubelike vessels can be simplified using new materials-processing technologies. Virtually all scaffolds used in tissue engineering are intended to degrade slowly after implantation and ultimately be replaced by new tissue.

Many epithelial and connective tissues have a simple macroscopic architecture consisting of many 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 onto degradable scaffolds made from synthetic fibers of polylactide (or its derivatives) that are 10–20 µm in diameter. Unlike polylactide, polylactic acid (PLA), and other semicrystalline polyglycolide (or its derivatives) that are 10–20 µm in diameter. Unlike polylactide, polylactic acid (PLA), and other semicrystalline polylactide coglycolide (originally developed for use in surgical sutures), which breaks down gradually in the presence of water. 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 incorporates 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 both skin products naturally secrete extracellular matrix proteins and respond to growth-regulatory molecules secreted by their hosts. These products can persist for up to six months after implantation (27, 28).

**Fabrication of Scaffolds**

**Textile Technologies:** Earlier tissue engineering scaffolds of fibrous biodegradable polymer fabrics were produced using textile technologies. Polyglycolic acid (PGA), polylactic acid (PLA), and other semicrystalline polymers can be processed into fibers using textile technologies. One such scaffold widely used in tissue engineering research is PGA nonwoven scaffold, which has been used either alone or combined with other biodegradable polymers for the
engineering of cartilage, tendon, ureter, intestine, blood vessels, heart valves, and other tissues (29–37). However, there are several limitations with such scaffolds: low mechanical strength, fast degradation, difficulty in controlling pore shape, and limited fiber diameter variation.

**Particulate-Leaching Technologies:**
Another technique that has been widely used to fabricate scaffolds for tissue engineering is particulate leaching. In this relatively simple process, salt is first ground into small particles, and those of the desired size are transferred into a mold (38, 39). A polymer solution is then cast into the salt-filled mold. After solvent evaporation, salt crystals are leached away using water to form pores in the scaffold. Pore size is controlled by the size of the salt crystals and porosity by the salt/polymer ratio. However, certain critical variables such as pore shape and inter-pore openings are not controlled.

To overcome the shortcomings of textile technologies and particulate leaching, new techniques are being developed involving phase separations.

**Solid–Liquid Phase Separation:**
Phase separation can be achieved by lowering the temperature of a polymer solution to induce solvent crystallization, a process termed solid–liquid phase separation (that is, solid phase formation in a liquid phase). After removal of the solvent crystals (sublimation or solvent exchange), the space originally taken up by them becomes pores. This technique can be used to fabricate scaffolds from many types of polymers and polymeric composite materials (40–41).

Through manipulation of the phase separation conditions, various pore structures can be achieved. For example, many tissues (e.g., nerve, muscle, tendon, ligament, dentin, and so on) have specifically oriented tubular or fibrous bundle architectures. To facilitate organization and regeneration of such tissue types, a high–porosity scaffold with an oriented array of open microtubules may be desirable. A novel phase separation technique has been developed to grow oriented, rod-shaped crystals from a polymer solution. After their removal, a parallel array of microtubules is formed. The resulting scaffold has anisotropic mechanical properties similar to fibrillar and tubular tissues and has been shown to facilitate cell organization into oriented tissues (42).

**Liquid–Liquid Phase Separation:**
Lowering the temperature can induce the liquid–liquid phase separation of a polymer solution that has an upper critical solution temperature. When such a process leads to formation of a bicontinuous structure (both the polymer-rich and polymer-lean phases being continuous), an open-pore scaffold is formed after solvent removal. For example, a dioxane–water mixture has been used for liquid–liquid phase separation to fabricate PLA and poly(lactic-glycolic acid) (PLGA) scaffolds (42, 43).

**Other Types of Scaffold**

**Nanocomposites:** Polymer/inorganic composite materials have been developed for mineralized tissue engineering applications. To mimic the size scale of mineral crystals in bone and other mineralized tissues, nanometer-scale hydroxyapatite (nano-HAp) is compounded with synthetic polymers or natural macromolecules to fabricate nanocomposite scaffolds. Such scaffolds have not only improved on the mechanical properties of polymer scaffolds, but also significantly enhanced protein adsorption over micrometer-scale HAp/polymer scaffolds. Enhanced protein adsorption improves cell adhesion and function (44–46).

**Bioactive Scaffolds:** The ideal tissue engineering scaffold would positively interact with cells: enhancing cell adhesion, growth, migration, and differentiated function. Surface or bulk modification of polymers are often the means of achieving such positive cell–scaffold interactions. Bulk modification is typically realized by copolymerization or functional group attachment to polymer chains before scaffold fabrication, which usually changes their mechanical and processing properties (47–50).

Surface modification can be carried out after a porous scaffold has been fabricated. For example, plasma treatment alone (or followed by chemical modification) has been used to modify polymer thin films and porous scaffolds — most effective on 2D film surfaces or very thin 3D constructs. In a complex, porous 3D scaffold, the surface is not just the outside surface, but also the internal 3D surfaces.

Simulated body fluid has been used to modify the chemical composition of the internal three-dimensional pore surfaces of polymer scaffolds. This biomimetic process was effective at introducing nanometer-scale, bone-like apatite into those internal pore surfaces in situ, which may lead to improved scaffolds for bone tissue engineering (51–54). More 3D surface modification techniques will be needed.

To program scaffolds with biological instructions, delivery of bioactive molecules and genes has been integrated into some scaffold designs for tissue engineering (55–62).

**Future View**
Tissue engineering has significant market potential, so financial investment continues at a healthy pace. Technical advances in the various components of the industry will contribute to market growth. One requirement is the availability of biomaterials that act as scaffolds for tissue repair and reconstruction or for the deposition of engineered tissues and cells before implantation. An increasing amount of research and development is being directed toward addressing the properties of such scaffolds with a goal of creating materials that have certain desired functional profiles for specific applications.

For example, so-called blended–polymer scaffolds have an extended lifetime in vivo that is more suitable for orthopedic applications than are nonblended scaffolds (63). Another study showed how collagen-based scaffolds used to grow keratinocytes in artificial skin preparations can be manipulated by cross-linking collagen with glycosaminoglycan (64). The result was increased biological stability, which augmented the likelihood that keratinocytes would “take” and grow out of the scaffold. In yet another application (65), a sacchitin glycolipid-type membrane prepared from the residue of a fungal fruiting body was shown to have significant promise in skin-damaged animals as a skin repair treatment alone (or followed by chemical modification) has been used to modify polymer thin films and porous scaffolds — most effective on 2D film surfaces or very thin 3D constructs. In a complex, porous 3D scaffold, the surface is not just the outside surface, but also the internal 3D surfaces.

Simulated body fluid has been used to modify the chemical composition of the internal three-dimensional pore surfaces of polymer scaffolds. This biomimetic process was effective at introducing nanometer-scale, bone-like apatite into those internal pore surfaces in situ, which may lead to improved scaffolds for bone tissue engineering (51–54). More 3D surface modification techniques will be needed.

To program scaffolds with biological instructions, delivery of bioactive molecules and genes has been integrated into some scaffold designs for tissue engineering (55–62).
substitute to facilitate wound healing and fibroblast growth.

Development of such materials that enable different applications of tissue engineering is likely to be the focus of considerable future research. For the biological component of tissue engineering, rapid advances are being made in identifying new cell types for use in tissue regeneration (66). For example, undifferentiated stem cells are attracting intense interest because of their capacity to be transformed into almost any cell type that may be needed. Even fat cells can be directed to produce appropriate tissues (67). In addition, promising artificial nerve grafts or nerve guidance channels are being developed for nerve regeneration (68).

In the future, efforts are likely to focus on the development of tissue-engineered products under consensus safety and efficacy standards for cell and tissue sources, characterization and testing of materials, quality assurance and control, and preclinical and clinical evaluation. The FDA has already provided some regulatory guidance (72) concerning specific materials, such as certain marketed artificial skin products. In the next few years, guidelines are likely to be formalized and structured, ensuring that not only do tissue engineering products work but that they are also safe to use.

Significant future developments are likely to include artificial organs that use cells embedded into appropriate support structures. A recent report describes the use of polyurethane foam as a matrix into which liver cells grew as spheroids, a system showing promise as an artificial liver. The future will also see significant efforts to develop engineered vascular grafts. An approach that will see increasing attention is that of using as a scaffold a structurally intact xenogeneic vessel, such as a pig’s aorta. After all porcine cells are removed, it can be repopulated with human autologous cells. A recent report showed how that could be done over a two to three-week schedule, showing the way to a possible alternative to vascular engineering.

The range of human tissue that can be engineered will also increase dramatically in the future. A great deal of excitement in clinical circles is surrounding the concept of artificial human thyroid tissues capable of producing T cells, which will be a major area for continued R&D. Finally, stem cells will continue to be a major area of development because of their pluripotency. For example, bone marrow stem cells contained in resorbable artificial tubes have been used for effective healing of nonunion defects in rabbit radii, which suggests significant surgical alternatives for organ and tissue damage (69–71).

Tissue engineering is one of the most exciting interdisciplinary and multidisciplinary research areas today, and the field is growing into a vibrant industry with a huge potential market. Scaffold materials and fabrication technologies play a pivotal role and are fast evolving. The biomaterials, scaffolds, artificial organs, and differentiating cells combined to create a tissue engineering product address significant medical needs, such as major tissue and organ damage or failure. Efforts to overcome the various challenges are under way. If past success is any indication, this technology will have a major impact on the future of health care.

REFERENCES