CME

Tissue Engineering: Progress and Challenges

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Learning Objectives: After studying this article, the participant should be able to: 1. Identify the current trends in tissue engineering. 2. Determine the limitations in the development of clinical products. 3. Gain further knowledge on specific approaches to engineered tissue types.

Tissue engineering is the interdisciplinary field of principles of engineering and life sciences. Most of the financial support for this field originates in the private sector, creating a relative economic handicap. Despite initial enthusiasm for the field, limited products for clinical use have been developed. This article evaluates the current progress in the field of tissue engineering, the current limitations to engineering tissue and organ-specific sites, and the current regulatory questions that need to be defined and answered to permit progress in this field. (Plast. Reconstr. Surg. 114: 26e, 2004.)

In 1933, Bisceglie et al. implanted mouse tumor cells encased in an immunoshielding, biocompatible polymer membrane and laid the foundation for tissue engineering.1 This nascent technology developed slowly, as investigators developed the tools and pooled the multidisciplinary talent necessary to advance the field. Discoveries such as the growth factor superfamilies and advances in microfabrication techniques, material science, and surface chemistry have allowed exponential growth in investigational models, with promising results. Fueled by private and public sector funding, tissue engineering has the potential to deliver significant accomplishments in the life sciences and engineering fields that could become landmarks in the timeline of human evolution. It may revolutionize clinical practice protocols, as current conventional paradigms of tissue repair and transplantation are displaced by superior innovations with demonstrated efficacy. But will the hype live up to the expectations? This article focuses on the current progress in the development of tissue-engineering constructs, their limitations, and the future direction of the field.

Overview

Tissue engineering is the interdisciplinary field of principles of engineering (material science, surface chemistry, physical chemistry, mathematics, and computer engineering) and life sciences (molecular biology, cell biology, transplant medicine, genetics, and surgery) directed to the development of biomimetic constructs that augment, restore, or maintain existing biologic systems. The approaches can be stratified as substitutive, histioconductive, or histioinductive.2 Substitutive approaches are essentially organ replacement systems. Histioconductive systems feature the replacement of missing or damaged parts with ex vivo constructs. An alternative approach is to direct reconstruction of tissue defects using harvested and ex vivo expanded cells delivered in an organized scaffold. Histioinductive approaches are facilitated self-repair and may involve the delivery of DNA through plasmid vectors for gene therapy or growth factors via microsphere technology. Biologic systems may be modulated by both these methods to regenerate missing tissue.

Épidermis and cartilage were the first two constructs approved by the U.S. Food and Drug Administration and are currently used in clinical applications. Despite years of research, today there are fewer than five products approved by the Food and Drug Administration and in routine clinical use. This small number

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is attributable to a variety of reasons, including limitations in the science, regulatory questions, and the economics of large-scale production.

**CURRENT ECONOMICS**

In 2001, there were 3300 individuals employed by 73 companies actively engaged in tissue engineering research. During the past 10 years, an estimated $3.5 billion has been invested in this sector, with recent expenditures of $610 million per year. This annual rate of spending has been increasing by a compounded 16 percent. Despite substantial investment, the industry has yet to produce a profitable product. This fact is best appreciated in the context of the cost for research and development to deliver products from this nascent technology.

Of the 73 companies involved in tissue engineering research, 16 are located outside the United States, and 16 are traded globally. Market dynamics, however, have forced several rounds of mergers and acquisitions as the industry consolidated around the leaders. Several companies with diversified investments may weather the inevitable rounds of consolidation and collapse. These include Organogenesis, Modex, and Genzyme Tissue Repair.

For purposes of market analysis and industry evaluation, these companies are divided into three groups. The first group is structural and is involved with the production of skin equivalents, bone substitutes, and vascular grafts. Organogenesis (Canton, Mass.), the makers of Apligraf, and Genzyme Surgical Prod (Cambridge, Mass.), the makers of Carticel, are examples. The structural group represents 60 percent of the aggregate industry, which has expanded by 85 percent during the last 3 years. The second group is focused on the development of cell-based technologies, such as stem cell research. Activity in this area has increased by 70 percent in the past 3 years and now accounts for 30 percent of the field. The third and final group is metabolic, involved in the production of histiosubstitute products such as bioartificial organs and encapsulated cell therapy. The technical difficulties inherent in engineering solid organ substitutes have left this area fraught with failures. The lack of a marketable product has led to a 35 percent reduction, and currently this industry represents only 10 percent of the overall tissue engineering initiative.

Unlike the development of transplantation, tissue engineering has relied primarily on private funding for research and development. Recently, however, the National Heart, Lung, and Blood Institute established the “SPARK” working group, a scientific panel intended to explore promising developments that deserve funding. This focus group is divided into four areas: functional genomics, gene-gene and gene-environment interactions, tissue genesis/organogenesis, and immunobiology. The vision of the group was fulfilled with the establishment of six national service cores designed to allow free access to and sharing of information among participating research laboratories. Governmental support is essential to reduce start-up costs for satellite facilities as well as to reduce efforts in duplication.

**CONSTRUCT MATERIALS**

Materials utilized in tissue engineering have defined prerequisites, such as the absence of toxicity, biocompatibility, absence of carcinogenicity, sterilizability, and surgical pliability. In addition, their mechanical properties should include permeability, stability, elasticity, flexibility, plasticity, and resorbability at a rate congruent with tissue replacement. They should allow cell adhesion and the potential for delivery of biomodulating agents such as growth factors and genetic material.

Construct materials may be divided into three groups: biogenic, semisynthetic, and synthetic. Biogenic materials include acellular matrices, collagens, alginate, glycosaminoglycans, and fibrin. Synthetic polymers include polylactic acid, polyglycolic acid, polylactic and polyglycolic acids, polyethylene glycol, polyphosphazenes, polyanhides, and various hydrogels. Semisynthetics include a combination of biogenic materials and synthetic polymers. The material properties of individual components influence the attributes of the engineered construct and should be tailored to simulate the properties of the native tissue being replaced. Polylactides are thermoplastic natural polymers with remarkable flexibility yet high tensile strength and minimal inflammatory stimulus. These properties are ideal for use in heart valve and blood vessel engineering. Hydroxyapatite and tricalcium phosphate have utility in bone replacement. Polylactic and polyglycolic acids appear to have intrinsic vascular inductive properties independent of the addition of exogenous angiogenic growth factors. Their structural properties can also be tailored by varying the ratio of the constituent
polymers. Constructs can then be engineered in the form of sheets, gels, or free-form, solid, three-dimensional scaffolds. The use of a three-dimensional, solid, free-form fabrication technique may allow the creation of scaffolds with an intricate microarchitecture. Only recently has this technique produced constructs with the required porosity to permit microcapillary ingrowth.

Current advances in scaffold engineering feature the use of “smart materials.” These constructs are enhanced to enable the manipulation of endogenous host systems to facilitate the repair process. This may be accomplished via DNA plasmid vectors, cells, or growth factors. Supercritical carbon dioxide conditions or salt-leaching techniques can be used to engineer scaffolds with these factors incorporated into the structure. The use of adhesion peptides RGD (Arg-Gly-Asp) to coat scaffolds permits directed tissue ingrowth into the scaffold or, alternately, a method to bind cells for tissue enhancement.

CELLS

The source of cells utilized in tissue engineering may be autologous, allogenic, or even xenogenic. The cells can be injected, or they can be incorporated into ex vivo engineered constructs and implanted for directed tissue repair. There are immune advantages to the use of autologous tissue. This is neither cost effective nor batch-controlled for universal clinical use, however. Although the economics of scale suggest that the use of a few cell lines would allow greater cost control and product uniformity between batches, the use of freshly isolated cells may allow survival advantages over those that are expanded in vitro.

The variety of cell lines with potential applications in tissue engineering include embryonic stem cells, bone marrow–derived stem cells, somatic pluripotent stem cells, adipose-derived stem cells, mesenchymal stem cells, and progenitor cells. There is some uncertainty regarding the origin of some peripheral stem cells, and it is postulated that they may reflect cells of bone marrow origin that have migrated to various locations. The current profile of stem cells and cell line differentiation will need revision and improved methods for identification. It is postulated that this understanding can lead to the development of techniques to direct the process of their differentiation.

GROWTH FACTORS

Protocols involving the use of growth factors must address the sequence, timing, dosage, delivery vector, and duration of therapy. Incorporation of growth factors into scaffolds must include consideration of appropriate loading capacity, uniform load distribution, binding affinity, dose delivery, release kinetics, and targeted delivery to the specific cell populations. Microspheres have been used to deliver growth factors. The delivery of growth factors has also been accomplished by the use of impregnated scaffolds that release factors during resorption. These systems accomplish site-directed drug delivery, thereby avoiding potentially undesirable side effects from systemic delivery. Stryker Biotech (Kalamazoo, Mich.) has developed a collagen-based scaffold impregnated with osteogenic protein-I and BMP-7. T-cell growth factor-B1/fibroblast growth factor-2 was demonstrated to promote chondrocyte proliferation, as evidenced by Western blot and immunohistochemistry assays. Other groups have used gene therapy to deliver plasmids by ex vivo transfection of cells followed by implantation within the construct or by in vivo transfection of responding regional cells.

BIOREACTOR SYSTEMS

In vitro bioreactor systems must provide the appropriate conditions for cell adhesion, growth, and differentiation within the construct. These conditions are determined by the particular cell lines and the properties of the scaffold. Investigators have determined that dynamic systems simulating the biologic stress factor appropriate for the cell line resulted in improved matrix formation and tissue architecture stability. Pulsatile forces improved construct mechanical properties in engineered blood vessels, whereas magnetic and compressive forces resulted in improved bone replacement constructs. Essentially, the bioreactor conditions can be tailored to the cell line or tissue type to generate the optimal product. Alternately, other investigators have focused on utilizing the body as the ultimate bioreactor. Having determined the ideal culture conditions for individual cell lines, the next challenge is the automa-
tion of the process to allow large-scale production in an aseptic environment.

**Review of Specific Tissues**

Previous accomplishments in tissue engineering represent the first generation and are relatively simple constructs engineered at the cellular and basic tissue levels. The following are examples and reflect the current status of engineering specific tissue types.

**Bone**

Tissue-engineered attempts to reestablish bony continuity to bridge a defect presently involve autografting or allografting cancellous bone, the use of vascularized bone grafts, or distraction osteogenesis. Ex vivo expansion of bone marrow cells followed by reimplantation has been used to repair bony defects. Osteoprogenitor stem cells have been used in the treatment of osteogenesis imperfecta in the preadolescent population. Recent literature reported successful retroviral transduction of BMP-7 into periosteal cells coupled with a polyglycolic acid scaffold for repair of calvarial defects in a rabbit model. Other investigators described the use of adenovirus vectors to transfect target area cells and affect bone production under the influence of BMP-2 elaboration. A sheep model was used to demonstrate the efficacy of bone generation via matrix delivered DNA transfection of locoregional cells to elaborate BMP.

One major limitation to the creation of a system for bone repair is the development of a scaffold with the requisite mechanical competence as well as porosity and resorbability. Many biocompatible scaffolds utilized in soft-tissue repair are unsuitable for use in bone tissue repair. They often lack the structural integrity required for load bearing. Those materials that are appropriately strong are often not resorbable. The ideal construct should have the ability to withstand the load-bearing capacity of bone as well as the characteristic of bioabsorbability. Hydroxyapatite, tricalcium phosphate, and the polymers DegraPol-foam and Polyactive have demonstrated potential for use in bone replacement constructs. Their suboptimal structural integrity during resorption has led to the design of surface-eroding composites that have a nonresorbable biocompatible core to maintain weight-bearing capacity during the process of surface resorption.

**Tendon**

The feasibility of in vivo repair using tissue-engineered tendons in a hen model was recently reported. Tenocytes were isolated, expanded in vitro, and then seeded onto polyglycolic acid scaffolds enveloped in intestinal submucosa. Dynamic studies demonstrated mechanical properties similar to normal tendons, with approximately 70 percent normal strength at 14 weeks. Histologic analysis noted tenocytes and collagen arrangement similar to normal tissue configuration. This suggested that in vivo systems could potentially bridge tendon defects.

**Cartilage**

More than a million procedures involving cartilage replacement are performed each year in the United States. Autografts as well as allografts have been used, with acceptable results. The lack of intrinsic vascularity renders cartilage less susceptible to ischemic insult than tissue with greater dependency on direct vascularity. This quality is ideal for use in tissue engineering. Results from a series of experiments using pig models to study cartilage repair suggest that polyglycolic acid–chondrocyte–pluronic systems produce meniscus similar to that of autogenous tissue.

The U.S. Food and Drug Administration has approved the use of an autologous cell product for cartilage repair. Chondrocytes are harvested from a normal section of the injured knee. In vitro expansion produces chondrocytes, which are then reimplanted into the area of injury. Data from 9 years of follow-up support its clinical efficacy with good long-term outcomes. Alternately, mesenchymal and embryonic stem cells have been induced to differentiate into the appropriate cells for repair of damaged bone, cartilage, and tendon. It was noted that the removal of extracellular matrix induced chondrocytes to resume their cell cycle of replication and division. Proteoglycan removal via trypsin digestion of explanted cartilage was performed and the tissue properties were assessed. When placed adjacent to tissue-engineered composite cartilage grafts in ex vivo bioreactors, the engineered grafts and the explants demonstrated increased adhesion strength, suggesting greater tissue integration.
Esophagus

Intestinal interposition grafts have been the mainstay of esophageal replacement. While often successful, these reconstructions can be plagued with strictures and leakage. Previous attempts at replacement using materials such as rubber, Teflon, and polytetrafluoroethylene were complicated by erosion, extrusion, leakage, and stenosis. The use of soft tissue, including skin, fascia, and trachea, has been explored previously with similar unsatisfactory results. Pioneering tissue engineering efforts have utilized a collagen matrix with an inner silicone stent to bridge 5-cm esophageal defects that allowed autologous tissue replacement. Attempts to engineer esophageal replacement constructs using cultured esophageal keratinocytes layered onto polyglycolic acid–reinforced collagen gel sheets seeded with fibroblasts are now being conducted. The sheets are first folded into a tubular shape and placed on the latissimus dorsi muscle or omentum and then used as an in vivo bioreactor to allow maturation of the basement membrane. Minimal success has resulted from these constructs. As with most multilayered constructs, vascularity is a limiting factor. Constructs preseeded with endothelial cells and allowed to form capillary networks in vivo by preimplantation, or in bioreactor systems in vitro, may ameliorate this problem.

Trachea

Tracheal replacement using nonbiologic materials, autogenous tissue, cadaveric allografts, and, recently, tissue engineering constructs has been described. A rabbit model was developed to demonstrate heterotopic tracheal transplantation of a vascularized fascial flap delayed 2 weeks before orthotopic transposition. The results were not encouraging. Other investigators have attempted direct vascularization of thyrotracheal grafts. Overall, the majority of replacements have had dismal clinical outcomes featuring early stenosis, stricture, high leak rates, and segmental graft necrosis. Tissue engineering may provide more successful replacements than those utilized thus far.

Heart Muscle

Cardiac myocytes are incapable of regeneration; therefore an infarcted area heals by fibroblast infiltration and scar formation. Cardiac myocytes have been demonstrated to survive when transplanted into this scar tissue, a phenomenon described as cellular cardiomyoplasty. The use of stem cells in this capacity is being investigated. Current investigations involve the use of biodegradable scaffold constructs implanted onto the myocardial scar tissue. Results to date indicate neovascularization of the grafts.

Heart (Valve)

More than 89,000 valve replacement procedures were performed in 1998, with an estimated 300,000 procedures performed worldwide annually. Therapeutic approaches to valve replacement currently utilize prosthetic, bioprosthetic, or cadaveric homografts with their attendant limitations and complications, including the requirement for antithrombotic therapy or anticoagulation. The use of these devices in the pediatric population is limited by a lack of growth potential. Initial success with single-valve constructs paved the way for the development of a trileaflet cardiac valve. A composite scaffold of polyglycolic acid and a thermoplastic polyhydroxyalkanoate was preseeded with myofibroblasts and then seeded with autologous ovine endothelial cells. They were then implanted into donor lambs at the pulmonary valve position with no postoperative anticoagulation. Echocardiographic studies revealed functional analogy to the native valve with good leaflet closure, minimal regurgitation, and no thrombogenic activity. Exploration and examination at defined intervals to 24 weeks revealed tissue histologic architecture and mechanical properties similar to native valves.

Bladder

Recent advances have resulted in the construction of a three-dimensional polyglycolide scaffold dipped in a bath of polylactide in chloroform solvent and then molded to simulate the structure of a bladder. The chloroform evaporates, leaving the polylactide “glue” to retain the scaffold in the contoured shape. Urinary epithelial cells and smooth muscle cells have been seeded onto the construct. Uro-
dynamic in vivo studies using dog models demonstrated function analogous to the normal bladder.

Nerve

Before the era of tissue engineering, the only repair available to bridge nerve injury gaps was autografting. This concomitantly produced a neurologic deficit at the donor site. Early tissue engineering efforts attempted to guide neural repair by entubulation methods. Initial results demonstrated proof of concept, but they failed to generate a reliable and commercially viable product for distances greater than 3 cm.

Groove micropatterned biodegradable scaffolds coated with laminin and preseeded with Schwann cells have been used to promote neurite alignment and to augment axonal regeneration.32 Schwann cells in constructs lacking a defined capillary network remained viable for approximately 1 week, during which time their survival was dependent on the diffusion kinetics of the required nutrients and waste removal and on distance from the circulatory pool.

Work in our laboratory has focused on inducible time delivery of growth factors for nerve regeneration as well as the utilization of adult stem cell technology.34 The use of induction agents to promote nerve growth factor expression has been demonstrated in vitro.35 Future investigations will determine the dose-response curve for both induction agent and nerve growth factor activity.

Cornea

In 1995, an innovative procedure developed by De Luca and Pellegrini resulted in improved vision for two patients with severe unilateral ocular alkali burns.36 Using autologous limbal stem cells expanded ex vivo in a fibrin culture; corneal sheaths were produced which were then grafted onto the injured eye. The 2-year follow-up demonstrated a stable corneal graft with preservation of visual improvement. The investigators are currently preparing for publication a multicenter study involving 18 patients. This protocol was later refined by using even smaller limbal donor grafts, making the procedure available to patients with bilateral corneal injury.37 Corneal tissue engineering is undergoing U.S. Food and Drug Administration evaluation and may be added to the list of commercially available tissue-engineered products in the future.

Blood Vessels

Previously, synthetic large-vessel replacement was accomplished using Dacron or polytetrafluoroethylene. These grafts are susceptible to infection, thrombosis, and stenosis. The availability of autologous donor vessels is limited in many patients, and patency results from cadaveric vascular grafts are poor in comparison.

First-generation large-vessel constructs involved the use of Dacron or polytetrafluoroethylene seeded with endothelial cells. A 75 percent patency rate was reported for carotid interposition grafts consisting of a polyurethane scaffold complexed with collagen and dermatan sulfate with an autogenous endothelial cell monolayer.1 More recently, polyhydroxyalkanoate and polyglycolic acid copolymer constructs were demonstrated to effectively replace the pulmonary artery and abdominal aorta in lambs. Dynamic and histologic studies at 5 months revealed similar mechanical properties and tissue content in comparison to native vessels.

A significant advance in small-vessel construction was achieved with the use of a trilayered, 3-mm synthetic blood vessel with 200 mmHg of burst strength, comparable to medium-sized arterioles.39 Human vascular smooth muscle cells were cultured with ascorbic acid to produce a cellular sheet. A temporary tubular support was used to provide a scaffold medium. A sheet of human fibroblasts wrapped around the medium simulated the adventitia. The tubular support was removed after maturation and endothelial cells were seeded in the lumen. This was a trilaminate-engineered vessel devoid of artificial structural support. These were grafted into the femoral artery of dogs; postoperative follow-up at 1 week revealed a 50 percent patency rate. One group successfully demonstrated the construction of a functional 4-mm-diameter, host-directed bioresponsive vessel.40 The vessel was created using small intestinal submucosa and type I bovine collagen. Investigations revealed appropriate reactivity to norepinephrine, serotonin, and bradykinin stimuli. Recently, investigators reported the implantation of a tissue-engineered pulmonary artery into a 4-year-old child.41 The implant was created using a tubular scaffold of a copolymer (polycaprolactone–polylactic acid) with a reinforcing layer of polyglycolic acid. Vascular cells isolated from an autologous peripheral vein and expanded in vitro were then seeded on the
construct and cultured for 10 days. The graft was then used to reconstruct the pulmonary artery. Postoperative angiographic and clinical studies revealed patency and function at 7-month follow-up. This was a historic milestone in tissue engineering, the first tissue-engineered blood vessel implanted in a human being.¹

Capillaries and microvascular constructs prove elusive, and this remains a major barrier to creating multilayered and solid organ structures. Using MicroElectroMechanical Systems technology, bioengineers have succeeded in creating a capillary network.⁴² Initial studies in rat models have successfully demonstrated no leaks or obstruction to flow in these fabricated capillary networks.

In clinical practice, magnetic resonance imagining or high-resolution, three-dimensional, reconstructed computed tomographic images may be used as the data source for scaffold construction. The creation of scaffolds with preseeded endothelial capillary networks may allow improved vascular incorporation and survival of complex multilayered constructs. This would remove one significant barrier to solid organ replacement. Tissue engineering of large vessels may answer the demand in the athero-occlusive and trauma populations, whereas small-vessel constructs may find use in some of the 600,000 coronary bypass procedures performed annually in the United States. At the very least, this would eliminate the donor-site morbidity frequently seen in bypass procedures.

**Skin**

Skin defects can be treated by the application of artificial skins. The use of collagen–glycosaminoglycan artificial dermal constructs in burn reconstruction dates back 20 years.³¹ Dermal fibroblasts were obtained from neonatal foreskin and expanded in vitro and then seeded onto a scaffold of polylactic and polyglycolic acids. They were then cultured in a bioreactor system to allow generation of a dermal layer.⁴³ This product was then packaged, frozen, and shipped for use as an allograft. Another product featured both dermal and epidermal elements. It was constructed from dermal fibroblasts placed in a collagen solution that gelatinizes at body temperature. A bilaminate construct was produced by coating with multiple layers of keratinocytes. Implant viability was reported at up to 6 months, with elaboration of extracellular matrix proteins and host-regulatory responsiveness.

Some laboratories reported using fresh split-thickness skin grafts from tissue banks preserved in 98% glycerol solution for 24 hours.⁴⁴ Enzymatic digestion produced dermal remnant free of epidermal cells and basement membrane components. When seeded with (autologous) keratinocytes, these constructs in vitro and in nude mice models demonstrated features of hemidesmosomes with reorganization of the lamina densa, suggesting reestablishment of the dermal epidermal junction. Genesis of a basement membrane with cellular differentiation and formation of a true stratum corneum was also noted. Additional evidence of physiologic function was demonstrated by random melanocyte production of pigment granules.⁴⁴

It has been noted that the long-term viability of skin grafts is dependent in part on the population of engrafted keratinocyte stem cells.¹⁷

**Solid Organs**

The mainstay of organ replacement is transplantation or external mechanical devices with functional analogy. Data published in February of 2001 indicated that 74,800 individuals were awaiting organ transplantation. This reflects the most extreme potential application for the products of tissue engineering. The risks and side effects of transplantation, including immunosuppressive therapy, tumorigenesis, and infections, spurred the search for appropriate alternatives.¹

The architectural complexity of solid organ structures relative to tissue renders solid organ engineering exponentially more challenging. The construction of a solid organ requires multiple cell lines with tissue thickness that may exceed the ability to deliver nutrients and remove waste. Advances in tissue synthesis and biomodulation suggest that eventually it may be possible to accomplish the production of histiosubstitutive organ constructs. The Living Implants from Engineering Initiative was established as a multi-institutional affiliation of researchers dedicated to designing tissue-engineered organs as a source for organ transplantation.⁴⁵

Xenografts from immunoengineered animal donors are also being evaluated. Coleman et al. presented data at the Nineteenth International
Congress of the Transplantation Society reporting cloning of double “knockout” pigs. These pigs lack the gene for the enzyme galactose-alpha-1 galactosyl transferase, a primary xenoantigen provoking the hyperacute rejection. This is a significant advance toward removing the immunologic barrier to xenotransplantation.

Liver

Each year 26,000 individuals in the United States die as a result of end-stage liver failure. The estimated annual cost is $9 billion. The only successful treatment modality is liver transplantation. Previous alternatives produced via tissue engineering featured transplantation of hepatocytes. Other approaches included injection of cells, including cells complexed with microcarrier beads. These approaches have had little success with no commercial promise. Despite their inherent propensity for regeneration in vivo, hepatocytes proliferate poorly in vitro, making culturing efforts difficult. The functional nature of this cell line mandates immediate access to a vascular pool for nutrient provision and metabolic waste removal. This imposes limitations on engineering histiosubstitutive constructs. Currently, the most successful constructs feature the use of highly porous biodegradable discs to deliver mass quantities of hepatocytes. This approach is limited by the suboptimal cell survival ratio.

Heart

As with most solid organs, replacement for the terminal failed heart is primarily by transplant surgery. The recent creation of “knockout” pigs promises the possibility of xenotransplantation of organs that will be immunocompatible. Currently, the substitutive products available are essentially temporary bio-compatible nonbiodegradable mechanical devices with functional analogy to the heart. The dynamic complexity and specialized conduction infrastructure of the heart have proven difficult to duplicate in engineered systems. The possibility of the development of a tissue-engineered heart is promised by the successful fabrication of functional subunits, such as tissue-engineered valves and myocardial infarct scar remodeling. The next step is to engineer a dynamic, functional analog of the conduction system. The following step will be the addition of the endocardial and myocardial layers. Advances with the incorporation of a capillary network suggest that such multilaminate constructions would be viable.

Pancreas

With the exception of pancreas transplantation, there is no reasonable replacement for endocrine and exocrine pancreatic function. Exogenous supplementation is subject to poor compliance and the need for constant monitoring due to the loss of intrinsic feedback control mechanisms. Many laboratories have scaled down their tissue engineering efforts toward developing replacement pancreatic tissue; others have entirely ceased bioartificial pancreas research.

Small Intestine

Researchers previously demonstrated the survival feasibility of transplanted mixed enterocyte and mesenchymal stromal cell populations arranged into a villus structure. Subsequent experiments using small-bowel resection and neointestine constructs provided trophic effects on the neointestinal mucosa, resulting in increased villus height, expanded mucosal surface area, and increased crypt area. Experiments are pursuing establishment and demonstration of functional activity in absorption, motility, and innervation.

Current Limitations

The current successful constructs for cartilage and skin repair reflect their relative independence from an intrinsic blood supply. The absence of an appropriate capillary network to provide nutrient support and removal of metabolic waste is a major limitation on the construction of multilayer or simulated organ system constructs. Multilayered solid organ synthesis is restricted to the diffusion distance of the cell from an available vascular pool. For optimal metabolic exchange, cells need to be within 300 μm of their vascular supply. Transplanted cells remain viable for a defined duration of time before exhausting their intrinsic adenosine triphosphate stores and regional oxygen supply within the construct. Prolonged survival must be via inosculation of nutrients and oxygen diffusion. This necessitates a capillary network or the creation of a tubular system with a diameter of 10 μm to allow vascular inflow. An alternative attempt to provide a vascularized construct involves implantation in an ectopic, well-vascularized location, such as the omentum. After establishment of capillary in-
growth, the construct is then harvested as a prevascularized graft, and additional cell layers may then be added before reimplantation in the intended area.\textsuperscript{17}

The influence of the local milieu must not be underestimated. Dedifferentiation can be observed when terminally differentiated cells are cultured in flasks.\textsuperscript{14} This observation, in addition to the phenomenon of contact inhibition, suggests that cellular development is directed in part by its locoregional tissue architecture. Different cell types often require unique culture environments, making it difficult to design a multilayered organ system construct. A future challenge is to create a scaffold capable of supporting a variety of cell types through the histioconductive process.

When embryonic or pluripotent stem cell lines are used in such constructs, their differentiation pathways must be controlled to accomplish permanent differentiation into the desired cell type. This may present a more significant problem for ex vivo systems.

There is much to learn regarding the efficient use of growth factors in the clinical setting. The current use of growth factors in tissue engineering does not address the appropriate dosing or the complex cascade and coordinated sequences of their elaboration. Many growth factors have pleiotropic effects on cells. Basic fibroblast growth factor systemically delivered may induce intimal thickening of blood vessels resulting from exuberant smooth muscle cell proliferation. This may predispose to stenosis or occlusion of critical vessels. This illustrates the importance of defining precisely the appropriate expression sequence, dosing, and duration for growth factors. In particular, growth factor therapy designed to stimulate neovascularization may risk tumor induction as a possible side effect.

The challenge will be to determine the life profile of each human cell line, with the deterministic character of synchronized gene expression. The use of the gene chip may allow this temporal genetic activity profiling. In particular, the expression of growth factor activity in healing wounds could be evaluated for the orchestrated sequence of multiple growth factor activity. A quantitative assay control using mRNA or peptide product would help determine the appropriate dose regimen for growth factor therapy in histioinductive constructs attempting to simulate the peptidochemical milieu.

In the process of tissue engineering, it must be considered that there are important differences between in vitro and in vivo systems. Proof of concept models that demonstrate in vitro success may not be practical for in vivo systems. Furthermore, there are often difficulties in extrapolating successful in vivo animal models to human systems. This may be improved by finding appropriate animals with systems or structures analogous to those in humans. Although the use of autologous cells limits immune complications, the use of allogenic cells offers significant advantages in uniformity and standardization of procedures, quality control for batch units, and cost effectiveness. The use of allogenic tissue lowers costs while increasing reliability, reproducibility, and accessibility to the therapy. Additional issues regarding safety, efficacy, preservation, storage, and delivery will take prominence once a product has demonstrated clinical efficacy and is considered for mass production. The final phase of commercially prepared tissue-engineered products would involve protocols for storage and delivery. Some products have demonstrated a longer shelf life and decreased immunostimulation with cryopreservation.\textsuperscript{6}

All current modalities for postnatal wound healing rely on the repair of connective and epithelial tissue layers. The final product is usually the formation of fibrous tissue or healing by scar formation, which does not contain all the components of the normal tissue. For example, there is a distinct lack of the normal components of uninjured skin in skin wounds. If the process of healing could be truly defined and controlled, then we may be able to design a construct incorporating all the elements and cell lines of native uninjured skin. The field of developmental biology may yield great strides in future tissue engineering approaches.

**Regulatory Questions**

The plight of Dermagraft (Advanced Tissue Science, La Jolla, Calif.) illustrates some of the regulatory hurdles that impede the delivery of products to market. This in turn adversely affects the profitability of the company and may be counterproductive in the long run if the assets of the company are insufficient to sustain it during this transitional period. These regulatory constraints are significant handicaps to U.S. companies. In contrast, European companies face fewer obstacles on the road to the marketplace. This has prompted the creation
of several upstart companies nimble enough to move products to market expeditiously, though with marginal profitability. One area of regulatory impact involves the recent restrictions on embryonal stem cell research. Further, the European ban on certain animal research could potentially stall product development. It is conceivable that the cell systems being developed by Griffith and Naughton may resolve this issue.\(^2\) The use of engineered human skin and DNA microarrays to study hepatocyte detoxification processes may allow greater independence from animal studies in pharmaceutical protocols.

As with any technology introduced into society, accompanying regulations must guide its use. Technology poses no inherent threat other than the purposes for which humans choose to use it. Therefore, the danger lies not in the development of the technology but in the manner of its deployment. We must regulate the manner of its use, not its development. Perhaps the National Heart, Lung, and Blood Institute, the President's Council on Bioethics, and the President's Council of Advisors on Science and Technology can outline regulatory parameters for this fledgling industry. Governing ordinances should be established now, as this industry is expected to enlarge rapidly and will require guidelines to direct its growth and regulation.

**SUMMARY**

Numerous promising therapies are evolving in the field of tissue engineering. They range from improved wound-healing technologies to possible organ replacement constructs. Someday these constructs may reduce or obviate the need for donor organ transplantation, enhance the healing process, and improve our quality of life.

Advances in microelectromechanical systems technology may remove a major barrier to producing functional histiosubstitutive constructs. The requirement for a vascularized construct may be fulfilled by the creation of a scaffold incorporating a prefabricated capillary network. These may be placed in layers throughout the pseudo-organ to facilitate circulation. An alternative approach is using growth factor therapy to coax angiogenesis and neovascularization from the surrounding tissue into endothelial cell–preseeded scaffolds.\(^2\)

The use of DNA microarrays will have a significant effect on how we evaluate systems, and it could possibly change how we interpret and treat diseases. DNA microarrays have the potential to allow genome-specific characterization of individual tumors and ultimately may require revision of current tumor classification standards to allow inclusion of DNA derangements for directed gene therapy or immunotherapy. The molecular biology of tumor profiling will take on an increasingly important role in designing appropriate treatment protocols.

As our understanding of tissue repair and the cytokine response cascade increases, tissue engineering constructs may begin to resemble the complex architecture of native tissue. Multiple cell lines may be included to complete the construct, as incorporation of capillary networks allows multilayered systems. With future advances, the line between repair and regeneration may begin to blur as we strive to return tissue to its preinjury state. Tissue regeneration is the ideal outcome of tissue engineering. Insight may be gained from the study of mammalian organisms capable of such self-repair. In addition, the process of fetal regenerative wound healing suggests the possibility for this in the postnatal human. The regulatory systems of the active in utero wound-healing process would have to be defined, and then it must be determined whether this system could be recruited in the postnatal wound-healing process. The next decade will be exciting and challenging for this emerging field.

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