

# Tissue engineering

Despite technical and regulatory challenges, the prospects for tissue engineering are good.

Tissue and organ failure, produced as a result of injury or other type of damage, is a major health problem, accounting for about half of the total annual expenditure in health care in the US<sup>1</sup>. Treatment options include transplantation (human or xenotransplantation), surgical repair, artificial prostheses, mechanical devices, and in a few cases, drug therapy. Ultimately, however, major damage to a tissue or organ can neither be repaired nor long-term recovery effected in a truly satisfactory way by these methods.

Tissue engineering is emerging as a significant potential alternative or complementary solution, whereby tissue and organ failure is addressed by implanting natural, synthetic, or semisynthetic tissue and organ mimics that are fully functional from the start, or that grow into the required functionality. Initial efforts have focused on skin equivalents for treating burns, but an increasing number of tissue types are now being engineered, as well as biomaterials and scaffolds used as delivery systems. A variety of approaches are used to coax differentiated or undifferentiated cells, such as stem cells, into the desired cell type. Notable results include tissue-engineered bone, blood vessels, liver, muscle, and even nerve conduits. As a result of the medical and market potential, there is significant academic and corporate interest in this technology.

## Historical perspective

An important component in the early development of tissue engineering was the parallel development of artificial biomaterials. In the mid-1960s, artificial skin for burn victims was being pursued as a symptomatic therapy<sup>2</sup>, and later, synthetic fibers were being tried as artificial skin grafts for burn treatment<sup>3</sup>. In the early 1970s, there were concerted efforts to treat artificial surfaces to be used in implants in ways that would enable them to avoid causing blood coagulation, by applying special heparin complex coatings, for example<sup>4</sup>. Other efforts focused on the toxicology profiles and biocompatibility of a variety of organic polymers considered for implants or tissue engineering<sup>5</sup>, and the development of novel gels as the basis for artificial skin<sup>6</sup>. In the late 1970s, researchers experimented with collagen-based artificial skin for use in oral mucosa injuries<sup>7</sup>.

In the 1980s, R&D in tissue engineering and biomaterials took off. As part of this interest, several biomedical engineering departments were established at major universities around the world. In 1981, a skin equivalent

consisting of a silicone cover over a sponge of porous collagen cross-linked with chondroitin was used successfully to treat severe burns<sup>8</sup>.

In this decade, several products reached the market. Interpore's Pro-Osteon coral-derived bone graft material was introduced in 1993. In 1996, Integra's Artificial Skin was approved for as an in vivo, nonbiological tissue regeneration product. Then, in 1998, the General and Plastic Surgery Devices Advisory Panel to the US Food and Drug Administration recommended unconditional approval of Apligraf (Graftskin) Human Skin Equivalent for the treatment of venous leg ulcers. Apligraf, produced by Organogenesis, is the first manufactured living human organ, specifically multilayered skin, to be recommended for approval by an advisory panel to the FDA. Apligraf was approved for the treatment of venous leg ulcers in Canada in 1997, and was launched there in August 1997 by Novartis Pharmaceuticals Canada (Dorval, Canada).

## Current state

Table 1 lists selected companies involved in tissue engineering. Interestingly, the types of

collaborations involving tissue engineering companies can differ slightly from those done between traditional drug discovery companies and big pharma. For example, LifeCell develops tissue grafts for transplantation and the preservation of transfusable blood products. The company has developed engineered porcine heart valves for replacement surgery in humans in a 1993 partnership with Medtronic (Minneapolis, MN).

Medtronic, a leading medical device company with a cardiac surgery business, offers a complete line of mechanical and tissue prosthetic heart valves, and the alliance with LifeCell gives it access to a significant tissue-engineered product to complement its own lineup. This type of alliance illustrates an alternate route to the big pharma path for commercializing tissue engineering products—that is, through more specialized medical device companies.

Part of the interest and support for tissue engineering comes from the armed forces, in that numerous battlefield-related medical applications exist for tissue-engineered products and biomaterials. For example, Advanced Tissue Sciences had part of its clin-

Table 1. Selected companies with tissue engineering programs.

Company	Program
Advanced Tissue Sciences (La Jolla, CA)	Growth of human tissues and organs, including skin, cartilage, bone, and liver
Alertek/Bio (Quebec, QC, Canada)	Blood vessels
Cell Based Delivery (Providence, RI)	Bioartificial muscle
CellSource (Pittsburgh, PA)	Vascularized fat cells
Collagenesis (Beverly, MA)	Tissue matrix system
Creative Biomolecules (Hopkinton, MA)	NOVOS bone graft material
Cytomatrix (Cambridge, MA)	3-D hematopoietic stem cell engineering
CytoTherapeutics (Providence, RI)	Cell encapsulation; progenitor cell transplantation
ETEX (Cambridge, MA)	Bone substitute material
Genetics Institute (Cambridge, MA)	Bone morphogenetic protein
Genzyme Tissue Repair (Cambridge, MA)	Wound repair, tissue regeneration
Geron (Menlo Park, CA)	Telomerase-immortalized stem cells
Integra LifeSciences (Plainsboro, NJ)	Artificial Skin non-biologic material
Interpore International (Irvine, CA)	Pro-osteon bone graft from coral
LifeCell (The Woodlands, TX)	AlloDerm for skin replacement
MorphoGen Pharmaceuticals (New York)	Pluripotent mesenchymal stem cells
Organogenesis (Canton, MA)	Apligraf (Graftskin) human skin equivalent
Osiris Therapeutics (Baltimore, MD)	Cartilage tissue generation mechanisms
Progenitor (Menlo Park, CA)	Stem cell engineering
Protein Polymer Technologies (San Diego, CA)	Recombinant protein polymer hydrogel
Regeneron (Tarrytown, NY)	Vascular tissue engineering
Reprogenesis (Cambridge, MA)	Local and systemic tissue-engineered products
Selective Genetics (San Diego, CA)	Matrix-based delivery of DNA for tissue repair
Stratum Laboratories (La Jolla, CA)	Skin2 living human skin tissue in vitro laboratory testing kit
Stryker (Kalamazoo, MI)	Bone regeneration
Terumo (Japan)	Terudermis bilayer artificial dermis

Source: Biovista ([www.biovista.com](http://www.biovista.com))

ical trial for Dermagraft-TC in the treatment of chemical burns funded by the US Army Institute of Chemical Defense. Dermagraft-TC is an engineered human dermal tissue combined with a synthetic epidermal layer. It covers and protects burns, helping to minimize infections and retain fluids until a sufficient amount of the patient's own skin is available for autologous grafting. The principal alternative is cadaver skin, but the problems here include a limited supply, acute immunological rejection, and potential pathogen transmission.

Another application of tissue-engineered products is in the toxicology testing and in vitro markets, as alternatives to certain types of animal testing. A good example of this approach was the acquisition in 1995 by Stratum Laboratories (La Jolla, CA) of the In Vitro Laboratory Testing (IVLT) business of Advanced Tissue Sciences. Stratum received license rights to manufacture and sell Skin2 in vitro laboratory testing kits, in addition to an option to extend rights for up to six additional tissues. Skin2 is living human skin tissue used to test skin care, household, chemical, and pharmaceutical products for a variety of indications. The material is already approved by the US Department of Transportation and Transport Canada for use in a corrosivity test, demonstrating significant regulatory acceptance of this technology.

The use of progenitor-type cells as the starting point for developing differentiated tissue material is the focus of considerable research. For example, it is possible to take mesenchymal stem cells that reside in the adult bone marrow and induce them to differentiate into chondrocytes by using specific tissue culture media that include transforming growth factor  $\beta^9$ . Chondrocytes are constituents of cartilaginous tissue, and the possibility of generating them in a controlled fashion creates possibilities for the development of appropriate cartilage tissue for surgical procedures. Stem cells are also used as a starting point for a multitude of other cell types used in tissue engineering.

Wound repair is a key application for tissue engineering products. Although most applications focus on the use of artificial skin to treat burns, different disease conditions are also benefiting. For example, Advanced Tissue Sciences' Dermagraft is a three-dimensional human neonatal dermal fibroblast culture that has been grown on a biodegradable scaffold and cryopreserved. It has been applied to foot ulcers that develop as a side effect of long-term diabetes. In clinical trials, significant healing occurred with this material, especially when the Dermagraft cells were alive and functioning properly<sup>10</sup>.

Finally, increasing effort is being focused on correlating the actual physics of engineered cells that are to be used therapeutical-

ly. One recent report describes how the pressure that is applied to chondrocytes transplanted into articular cartilage defects is likely to inhibit the growth of these cells<sup>11</sup>. Thus, the actual mechanics of the environment into which bio-engineered cells are used needs to be carefully considered to ensure that optimal benefit is derived from these approaches.

### Industry challenges

Quality control of the materials used in various surgical applications is a key challenge for the tissue engineering industry. For example, living human cells are being used in scaffolds to repair structural tissue damage. These materials need to be produced and cultured under good manufacturing practice (GMP) conditions to meet FDA standards—especially the cells that are grown *ex vivo*. As a result, the tissue engineering industry is striving to create appropriate quality control standards and evaluate them.

A good example is that of autologous cultured chondrocytes used to repair knee damage. Recently, Genzyme Tissue Repair reported on a quality control program for this material that was based on evaluating 303 patients who had received the material<sup>12</sup>. The program was based on the analysis of several quantifiable parameters, including viability and freedom from pathogens; results showed that the materials were, indeed, appropriate for their use, and demonstrated one way to follow up with quality control monitoring of tissue-engineered products.

Another challenge concerns acquiring a fundamental understanding of tissue differentiation mechanisms that might be harnessed for the development of tissue-engineered products. One product that the tissue engineering industry is pursuing is “bone-on-demand,” to be used in cases where new bone formation is needed. An important component here is the bone morphogenetic protein (BMP) complex, which is capable of inducing extraskeletal bone formation at a concentration 1,000-fold lower than each of its constituents alone. Researchers are trying to concentrate BMP complex locally, using appropriate implants rather than the individual constituents, which could potentially lead to bone formation<sup>13</sup>.

Finally, the industry is challenged to develop tissue-engineered products for a number of surgery-related applications, such as vasculature. For example, a recent report describes the generation of a tissue-engineered blood vessel without any synthetic or exogenous materials. The vessels were produced by wrapping a sheet of human vascular smooth muscle cells around a tubular support, followed by wrapping a sheet of human fibroblasts around the first sheet. After cell maturation, the tubular support was removed and endothelial cells were seeded in the

lumen of the putative blood vessel. The vessel showed all the necessary key markers of activity, and also had a burst strength comparable to human vessels. In short-term grafts in a dog model, this engineered vessel demonstrated good handling and saturability characteristics<sup>14</sup>.

### The future

Tissue engineering has significant market potential and financial investment continues apace. A 1997 survey<sup>15</sup> of the field reported that in that year alone, R&D expenditure directly linked to corporate tissue engineering projects was about \$0.5 billion, with a growth rate of about 22% per year. This demonstrates the sustained interest in this area, driven in part by positive results regarding specific products and processes in clinical settings.

Technical advances in the various components of the industry will contribute to market growth. One component is the availability of biomaterials that act as scaffolds for tissue repair and reconstruction, or for the deposition of engineered tissues and cells preceding implantation. An increasing amount of R&D is directed toward addressing the properties of these scaffolds with the goal of creating materials that have the desired functional profiles for various applications.

For example, so-called blended-polymer scaffolds have an extended lifetime in the body that is more suitable for orthopedic applications than nonblended scaffolds<sup>16</sup>. Another study has shown how collagen-based scaffolds, used to grow keratinocytes in artificial skin preparations, can be manipulated by cross-linking collagen with glycosaminoglycan<sup>17</sup>. The result was increased biological stability, which augmented the likelihood that the keratinocytes would “take” and grow out of the scaffold. In yet another application<sup>18</sup>, a sacchitin glycolipid-type membrane prepared from the residue of a fungal fruiting body has been shown to have significant promise in animal models of skin damage as a skin substitute that facilitated wound healing and fibroblast growth. Development of new materials of this type 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. 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, and even fat cells can be directed to produce appropriate tissues (see Table 1). In addition, promising artificial nerve grafts or nerve guidance channels are being developed for nerve regeneration<sup>19</sup>.

In the future, efforts will likely increasingly focus on the development of tissue-engi-

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neered products under consensus safety and efficacy standards, including sourcing of cells and tissues, characterization and testing of the materials, quality assurance and control, and preclinical and clinical evaluation<sup>20</sup>. The FDA has already provided some regulatory guidance concerning specific materials, such as certain marketed artificial skin products; in the next few years, these guidelines will likely be increasingly formalized and structured, ensuring that tissue engineering products not only work but are also safe.

Significant future developments will include the continued development of artificial organs that use cells embedded into appropriate support structures. A recent report describes the use of polyurethane foam as a good matrix into which liver cells grew as spheroids, the system showing promise as an artificial liver<sup>21</sup>.

The future will also see significant efforts to develop engineered vascular grafts. An approach that will see increasing attention is that of taking a scaffold that is a structurally intact xenogeneic vessel, such as a pig aorta, removing all cells, and re-populating this with human autologous cells. A recent report showed how this could be done in 2–3 weeks, opening up the way for a good alternative to vascular engineering<sup>22</sup>.

The range of human tissue that can be engineered will also increase dramatically in the future, so in addition to the traditional targets, such as skin and liver, other tissues and organs will see their day. A great deal of excitement in clinical circles is that of developing artificial human thyroid tissues which are capable of producing T cells, and this will be a major area for continued R&D<sup>23</sup>.

Finally, stem cells and their manipulation for therapeutic purposes will continue to be a major area of development, because of the pluripotency of these cells. For example, bone marrow stem cells contained in resorbable artificial tubes have been shown to lead to effective healing of non-union defects in rabbit radii<sup>24</sup>, and this opens up significant surgical alternatives to organ and tissue damage.

### Conclusions

Tissue engineering is emerging as a vibrant industry with a huge potential market. The biomaterials, scaffolds, artificial organs, and differentiating cells that are combined to create a tissue engineering product address significant medical needs, such as major tissue and organ damage or failure. The industry faces numerous technical challenges, not the least of which is the establishment of a consensus quality control program to ensure that tis-

sue engineering products work and are safe to use. Efforts to address these issues are underway, and if past success is any indication, this technology is certainly one that will have a major impact in future health care practice.

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