Tissue Engineering and Regenerative Medicine: History, Progress, and Challenges

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Abstract

The past three decades have seen the emergence of an endeavor called tissue engineering and regenerative medicine in which scientists, engineers, and physicians apply tools from a variety of fields to construct biological substitutes that can mimic tissues for diagnostic and research purposes and can replace (or help regenerate) diseased and injured tissues. A significant portion of this effort has been translated to actual therapies, especially in the areas of skin replacement and, to a lesser extent, cartilage repair. A good amount of thoughtful work has also yielded prototypes of other tissue substitutes such as nerve conduits, blood vessels, liver, and even heart. Forward movement to clinical product, however, has been slow. Another offshoot of these efforts has been the incorporation of some new exciting technologies (e.g., microfabrication, 3D printing) that may enable future breakthroughs. In this review we highlight the modest beginnings of the field and then describe three application examples that are in various stages of development, ranging from relatively mature (skin) to ongoing proof-of-concept (cartilage) to early stage (liver). We then discuss some of the major issues that limit the development of complex tissues, some of which are fundamentals-based, whereas others stem from the needs of the end users.

A BRIEF HISTORY OF TISSUE ENGINEERING

Tissue engineering is a relatively new field that uses living cells, biocompatible materials, and suitable biochemical (e.g., growth factors) and physical (e.g., cyclic mechanical loading) factors, as well as combinations thereof, to create tissue-like structures. Most frequently, the ultimate goal is implantation of these tissue constructs into the body to repair an injury or replace the function of a failing organ. The critical functions may be structural (e.g., bone, cartilage), barrier- and transport-related (e.g., skin, blood vessels), or biochemical and secretory (e.g., liver and pancreas). Tissue engineering also applies to the development of specialized extracorporeal life support systems containing cells (e.g., bioartificial liver and kidney) as well as tissue units that may be used for diagnostic screening. In addition to clinical applications, other uses include drug testing for efficacy and toxicology as well as basic studies on tissue development and morphogenesis. The term regenerative medicine is often used synonymously with tissue engineering, although regenerative medicine often implies the use of stem cells as a cell source.

Some historical highlights related to tissue engineering and regenerative medicine are shown in **Table 1**. The first tissue-based therapies developed were skin grafting techniques. Then came techniques to preserve cells and tissues that enabled allograft skin banking, making these skin grafts an off-the-shelf product. The first synthetic skin substitute reportedly used by more than one investigator was developed in 1962; however, the first successful tissue-engineered skin products were made in the late 1970s and early 1980s. Most would agree that this is when modern tissue engineering really started, although the term "tissue engineering" was apparently coined later, around 1987.

Among the first tissue-engineered skin constructs was the product developed by Howard Green and colleagues (1–3) at Harvard Medical School, who described techniques to grow skin epidermis starting with a skin biopsy harvested from a patient. Keratinocytes isolated from the biopsy could be proliferated by coculturing with a feeder layer of mouse mesenchymal cells, thus expanding

Table 1 Some historical landmarks in tissue engineering

Year	Technology/accomplishment	Reference
3000 BCE	Skin grafting described in Sanskrit texts of India	(25)
1794	Autologous skin grafting in Europe by Bunger, Reverdin, and Baronio	(25)
1881	Cadaveric skin allograft by Girdner	(25)
1944	Refrigerated skin allografts by Webster	(129)
1949	Cell cryopreservation at subzero temperatures developed by Polge	(130)
1952	Skin cryopreservation developed by Billingham	(131)
1962	Ivalon sponge developed as "synthetic substitute for skin" by Chardack	(132)
1975	In vitro cultivation of keratinocytes by Rheinwald and Green	(1)
1979	Cultured autologous epithelium, later commercialized as Epicel by Genzyme	(2)
1981	Composite living skin equivalent by Bell, later commercialized as Apligraf by Organogenesis	(6)
1982	Collagen-glycosaminoglycans (GAG)-based dermal matrix by Yannas, later commercialized as Dermal Regeneration Template by Integra Lifesciences	(5)
1987	"Tissue engineering" term coined	(133)
1988	Cell transplantation in synthetic biodegradable polymers	(134)
1994	Chondrocyte culture and transplantation by Brittberg, later commercialized as Carticel by Genzyme	(54)
2006	Bioartificial bladder cultured in vitro and implanted in vivo	(135)
2008	Engineered trachea from decellularized matrix seeded with human cells derived from stem cells	(136)

the coverage area several thousand-fold within weeks. This technological breakthrough led to the first cell-based tissue-engineered product, Epicel, which was marketed by Genzyme (Cambridge, MA). Epicel consists of sheets of autologous (i.e., derived from the recipient) keratinocytes that are used to cover patients suffering from catastrophic cutaneous burn injuries who do not have enough viable skin remaining to be treated with traditional autografting techniques. The product does not have a dermis and is only a few cells thick; therefore, it is extremely fragile and is not commonly used (only approximately 60–70 patients per year on average). The U.S. Food and Drug Administration regulates Epicel as a xenogeneic (i.e., derived from another species, in this case nonhuman) product (because it uses a feeder layer of mouse cells), the first of its kind.

Another early product was developed by mechanical engineer Ioannis Yannas at the Massachusetts Institute of Technology (MIT) in collaboration with burn surgeon John F. Burke at the Boston Shriners Hospital for Children (4, 5) and their colleagues. It consists of a bovine type I collagen and shark chondroitin 6-sulfate mixture that is cross-linked and turned into a porous matrix by controlled freeze-drying. A silicone sheet attached to one side functions as a temporary epidermis-like barrier. Commercialized under the name Dermal Regeneration Template by Integra Life Sciences (Plainsboro, NJ), this product is used to cover severe burn wounds where the damage extends deep into the dermis. Under these circumstances, the wound bed may not support a skin graft, or the absence of dermis may lead to extensive contraction and scarring of the healed wound. The matrix is biodegradable and presumably dissolves as the host's cells—primarily fibroblasts, endothelial cells, and neural cells—migrate into it and deposit their own extracellular matrix (ECM). Ultimately, the matrix disappears and is entirely replaced with a neodermis made of the patient's own cells and matrix, thus promoting dermal regeneration while inhibiting wound contraction and leading to better function and appearance of the healed wound. At that point, the silicone film is removed, and the wound is covered with a skin graft. Interestingly, the product contains no living cells, and its main purpose is to guide and stimulate the body's repair and regenerative processes.

Also early on, Eugene Bell at MIT and colleagues (6) developed a composite skin product reconstituting both dermis and epidermis. The dermis is first made by seeding a collagen gel with dermal fibroblasts, which cause the gel to contract and form a neodermis. The keratinocytes are grown on top of the neodermis, initially submerged in culture medium, and then at some point in the manufacturing procedure exposed to the air-liquid interface to induce differentiation and formation of a keratinized layer. The entire process takes approximately 3 weeks and uses allogeneic (i.e., derived from donors of the same species) cells isolated from neonatal human foreskin, which provides the potential for off-the-shelf availability, but with the caveat that the allogeneic skin substitute can provide only temporary coverage, as the patient will eventually reject it. The current product based on this technology, Apligraf, marketed by Organogenesis (Canton, MA), is used to stimulate the host's wound healing response in recalcitrant venous leg ulcers and diabetic foot ulcers. Analogous skin constructs are also used for in vitro tests to measure transdermal transport and chemical corrosive properties.

During the 1990s, several of these and other tissue-engineered skin and subsequently cartilage products were successfully commercialized. These early successes fueled much enthusiasm, and many research laboratories embarked on applying tissue engineering to nearly every tissue in the body. Several new companies were spun off with great fanfare and the hope that, as some prominent spokespeople predicted just 15 years ago, tissue engineers would be making complex body parts by now (7). The strategy of simply combining cells and matrix worked for skin and cartilage because these tissues do not require extensive vascularization and other significant tissue processes. Furthermore, technologies to grow and differentiate keratinocytes made it possible to adequately source the needed cells for these products. This was not the case for other tissues. As

the same prominent spokespeople recently acknowledged (8), there remain significant hurdles to overcome, such as providing a functional vascular supply, controlling the complex arrangement of different cell types in a 3D tissue, and identifying qualitatively and quantitatively reliable cell sources to make those tissues.

In the early 2000s, the high-tech bubble burst, and weary investors stopped funding high-risk ventures including tissue-engineering companies, which led to a decline in the industry (9). A study conducted in 2004 found that activity in skin, cartilage, and other structural applications declined by more than 50% with a loss of 800 full-time employees (10). The decrease was partially offset by an increase in stem cell firms, which added more than 300 employees. Except for this transient resurgence fueled by the promise of stem cells, financing of startup activity since 2008 has been very limited. Although significant advances have occurred in some areas, such as bladder, cornea, and bronchial tubes, tissues such as blood vessels, heart, and liver—in spite of years of research efforts—are still far from offering clinically acceptable solutions.

During the maturation of tissue engineering over the past three decades, several technologies have been developed based on advances in molecular and cellular biology and micro- and nanosystems engineering. These technologies have been developed largely by basic scientists and engineers, who sometimes have a tendency to oversimplify the problem and do not always recognize the clinical issues. Nevertheless, some of these technologies have led to the development of molecular diagnostics, which as of 2002 comprised an industry market greater than $3 billion, growing at a rate of approximately 25% per year (11, 12). That nontherapeutic applications of tissue engineering are making strides may ultimately help support the development of new tissue-engineered therapeutic products, which are much more difficult to produce than enthusiastic advocates originally thought. As we reiterate at the end of this review, ultimately tissue engineers must focus their energy on solving clinical problems to have a real impact.

BASIC PRINCIPLES OF TISSUE ENGINEERING

The ability to reconstitute tissue function with therapeutic products at a clinically meaningful scale has a wide spectrum of applications. The main targets are those tissues that are prone to injury, disease, and degeneration (**Table 2**). Corresponding organs that have been the targets of tissue-engineered equivalents are listed in **Table 3**.

Most tissue engineering utilizes living cells, and supplying enough cells is obviously a critically important issue. Cells are typically derived from (*a*) donor tissue, which is often in very limited supply, or (*b*) stem or progenitor cells. Stem cells possess two major properties that make them attractive for deriving large cell quantities: (*a*) their high proliferative capacity and (*b*) their pluripotency, or ability to differentiate into cells of multiple lineages. Ethical concerns about the use of human embryonic stem (ES) cells are a significant impediment for industrial adoption, but recent advances in the use of adult stem cells, induced pluripotent stem cells (iPS cells), and stem cells from placental and umbilical sources have in part allowed these other stem cell types to replace ES cells as feasible sources.

A key need for effective tissue engineering is the cellular environment that allows the cells to function as they do in the native tissue. Often the environment mimics some critical aspects of the in vivo setting through proper control of the materials and mechanical setting as well as the chemical milieu. Cell scaffolds usually serve at least one of the following purposes:

1. cell attachment and perhaps migration;
2. retention and presentation of biochemical factors;
3. porous environment for adequate diffusion of nutrients, expressed products, and waste; and
4. mechanical rigidity or flexibility.

Table 2 Incidence of injuries and diseases in the United States

Indications	Procedures or patients	Reference
Skin		
Burns	2,000,000 total	(137)
Pressure sores	144,000 total	(138)
Venous stasis ulcers	2,500,000 total	(139)
Nervous system		
Spinal cord injury	259,000 total	(140)
Alzheimer's disease	5,300,000 total	(141)
Eye surgery	5,500,000/year	(141)
Ear surgery	900,000/year	(141)
Musculoskeletal		
Joint replacement (knee)	326,000/year	(142)
Joint replacement (hip)	165,000/year	(142)
Bone graft	500,000/year	(143)
Musculoskeletal (other)	6,300,000/year	(141)
Cardiovascular		
Heart disease	26,800,000 total	(144)
Respiratory system surgeries	1,500,000/year	(141)
Liver		
Liver cirrhosis	400,000 total	(145)
Liver cancer	16,260/year	(145)
Hepatitis C	3,200,000 total	(144)
Pancreas		
Diabetes	24,000,000 total	(144)
Digestive system surgeries	11,000,000/year	(141)
Urinary system surgeries	2,500,000/year	(141)

Many of the synthetic biomaterials that have been used in tissue engineering, notably collagen-based materials and the polylactic, polyglycolic, and polycaprolactone family of polymers, were already well known in the medical community, having already been employed as bioresorbable sutures. These materials were attractive initially because they already had regulatory approval, but they were far from optimal for many tissue engineering purposes, particularly because the hydrolytic biodegradation process releases acid, which can be toxic to cells. Other synthetic materials have been engineered with customizable properties such as injectability, transparency, and optimal porosity and resorption rates. One such biomaterial is PuraMatrix (3DM, Cambridge, MA), which consists of small (16 amino acids long) oligopeptide fragments that self-assemble into nanofibers on a scale similar to the in vivo ECM (13).

Natural scaffolds that use existing ECM materials are still extensively used, including protein-based materials (e.g., collagen, fibrin) and polysaccharide-based materials (e.g., chitosan, alginate, glycosaminoglycans, hyaluronic acid) (14–16). Cross-linking agents (e.g., glutaraldehyde, water-soluble carbodiimide) may be used with these and other materials to reduce degradation rates. Although biocompatibility with natural materials is obviously excellent, there remain issues with potential immunogenicity in some cases. Recently, there has been heightened interest in using decellularized tissue matrices obtained from processing discarded donor tissue. This approach to generating a tissue engineering scaffold has had some recent successes with the world's first whole

Table 3 Overview of tissue engineering thrusts

Tissue	Function	Approach	Challenges	Ref.
Skin	Barrier for the body	Matrix implanted to guide regeneration; implants with autologous or allogeneic cells	Lack of appendages, slow process for growing cells, slow vascularization	(27, 146, 147)
Cornea	Transparent barrier for the eye	Matrix implants; extracellular matrix generated by cells cultured ex vivo	Maintain transparency and barrier properties of the matrix	(148–150)
Liver	Detoxification, production of liver-specific proteins	Hepatocytes from xenogeneic, allogeneic or stem cell-derived sources, or immortalized hepatoma seeded in implantable matrices, extracorporeal bioreactor systems	Cell source, maintenance of hepatic function, high cell density, vascularization of implants	(151–153)
Pancreas	Secrete insulin to maintain glucose homeostasis	Free or encapsulated islet transplantation	Choice of transplantation site, vascularization, cell source, immune rejection	(154–156)
Cartilage	Critical component of joints	Matrix implanted to guide regeneration; implants with autologous or allogeneic cells	Slow process for growing cells, control of cell differentiation, host integration, long-term durability	(157–160)
Heart	Provides blood circulation	Materials, including decellularized organs, seeded with progenitor and stem cells differentiated into cardiomyocytes	Tumorigenicity, control of cell differentiation, electrical integration	(161–163)
Kidney	Regulates body fluid volume and pH, metabolite excretion	Stem cell–derived nephrons cultured ex vivo	Replicating glomerular selectivity while retaining high hydraulic permeability	(164–166)
Neurons and spinal cord	Send electrical stimuli to control bodily functions	Materials shaped as tubes for axonal guidance and regeneration, sometimes used in combination with anti-inflammatory strategies; neural stem cells	Reconnecting proper axons, controlling proinflammatory environment, preventing scar tissue formation	(167–169)

tissue-engineered organ transplant of an engineered trachea (17) and rapid recent advancements with heart, liver, and lung tissues (18–21).

Another consideration with tissue-engineered constructs is the presence of exogenous chemical and mechanical stimuli such as soluble growth and differentiation factors as well as mechanical forces (e.g., cyclic mechanical loading, fluid shear). Among the chemical factors that frequently have been applied are bone morphogenetic proteins (BMPs), basic fibroblast growth factor (bFGF or FGF-2), vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β). Although these are chiefly soluble factors, they can be incorporated into the ECM during scaffold fabrication. In fact, one of the key nonstructural functions of the natural ECM in vivo is to bind, retain, and present growth factors to cells attached to the ECM. Controlled delivery schemes can also be used to increase the longevity of the original soluble factor load. Applied techniques include encapsulation in small biodegradable particles, use of transfected cells to express and release the factors, and chemical conjugation to the scaffold material itself.

With the cells, scaffolds, and environmental needs identified, one must consider adequate means for assembly. Early work in this area, which just randomly mixed all the components together before implantation, was fraught with major failures. Many a study was published claiming success, only to have succeeded in implanting cells that eventually died. One problem with this approach is mass transport limitations. Tissues engineered in this manner lacked a preexisting vascular network, thus making it difficult for implanted cells to obtain sufficient oxygen and nutrients to survive and/or function properly. Potential solutions have been offered in which scaffolds are engineered to promote rapid vascular ingrowth or vascular endothelial cells are introduced and allowed to form a vascular network prior to, or concomitant with, the seeding of tissue cells. With the advent of technologies such as soft lithography, robotic printing, and laser tweezers, some investigators have attempted to "print" tissues and even organs. Recent methods include an assembly that uses an ink-jet mechanism to print precise layers of cells in a matrix of thermoreversible gel (22, 23). For example, endothelial cells, which line blood vessels, have been printed in a set of stacked rings. When incubated, these fused into a tube. Through this controlled integration approach, it may be possible to generate an emergent vascular network.

REPRESENTATIVE EXAMPLES OF ENGINEERED TISSUES

In this section, we describe in greater detail three applications for which engineered tissues have been developed. The first example is skin, for which tissue-engineered products were first established and the field is most mature. Second, we look at cartilage, for which some products are available but with limited therapeutic use. Third, we discuss liver tissue engineering, an area that has seen much laboratory investigation, many animal studies, and even clinical trials, but no successful translation to the clinical arena yet.

Skin

All skin wounds that extend deep into the dermis and are more than 1 cm in diameter require specialized treatment, as they cannot close (i.e., regenerate the epithelial lining) on their own and may lead to extensive scarring that may result in joint mobility limitations and severe cosmetic deformities (24). The gold standard for serious cutaneous wounds remains autologous skin grafts, a technique that originated several thousand years ago (25). The limitation for autologous skin grafting is inadequate uninjured donor sites remaining to harvest skin graft material. Although it is possible to extend coverage by meshing the skin (a technique in which the skin graft is uniformly perforated and stretched to cover greater areas of the wound), the lack of dermis in the interstices of the stretched meshed skin graft as well as slow epithelialization from graft margins across interstices results in greater graft contraction and a pronounced crocodile skin appearance of the scar. In general, areas where injuries extend deep into the dermis may not support the skin graft, and/or severe scarring may occur owing to the lack of functional dermis.

Skin substitutes were originally developed to address some of these limitations. In particular, biodegradable matrix materials can emulate the dermis, and keratinocyte and fibroblast culture techniques have led to live cultured skin substitutes.

According to Shakespeare (26), the functions that tissue-engineered skin products can offer are: (*a*) protection—by establishing a mechanical barrier to microorganisms and vapor loss; (*b*) procrastination—by providing some wound cover following early wound debridement until permanent wound closure can be achieved with serial skin grafts or cultured autologous cell applications, especially in extensive burns; (*c*) promotion—by delivering to the wound bed dermal matrix components, cytokines, and growth factors, which can promote and enhance natural

Table 4 Representative skin substitutes approved by the U.S. Food and Drug Administration

Product	Indications	Comments
Alloderm	Burns and full-thickness injuries	Incorporates into patient's skin, two-year shelf life
Apligraf	Venous and diabetic ulcers	Allogeneic, cryopreserved
Dermagraft	Diabetic ulcers, epidermolysis bullosa	Allogeneic, cryopreserved
Epicel	Deep partial- and full-thickness burns, congenital nevi	Incorporates into patient's skin, variable take rate, made to order
Integra Dermal Regeneration Template	Deep partial- and full-thickness burns	Incorporates into patient's skin, moderate shelf life
OrCel	Split-thickness donor sites, epidermolysis bullosa	Allogeneic, nine-month shelf life cryopreserved
TransCyte	Deep partial- and full-thickness burns	1.5-year shelf life, cryopreserved

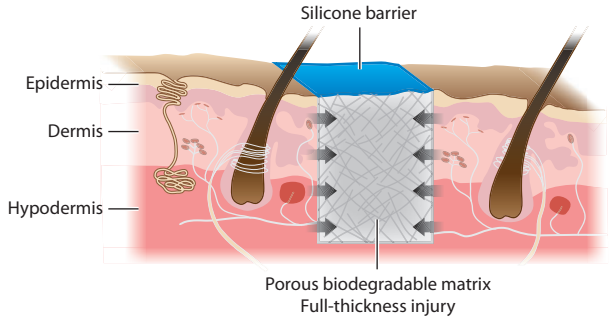
host wound healing responses; and (*d*) provision—of new structures, such as dermal collagen or cultured cells, that are incorporated into the wound and persist during wound healing and/or thereafter. Although none of the existing products can fully replace damaged skin (27, 28), they have been used to treat extensive acute wounds (especially burns) as well as to promote healing of chronic nonhealing wounds such as diabetic ulcers and venous ulcers.

A representative listing of engineered skin substitutes that are available on the U.S. market is in **Table 4**. **Figure 1** summarizes the current main approaches to skin tissue engineering. The simplest engineered skin substitutes, which are still in use today, consist of porous matrices that function as templates for dermal regeneration. The matrices are placed on the wound bed and allowed to integrate and vascularize. After sufficient revascularization of the matrix, these products must be covered with autografts (29). Integra Lifesciences's Dermal Regeneration Template™, which we described earlier, is primarily used for the treatment of deep burn wounds, which are prone to forming undesirable scars. The matrix degrades while the host's cells invade and proliferate within it, thus promoting dermal regeneration while inhibiting wound contraction, leading to better function and appearance of the healed wound (30). Another skin substitute, Alloderm (LifeCell, Branchburg, NJ), is made from decellularized donor skin. Removing all the cells and keeping only matrix components prevents an allogeneic immunological response and also reduces the risk of disease transmission (31, 32). Alloderm is used for both wound repair and reconstructive surgery. As with Integra, an autograft must be applied eventually to re-epithelialize the wound. Another tissue-engineered dermal analog consists of allogeneic neonatal dermal fibroblasts cultured in a polyglactin mesh. The cells produce ECM proteins as the mesh degrades, thus producing a matrix usable on the wound (31). This product, called Dermagraft (Advanced BioHealing, Westport, CT), has been used to cover diabetic foot ulcers. Although Dermagraft is eventually rejected, it appears to help restore the dermis and promote keratinocyte migration to close the wound (33).

Figure 1

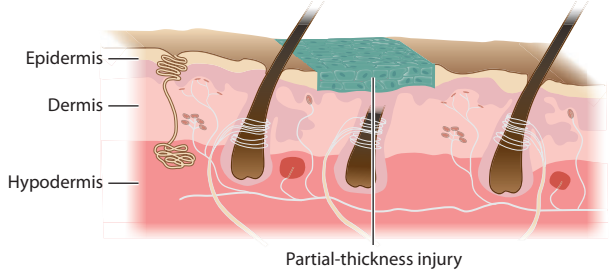
Current approaches to skin tissue engineering. One approach consists of placing a biodegradable matrix in the wound to promote the regeneration of the skin dermis through a process of host cell migration and proliferation (*a*). Another approach focuses on regenerating the keratinocyte layer by putting on top of the wound cultured autologous keratinocytes or a temporary covering that contains extracellular matrix and growth factors that stimulate keratinocyte proliferation (*b*). These methods work best on partial-thickness wounds. Cultured autologous keratinocytes are used on full-thickness wounds as well, but the take is poor. The most comprehensive tissue-cultured skin incorporates both living dermis and epidermis, which are usually cultured from allogeneic sources (*c*).

a Dermal regeneration matrix

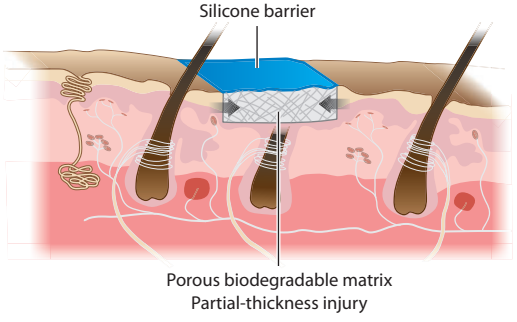


- Porous biodegradable matrix with no cells
- Host fibroblasts and endothelial cells migrate into matrix
- Promotes regeneration of dermis
- Improves scar appearance

b Epithelial regeneration

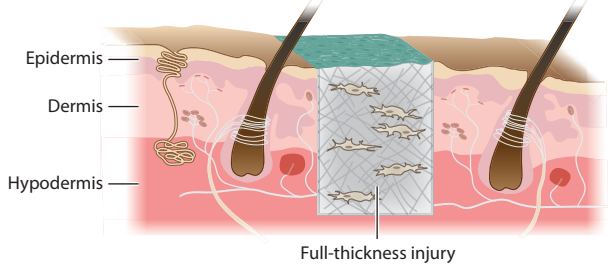


- Cultured autologous epithelial cells
- Permanent wound coverage



- Temporary matrix/growth factor cover
- Promotes host epithelial migration and proliferation

c Full-thickness composite skin



- Fibroblast-populated matrix covered with keratinocytes
- Allogeneic cells used, so coverage is temporary
- Attempts to develop technology using autologous cells are underway

The first cultured skin began with Epicel, a cultured autologous epidermis described earlier. Some of the major limitations of this product are the absence of a dermis and its lack of off-the-shelf availability because a patient biopsy is used as starting material. To overcome these limitations, other tissue-engineered skin products were developed to include both epidermis and dermis. The first full-thickness engineered skin product is the earlier-described Apligraf, a bilayered construct using fibroblasts in a collagen gel and keratinocytes to create a dermis and epidermis, respectively. Other analogous products have since been developed, such as OrCel (FortiCell Bioscience, Englewood Cliffs, NJ), which uses bovine type I collagen sponge as substrate. As is the case for any allogeneic tissue, Apligraf and Orcel ultimately are rejected (34). Similar products based on autologous cultured keratinocytes and fibroblasts may fulfill the role of true skin substitution; they are currently in research and development, and results of clinical trials seem encouraging (35). At the same time, other products that are simpler and less expensive have also become available. For example, TransCyte (Advanced BioHealing, Westport, CT) is a nylon mesh with a silicone membrane on one side and cultured foreskin human fibroblasts on the other side. The fibroblasts proliferate within the nylon mesh and deposit ECM as well as growth factors. The product is frozen and then thawed for application. Cells die in this process, but the ECM and growth factors remain essentially intact (36).

A relatively new approach involves distributing a minced micrograft over the wound area. This technique involves excision of a small area ($\sim$2 cm^2) of full-thickness skin from the patient, which is then minced. The resulting mixture, which contains both the dermal and epidermal components of skin, is combined with a hydrogel and applied to the wound. The distributed cells proliferate and participate in the wound healing process. This clever approach may provide a future alternative to traditional skin grafts, given its need for only a small donor area and its inherent simplicity when compared with full-fledged tissue-engineered products (37).

Even though tissue-engineered skin substitutes have been available for decades and arguably are the best-established tissue-engineered products, their practical role is limited to a specific niche within a complex approach to treating acute and chronic wounds. In most instances, they serve as temporary biologically active wound dressings until the patient's own skin regenerates and can be used for serial autografting (27). This reality exists because the reported benefits of skin substitutes tend to be modest, and most experts in the field would agree that no existing product can claim to be a complete solution. In general, these tissue replacements only partially address specific functional requirements, and surgeons tend to use different products to achieve different purposes.

One of the main limitations of engineered skin substitutes is slow revascularization and in some instances poor take (i.e., attachment to the wound bed). In fact, those systems that contain a dermal component, which is meant to help regenerate the dermis as well as provide a better surface for attachment of the epidermis, take a long time to vascularize and delay wound closure. Thus, the surgeon must balance the pros and cons of using a skin substitute to improve long-term scar appearance and function in the face of increased risk of infection owing to delayed vascularization and wound closure. However, new research is promising in this regard. For example, collagen has been used for some time in the design of skin substitutes and recently has been used to create a model of endothelialized, reconstructed dermis that promotes the spontaneous formation of a human capillary-like network (38).

Tissue-engineered skin also lacks several important structures and cell types, including sebaceous glands and sweat glands as well as melanocytes and dendritic or Langerhans cells (27, 28). Recently two research teams described the use of bulge cells from hair follicles to regenerate skin appendages. In one case, freshly isolated bulge cells from adult mice, when combined with neonatal dermal cells, formed hair follicles after injection into immunodeficient mice (39). In the

other case, a mixture of isolated neonatal dermal cells injected with epidermal aggregates (isolated hair follicle epithelial stem cells) into the dermis of nude mice led to hair morphogenesis and gave rise to cycling hair follicles within 8 to 12 days (40). These findings suggest that it may be possible to incorporate complex differentiated structures in a new generation of skin substitutes.

Genetically modified skin substitutes have also been developed. This area has been extensively explored with respect to correcting genetic defects (41, 42), augmenting the supply of deficient hormones (43), and enhancing the efficacy of a tissue-engineered product (44). The first two of these areas have received considerable attention but have not yet generated widely used therapeutic skin products. However, Stratatech (Madison, WI) is currently developing genetically engineered skin products to confer enhanced antibacterial, angiogenic, and antiproteolytic properties.

Cartilage

The demand for engineered and regenerative tissue approaches for cartilage has been growing in the face of the increasing prevalence of degenerative joint diseases (e.g., osteoarthritis) as the general population continues to age and become more overweight. The demand in young and healthy individuals is also high owing to the high incidence of sports injuries, given the limited spontaneous repair following articular cartilage injury (45). It is thought that the lack of vascularization of articular cartilage prevents the onset of an inflammatory response to tissue injury and resultant repair. The low cellularity and proliferative capacity of chondrocytes may also underlie an intrinsic inability to repair, leading to scar tissue of inferior mechanical properties and durability.

As will be discussed below, it is noteworthy that most cartilage repair technologies work best when used early after injury and in young, healthy individuals. Evolution of the injury toward a chronic state may create an environment that is hostile to tissue repair and regeneration, and delayed treatment universally results in poorer outcomes. Furthermore, the use of matrix materials to supplement surgical methods and cell transplantation techniques is more widespread than the use of cultured cells. The development of tissue-engineered grafts that are made in the laboratory for eventual implantation is a more recent advance that has undergone very limited clinical testing. Major issues pertaining to cartilage tissue engineering are depicted in **Figure 2**.

Current techniques to repair cartilage that are used to treat acute injuries generally fall into three categories: (*a*) marrow simulation–based techniques, (*b*) osteochondral transplantation techniques, and (*c*) cell-based repair techniques.

The most prevalent marrow-based technique is called microfracture; the damaged area is perforated below the subchondral plate, allowing blood to flow and clot in the microfractures. The blood clot contains a relatively high proportion of marrow-derived mesenchymal stromal cells (MSCs) with high chondrogenic differentiation potential (46), which subsequently produce a scar tissue more akin to fibrocartilage than true cartilage (47, 48). This technique is a first-line procedure in acute knee injuries for athletes younger than 40 years old. Favorable short-term outcomes have been observed, especially if treatment is applied early after injury; however, evidence suggests that the repair tissue undergoes significant deterioration after ~2 years, and in general the outcome is highly variable. It has been suggested that the imperfect integration of the scar with surrounding healthy cartilage, together with inferior mechanical properties of the scar itself, may be responsible for the observed long-term deterioration. Further development of this approach—still in an active research stage—involves locally applying growth factors and anti-inflammatory agents. Another improvement of the technique uses biodegradable scaffold materials that are inserted into the microfracture. Clinical studies are ongoing and suggest that the scaffold improves cartilage repair volume, composition, and stability.

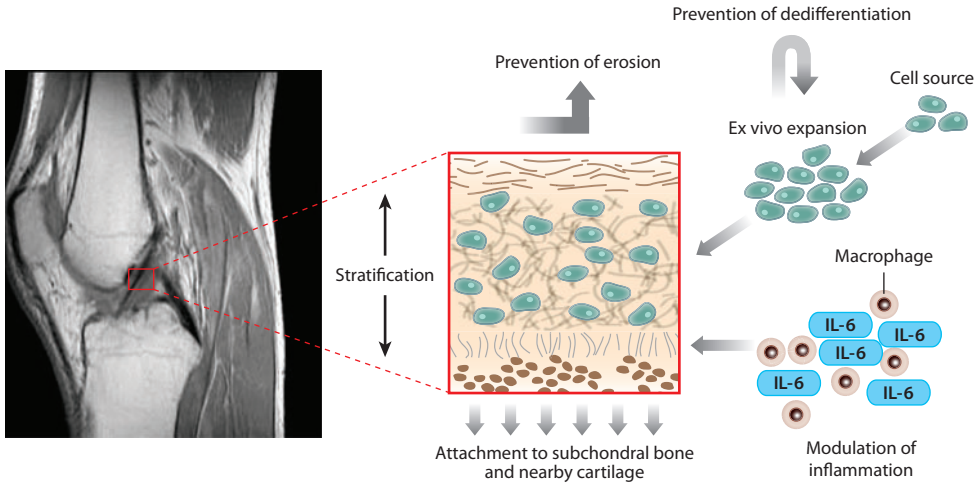


Figure 2

Critical issues in cartilage tissue engineering. The most advanced tissue-engineered cartilage constructs consist of matrices seeded with chondrocytes, although both have been used separately as well. These implants must be designed so that they firmly attach to the subchondral bone and nearby cartilage and have the appropriate type II–collagen density and orientation near the surface to withstand shear forces in the joint. In addition, stratification of the matrix is important to provide appropriate cushioning and an environment for chondrocyte survival and differentiation. Identification of the best source of cells and the ability to proliferate and differentiate these cells are also critical. Finally, one must take into account that the implant may be put in a hostile proinflammatory environment, and provisions to control the impact on implant performance must be taken. IL-6, interleukin-6.

Osteochondral transplantation techniques involve harvesting cartilage together with subchondral bone from nonweight-bearing regions of the joint and placing them in the weight-bearing area of the damage. Technical challenges are associated with this approach, mainly owing to mismatch in the surface shape (convexity) of the treated joint versus the donor tissue and fixation of the graft to the host tissue. This approach is used for mid-size defects (1–4 cm^2) and has shown excellent results, although evidence suggests that preexisting joint degeneration decreases favorable outcomes. Acute donor site morbidity is also an issue, and there is some controversy as to whether even a small injury to the donor site could be detrimental to the nearby tissue and increase the risk of osteoarthritis in the long term (49, 50).

This technique is obviously limited by the rather short supply of autologous donor tissue available, and for this reason, surgeons are looking toward allografts and cell-free osteochondral graft substitutes. In the former case, the technique takes advantage of the fact that chondrocytes are immunoprivileged in their surrounding ECM (51). The data show excellent results for the first 5 years but significant loss of viability at 15 years (52). The latter approach obviates the need for tissue and makes possible off-the-shelf products that can be used whenever needed. These implants have been developed recently as substitute grafts for treatment of focal chondral and osteochondral defects and include a bone phase and a cartilage phase, each designed to physically and mechanically match the layers of the adjacent cartilage and subchondral bone. These implants are composites of polylactide-glycolide copolymers, calcium sulfate, polyglycolic acid fibers, and surfactant (TruFit, Smith & Nephew Endoscopy, San Antonio, TX). Injectable materials (BST Cargel, BioSyntech, Montreal, QC, Canada) have also been described (53). Such implants are replaced with new tissue within 12 months; however, long-term data on performance are still

lacking. Besides their use as graft substitutes, these bioresorbable implants can be used successfully to backfill donor sites in osteochondral autograft transfers.

Cell-based cartilage repair techniques were first reported in 1994 by Brittberg and colleagues (54), who developed the first commercially available cell-based technology, now called Carticel (Genzyme, Cambridge, MA). This breakthrough technology successfully repaired articular cartilage lesions of the human knee by autologous chondrocyte transplantation. In this approach, autologous chondrocytes are harvested from a less weight-bearing area of the joint, extracted from the cartilage explant, and proliferated in culture before implantation on the defect is performed. This therapeutic approach has been successfully used for full-thickness cartilage lesions in the knee with long-term durability of functional improvement exceeding 10 years (55). Long-term functional results were best in athletes with single lesions, age <25 years, and short preoperative intervals. In many cases it appears that formation of fibrocartilage with inferior mechanical properties and limited durability could be correlated with persistence of symptoms (56), suggesting that improving the quality of the repair tissue is critical for long-term successful therapy. This technique also causes periosteal hypertrophy, which may lead to acute graft delamination, and requires long postoperative rehabilitation. Postoperatively, protected weight bearing is maintained for 6 to 8 weeks, and return to demanding sports is usually allowed by 12 months.

A critical but often ignored issue that plagues techniques relying on isolated chondrocytes is the tremendous variability in the quality of donor chondrocytes, which is dependent upon donor age (57), health status of the donor joint (58), and other yet unknown factors (59). Because in vitro culture and expansion of human chondrocytes for autologous chondrocyte transplantation causes some dedifferentiation of the cultured cells, with a shift from a predominantly type II collagen–containing hyaline matrix to a fibrocartilage-like type I collagen–rich repair cartilage (60), selection and expansion of specific chondrocyte subpopulations capable of producing more hyaline-like repair tissue have been proposed (ChondroCelect, Tigenix, Leuven, Belgium). This subgroup of chondrocytes is characterized by expression of specific marker genes and phenotypic characteristics (61).

Scaffold-associated chondrocyte implantation, also known as matrix-associated chondrocyte implantation, is a second-generation autologous cartilage transplantation technique that uses biodegradable scaffolds to temporarily support the chondrocytes until they are replaced by matrix components synthesized from the implanted cells (62). The technique has been used in Europe and Australia with promising results showing improved tissue morphology (i.e., hyaline-like tissue), defect filling, and function (63, 64). The use of scaffold theoretically should reduce chondrocyte leakage, provide a more homogeneous chondrocyte distribution, and lessen graft hypertrophy; however, one study found results similar to implantation without matrix (27). Successful arthroscopic matrix-associated chondrocyte implantation has been described (Hyalograft-C, Fidia Advanced Biomaterials, Bologna, Italy) as well (65). As in most cartilage repair therapies, better results were seen in younger patients (in this case <30 years) and athletes participating in higher-level competitive sports. Future developments focus on improving cellular matrix production by including growth factors in the scaffold and promoting a more natural spatial distribution of chondrocytes within the repair cartilage (66).

A natural extension of scaffold-associated chondrocyte implantation is to culture the cells in the scaffold to create a relatively mature cartilage in vitro before implantation. The presence of matrix around the cells is known to enhance donor cell retention at the repair site (67) and possibly to protect cells from inflammatory agents (68). Most importantly, the engineered tissue would be easier to handle and may withstand earlier mechanical loading (69). Some of the hurdles that have been encountered in generating this third generation cell-based cartilage repair technology, which is essentially tissue-engineered cartilage, include (*a*) inefficient and suboptimal redifferentiation

of the cells after prolonged culture to exhibit the collagen II–secreting phenotype (70), (*b*) poor understanding of the features of materials that promote this differentiation, (*c*) difficulty in scaling up culture systems to clinically relevant thicknesses of approximately 4 mm owing to transport limitations (which make it difficult to control the initial cell distribution as well as nutrient delivery and waste removal as the initial matrix is remodeled by the cells), and (*d*) limited cell source availability.

A fourth-generation approach is also emerging to circumvent the use of allogeneic cell sources, which are based on stem cells and gene therapy. Mesenchymal progenitor cells isolated from a variety of noncartilagenous tissues are under investigation for their chondrocyte differentiation ability, but it appears that they have a tendency toward producing hypertrophic chondrocytes, which often lead to matrix calcification and vascularization after implantation (71). Recently, it was found that coculture of mesenchymal progenitor cells with differentiated chondrocytes helped direct them toward a more normal mature chondrocyte phenotype (72, 73). Alternative autologous sources of chondrocytes, such as the nasal septum, have also been proposed (69, 74).

Bringing tissue-engineered cartilage to clinical use has been hindered by attendant costly, labor-intensive, and time-consuming processes that are difficult to control and standardize. To be attractive for routine clinical application, engineered cartilage will need to demonstrate (*a*) cost effectiveness relative to other existing therapies, (*b*) an excellent safety profile, and (*c*) reliable quality control, the last of which is essential to achieving reproducible results. To meet these targets and translate research-scale production into clinically compatible manufacture, the processes could be streamlined and automated within bioreactor systems implementing precisely monitored and tightly controlled conditions (75). One proposed concept is the on-site hospital-based ACTESTM (Autologous Clinical Tissue Engineering System) currently under development by Octane (Kingston, ON, Canada). In this fully automated bioreactor system the patient's cartilage biopsy is digested and the chondrocytes expanded before being seeded and cultured on an osteoconductive porous scaffold to generate a cartilage graft; all production phases are implemented within a single, closed bioreactor system. This concept could simplify logistical issues surrounding transfer of specimens between locations, reduce the need for large and expensive Good Manufacturing Practices tissue engineering facilities, and minimize operator handling, with the likely final result of reducing the cost of engineered grafts.

An alternative concept for bypassing the bottleneck of tissue manufacturing would be to develop a process whereby cell procurement, scaffold seeding, and transplantation back into the patient would occur during the same surgical operation. Clearly this concept would not generate mature cartilage tissue and would rely on the patient's regenerative capacities to develop a functional tissue equivalent from the grafted template. The difficulty of having a sufficiently large number of cells to be implanted could be overcome, for example, by combining a small number of chondrocytes, freshly isolated from a small biopsy, with resident or exogenously delivered mesenchymal progenitor cells. Indeed, it has been reported that nonexpanded chondrocytes can induce chondrogenic differentiation of other cell types (76). Recently, it was shown that undigested cartilage tissue, minced into small particles, can be used to repair experimental cartilage defects in large animal models (77); this technique obviates the need to isolate cells altogether.

Cartilage tissue engineering began approximately 15 years ago and has seen some success in its application in the clinic. However, still little is understood about the complex mechanism involved in cartilage repair and regeneration, which makes it difficult to develop rational ways to advance the endeavor. In the meantime, cartilage tissue engineering is a potentially powerful tool to model the biological and molecular processes of cartilage development and to identify the cues required to induce its regeneration.

Liver Tissue Engineering

Liver (or hepatic) tissue engineering has been driven by a clinical need to treat patients with acute and chronic liver failure, which together account for 30,000 deaths each year in the United States alone (78). Orthotopic liver transplantation (i.e., when the diseased liver is removed and a donor liver is put in its place, which is the normal anatomical location of the organ, as opposed to a different or heterotopic location) is currently the only viable option for end-stage liver disease, but donor scarcity allows only 6,000–7,000 liver transplants per year. Furthermore, many of the chronic liver failure patients are not eligible for transplantation, because they are too ill to tolerate the complex and invasive procedure. Two tissue engineering approaches are under development as alternatives to liver transplantation: (*a*) extracorporeal bioartificial liver (BAL) devices that would bridge the gap between the onset of liver failure and liver transplantation, and (*b*) a transplantable bioartificial liver based on decellularized liver matrix seeded with autologous or allogeneic cells (18, 79–81).

Early BAL concepts were modified dialysis systems that did not incorporate living cells and were limited in efficacy (82). Because the liver provides a host of biochemical processing and detoxification functions that are essential to life, it was thought that an effective device should contain liver parenchymal cells (e.g., hepatocytes). Although cells were added to the dialysis systems, poor results were obtained again, this time because the systems were not properly designed to enable sufficient metabolite transport (especially oxygen) to the cells; in particular, it was found that large cell aggregates with necrotic cores formed. Some investigators suggested that retrofitting dialysis cartridges with liver cells was a poor strategy and instead focused on the careful design of systems that allowed proper convective and diffusive transport of plasma metabolites to and from the cells (83). Many creative operational strategies were subsequently developed (84–86) including packed bed bioreactors (87, 88) and flat plate bioreactors (81, 89, 90). It was found that hepatocytes could withstand only low levels of fluid shear stress. Therefore, in a recent modification of the flat plate geometry, cells were seeded at the bottom of grooves to allow for higher flow rates, thus increasing mass transport within the device while keeping shear stress low (91, 92). The progress made in BAL design led to a few clinical trials, as seen in **Table 5**. Advances in hepatic tissue engineering also led to improved in vitro screening tools to investigate drug metabolism, pharmacokinetics, and hepatotoxicity (93–96).

Whereas the traditional BAL concepts use artificial support materials, recent studies have also shown that it is possible to seed hepatocytes in a decellularized liver matrix and obtain liver-specific function including albumin secretion, urea synthesis, and cytochrome P450 expression at physiological levels on a per-cell basis (18, 97–103). An example of a reseeded rat liver is shown in **Figure 3**. This process preserves the structural characteristics of the original microvascular network, which allows efficient recellularization of the liver matrix with adult hepatocytes and subsequent perfusion for in vitro culture. The next step is to introduce vascular endothelial cells to line the vascular luminal spaces with a functional endothelium, without which blood clotting will occur when the reseeded liver is implanted in vivo.

It was recognized early on that any BAL would require on the order of 10^{10} cells, assuming that 10% of the normal liver mass is sufficient to provide the biochemical and synthetic functional capacity required to treat a patient. However, human hepatocytes are in extremely limited supply, and there is no reliable and efficient method to propagate these cells ex vivo. Although several investigators have proposed the use of xenogeneic (e.g., pig) or immortalized cells (e.g., C3A hepatoma cells), the preferred cell type remains the adult human hepatocyte.

Thus, much research has been conducted to identify a renewable hepatic cell source, primarily through the use of stem cells. The first foray into the stem cell field was through the use of

Table 5 Current bioartificial liver devices in clinical testing phase

Device	Organization	Clinical Phase	Design	Cell Source
Liver Dialysis Unit™ (formerly BioLogic-DT)	HemoTherapies (formerly HemoCleanse)	FDA-approved multicenter	Membrane-separated hemodialysis unit	Noncellular (charcoal)
Molecular Adsorbent Recycling System (MARS®)	Teraklin	I/II approved multicenter	Hollow fiber bioreactor	Human albumin
Extracorporeal Liver Assist Device (ELAD®)	Vitagen	I/II multicenter	Hollow fiber membrane bioreactor	Immortalized human hepatocytes
HepatAssist 2000 System	Circe Biomedical	III	Hollow fiber membrane bioreactor	Porcine hepatocytes
Bioartificial Liver Support System (BLSS®)	Excorp Medical, Inc.	I/II multicenter	Hollow fiber membrane bioreactor	Primary porcine hepatocytes
LIVERX2000 System	Algenix, Inc.	I center	Hollow fiber membrane bioreactor	Primary porcine hepatocytes
Modular Extracorporeal Liver System (MELS®)	Charite Virchow Clinic-Berlin (Igor M. Sauer)	I/II multicenter	Hollow fiber membrane bioreactor	Human hepatocytes
Hepamate	Hepalife	III	Hollow fiber membrane bioreactor	Primary porcine hepatocytes

hepatoblasts, which are not only capable of expressing differentiated function, but also able to self-renew. A few hepatoblasts have been identified that have the capacity to differentiate into mature hepatocytes; these include the bipotential precursors for hepatocytes and biliary cells (oval cells) as well as hematopoietic stem cells (HSCs). In scenarios following severe hepatic injury, liver regeneration is attributed to a potential stem cell compartment located within the smallest branches of the intrahepatic biliary tree, which gives rise to the bipotential cells known as oval cells (104, 105). Oval cells have been shown to require growth factors such as TGF-α, epidermal growth factor, and hepatocyte growth factor for progression through the cell cycle as well as subsequent differentiation toward mature hepatocytes (106). Despite the many observations describing liver growth processes driven by oval cell proliferation and differentiation into hepatocytes, oval cells are difficult to isolate, and the molecular mechanisms behind these processes have yet to be sufficiently elucidated. HSCs have also been induced to differentiate along hepatocyte-specific pathways. For example, one experimental system utilized HSC transplantation to alleviate liver disease in fumarylacetoacetate hydrolase (FAH)-deficient mice (107); FAH deficiency leads to liver dysfunction and eventual lethality. Following HSC transplantation, liver function was reconstituted. However, it is unclear whether the HSCs or HSC progeny repopulated the liver. In addition, the mechanism that induces differentiation toward mature hepatocytes is also unclear. Even though these aforementioned hepatocyte precursors exhibit the potential to provide a renewable hepatocyte cell source, these cells are hard to isolate and exist in extremely low numbers (108). In addition, the full efficacy of utilizing these precursor cells is questionable, because the long-term functional stability of hepatocytes obtained from these systems has yet to be assessed.

The field then turned to the use of ES cells, which are derived from the inner cell mass of the blastocyst (109). ES cells are pluripotent and can be induced to differentiate into any cell type. When cultured in the presence of an antidifferentiation agent such as leukemia inhibitory factor (LIF), these cells can proliferate while maintaining pluripotency (110). Upon removal of the anti-differentiation agent, ES cells begin to spontaneously differentiate. Many paradigms currently

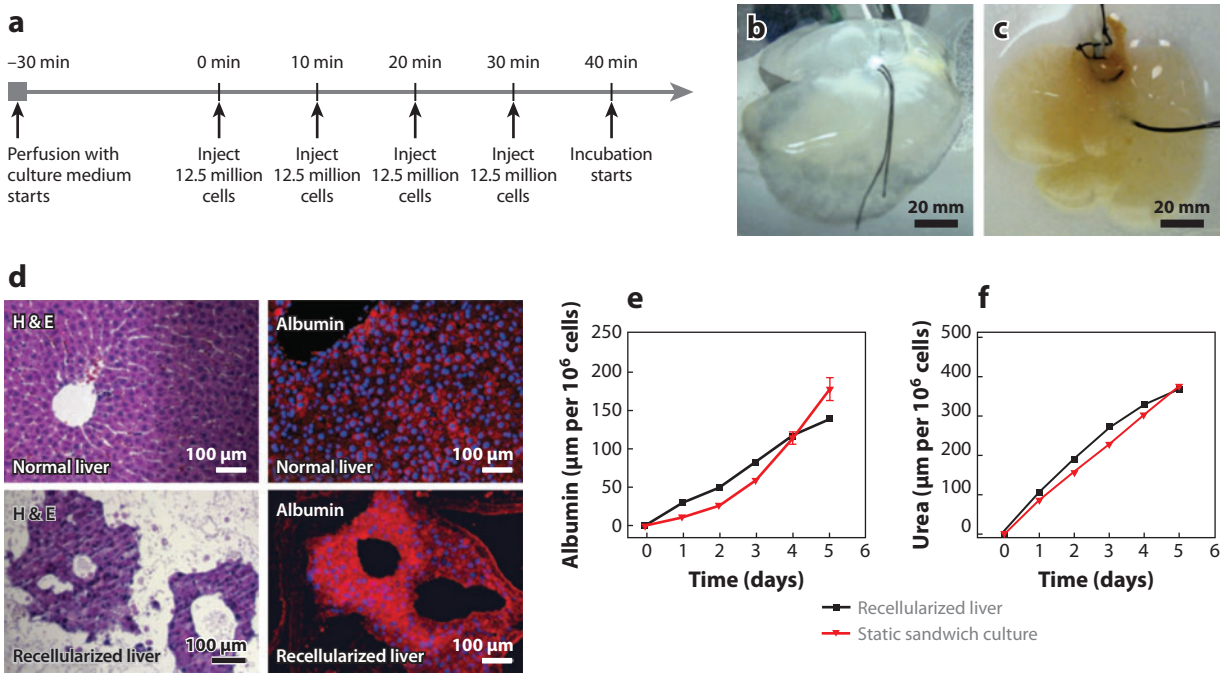


Figure 3

Tissue-engineered liver produced by seeding a decellularized liver matrix in a rat model. (*a*) Recellularization scheme. (*b*) Decellularized whole liver matrix and (*c*) the same liver after recellularization with approximately 50 × 10^6 hepatocytes. (*d*) Immunohistochemical staining of the recellularized liver graft (*bottom*) in comparison with normal liver (*top*); hematoxylin and eosin (H & E) stain (*left*), albumin (*right*, *red*). Sections were counterstained with nuclear blue dye. (*e*) Albumin synthesis and (*f*) urea secretion in comparison with static collagen sandwich cultures. Adapted from figures 3 and 4 of Reference 18 by permission.

exist to specifically direct the differentiation of ES cells toward a hepatocyte lineage, mimicking the aforementioned embryological pathways. These paradigms can be broadly grouped in terms of temporal regulation through cytokine addition or spatial regulation using various ECM configurations. For example, a hanging drop process has been combined with a temporally regulated addition of hepatocyte-specific growth factors to promote differentiation toward mature hepatocytes (111). In this system, differentiation is initially induced by aggregating the ES cells into an embryoid body (EB) via the hanging drop technique. Hepatocyte-specific differentiation is then accomplished through the addition of aFGF or FGF-1, hepatocyte growth factor, and a mix of oncostatin M, dexamethasone, and insulin, transferrin, selenium as early-, mid-, and late-stage hepatocyte specific growth factors, respectively. Differentiating ES cells expressed a variety of endoderm-specific genes in addition to late differential markers of hepatic development such as tyrosine amino transferase, glucose-6-phosphatase, and albumin. In another system, hepatocyte differentiation was induced by coculturing ES cells with embryonic cardiac mesoderm (CM) (112). The ES cells cocultured with CM cells created colonies that have an appearance similar to that of hepatic progenitor cells in vitro. In addition, ES cells cocultured with CM cells exhibited increased cytoplasmic granularity, polyploidy, endoderm-specific markers, and hepatocyte-specific markers as compared with an ES monolayer culture control. In a third system, homotypic interactions of differentiating cells were maximized through the use of alginate encapsulation, resulting in hepatocyte-like cells with a high degree of hepatic functionality (113, 114). It is noteworthy that

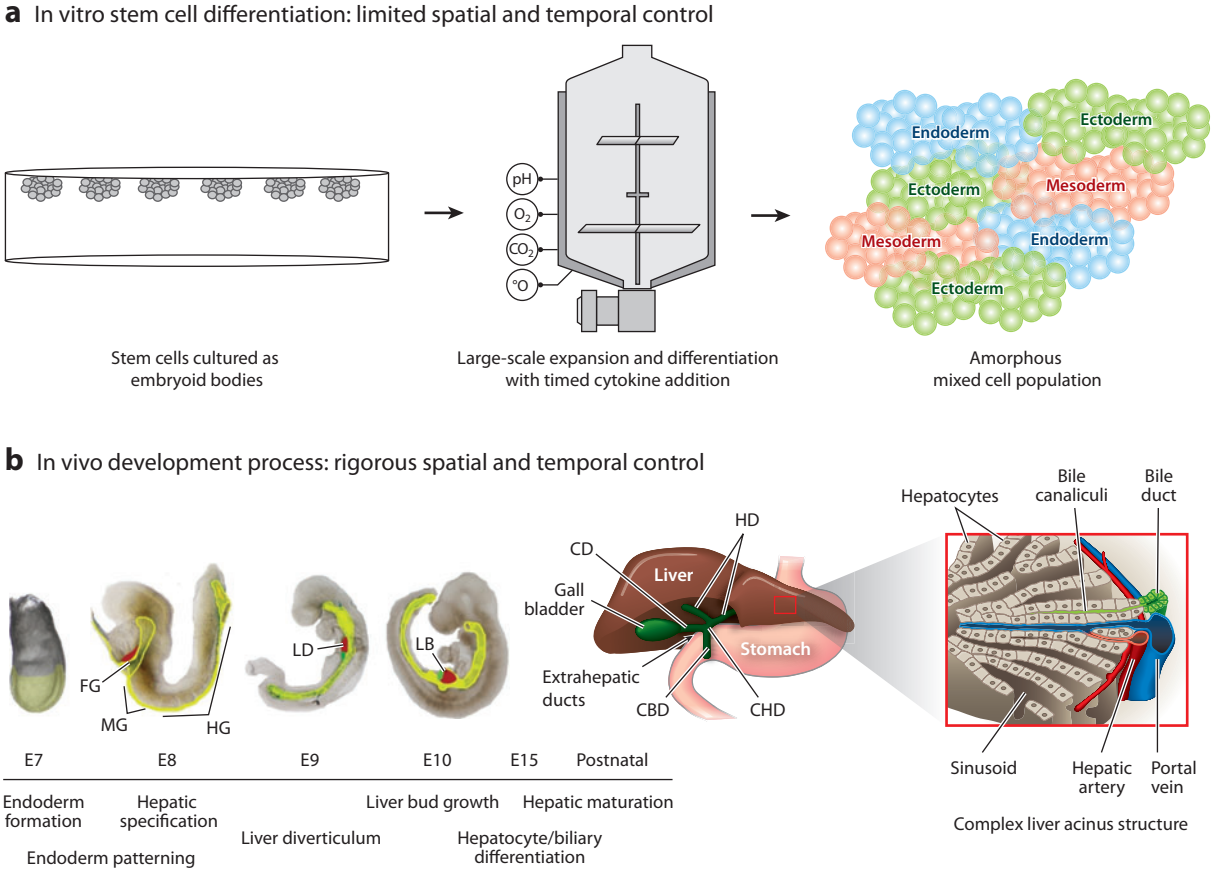


Figure 4

In vitro and in vivo stem cell differentiation and development. (*a*) Stem cells are often cultured in the form of aggregates called embryoid bodies that eventually differentiate into multiple cell types that appear in random patches throughout the culture. (*b*) In contrast, the developing embryo goes through distinct stages with extensive morphological reorganization and segregation of the germ layers. Ultimately, this process can generate intricate tissue structures. Embryonic development day 7, 8, 9. . . is shown as E7, E8, E9. . . Abbreviations: CBD, common bile duct; CD, cystic duct; CHD, common hepatic duct; FG, foregut; HD, hepatic duct; HG, hindgut; LB, liver bud; LD, liver diverticulum; MG, midgut. Panel *b* is adapted from Reference 170.

these techniques generally produce other lineages besides endoderm and hepatocytes as well as that the cell distribution is nowhere close to what the natural process of development can achieve in vivo (**Figure 4**). A better understanding of the development mechanisms, as well as techniques to more precisely control cell-cell interactions and the local environment of the cell in a dynamic fashion (such as the microfabrication techniques described below), will undoubtedly help improve the yield and functionality of stem cell–derived systems.

To translate these advancements to clinical applicability, many challenges lie ahead. These include identifying the most robust and potent cell source, determining reliable ways of expanding the cells, and growing them in 3D bioreactor environments (115–122). From a bioprocessing standpoint, the two major foci are (*a*) scalable culture systems, primarily those utilizing microcarrier systems optimized through decades of use in the pharmaceutical industry for large-scale mammalian cell culture, and (*b*) separation of the cell source of interest, namely through the use of

high-speed cell sorters. The eventual gates for liver therapies involving stem cell–based cell sources will be quality assurance of a homogeneous cell population and reproducible batch processing.

NEW TECHNOLOGIES

An exciting number of recently developed technologies may significantly impact the future of tissue engineering. As can be seen in **Table 6**, these technologies primarily focus on new methods for material and scaffold generation but also encompass high-throughput screening as well as stem cell technologies. Special attention should be given to microfabrication and microelectromechanical systems (MEMS) techniques, as they can provide features approaching the size scale and complexity of the in vivo environment. MEMS and nanolithographic techniques can be used to control features at length scales between 0.1 μm and 1 cm (123, 124). Techniques have been developed that are compatible with cells and are now being integrated with biomaterials to facilitate fabrication of cell-material composites that can be used for tissue engineering. In addition, microscale technologies provide an unprecedented ability to control the cellular microenvironment in culture and to offer cell-based and downstream assays for high-throughput applications.

In the past few years, microfabrication has been increasingly used in biomedical and biological applications, partly because of the emergence of techniques such as soft lithography to fabricate microscale devices without the use of expensive clean rooms and photolithographic equipment. Soft lithography is a set of microfabrication techniques that uses elastomeric stamps fabricated from patterned silicon wafers to print or mold materials at resolutions as low as several hundred

Table 6 Tissue engineering by critical technologies

Technology	Description/features
Solvent casting and particulate leaching (SCPL)	Makes porous structures with regular porosity but with a limited thickness. One may use a solvent with a low melting point that is easy to sublime for thermally induced phase separation (TIPS).
Emulsification/freeze-drying	A solution of synthetic polymer dissolved in a solvent (e.g., polylactic acid in dichloromethane) is mixed with water to form an emulsion.
Gas foaming	Uses gas as porogen, thus avoiding the need for organic solvents or solid porogens.
Nanofiber self-assembly	Molecules designed to self-assemble form biomaterials similar in scale and chemistry to those of the natural in vivo extracellular matrix.
Textile technologies	Techniques used to prepare nonwoven polymer meshes.
Electrospinning	Uses electrostatic repulsion to eject a thin fibrous stream to produce continuous fibers from nanometer to submicrometer diameters.
Computer aided design/ computer aided manufacture technologies	A 3D structure designed using CAD software is generated by ink-jet printing of polymer powders or polymer melt.
Soft lithography	Uses elastomeric stamps, molds, and conformable photomasks to fabricate structures in the range of 1–1,000 micrometers.
Microfluidics	Devices to manipulate fluids and suspended cells in geometries of submillimeter scale.
Decellularized matrices	Natural extracellular matrices generated by detergent-mediated removal of cells from explanted tissues. They can be reseeded with cells of the same organ or stem cells.
Bioreactors	Large-scale cell culture devices. In tissue engineering applications, they must be designed to provide efficient transport systems throughout the cell mass grown inside of them.
Stem cell technologies	Technologies of interest are isolation and separation techniques, control of proliferation, and differentiation in culture.

nanometers (125–127). Therefore, soft lithography can be used to control the topography and spatial distribution of molecules on a surface as well as the subsequent deposition of cells. Soft lithographic methods can also be used to fabricate microfluidic channels and scaffolds for tissue engineering in a convenient, rapid, and inexpensive manner. In addition, photolithography, a technique in which microscale features are fabricated on the basis of selective exposure of a material to light, can also be used for microfabrication of tissue engineering structures.

The additional power of these approaches comes when integrated with scaffold development. For example, by using lasers, specific regions within an agarose gel were tethered with RGD (Arg–Gly–Asp) peptide, which allowed for neurite extension within peptide-modified regions. By using similar techniques, multicomponent, spatially patterned, photocross-linkable hydrogels were fabricated to localize growth factors within hydrogels. Microfabrication approaches such as microfluidics provide an attractive alternative to these technologies because of their availability and cost effectiveness. Recently, the ability to pattern fluids within microchannels has been merged with photopolymerization chemistry to form spatially oriented hydrogels (128). As a result, gradients of photocross-linkable monomers were formed within microfluidic channels and subsequently gelled by exposure to UV light. Hydrogels were synthesized with gradients of signaling or adhesive molecules or with varying cross-linking density across the material in order to direct cell behavior such as migration, adhesion, and differentiation.

CHALLENGES AHEAD

Tissue engineering and regenerative medicine is an extremely interdisciplinary and complex field that requires a deep understanding of the effect of a myriad of factors on the development and sustainability of tissues and organs. The early successes with skin and cartilage encouraged many to posit that they could place any cell type in a matrix and then implant the resulting construct into the body with the hope of success. These simplistic and fanciful notions were a major factor in the so-called crash of the tissue engineering field in the 1990s, which was fueled by hype, overpromising, gullible early investors, and bad science. What worked with tissues that are relatively avascular and have low metabolic rates was doomed to fail when applied to more complex tissues. Furthermore, function of complex tissues is also dependent on proper homotypic and heterotypic cell-cell interactions, which requires spatial control of the various components (i.e., parenchymal cells, blood vessels, and nonparenchymal cells) at the micrometer scale.

Interestingly, industry suffered terribly with bankruptcies and other forms of failure, whereas in academia many of the prominent spokespeople who advanced simplistic and sometimes nonsensical notions went along their merry way, revising history and lacking introspection. The infusion of stem cell technology has provided a temporary hope that the field will gain some momentum, but it is still hard to see how this very expensive technology can have a short-term, meaningful impact that will reinflate the enthusiasm the field saw in the 1980s.

Microscale technologies, developed to position cells on surfaces and in matrices with a high degree of control, may also provide new pathways, but even here progress has been slow. It is time for tissue engineers to refocus their activities again toward the ultimate goal of creating new tissues and therapies with the modesty, clarity, and humble drive to excel that should characterize all good science. The requirements of the field are still the same for complex tissues and organs and include

1. scalability to a clinically relevant size, which may require the provision of a larger transport/vascular system;
2. strict control over the starting materials (e.g., cell source, scaffold material) and manufacturing protocol to guarantee reliability; and

3. ability to tolerate and even facilitate the process of integration with the host, which may involve, for example, modulation of the local immune response, use of a preconditioning regimen that eases the metabolic shock of going from culture medium to plasma, or stimulation of angiogenesis.

Basic tissue engineers also often fall in love with what they see as elegant solutions, whereas the end user, usually a medical practitioner, is looking to solve medical problems with products that show efficacy, reliability, ease of use, and cost savings. Physicians perceive the current cell-based tissue-engineered products as ones that require long, complicated, and expensive cultivation procedures; need specific (and expensive) transport and storage conditions; have a limited shelf life; are fragile and delicate; and in those cases in which autologous cells are used, require precise coordination between the tissue culture facility and the clinic. A surgeon faced with a choice between a partially effective tissue-engineered product that is both expensive and difficult to use and a more traditional approach or in some instances a new surgical method may opt for one of the latter two options. Market penetration for tissue-engineered products will remain small until they can be shown to lead to much better outcomes than generally cheaper alternative solutions. In addition to making highly functional tissue constructs, it is therefore critical that tissue engineers address the needs of end users and develop strategies to integrate their products into current clinical practices. This is the business end of the problem, which is ultimately going to make all the difference, because the financing of tissue engineering beyond academic laboratories will mainly come from private investors.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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