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Tissue Engineering: Toward a New Era of Medicine

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Abstract

The goal of tissue engineering is to mitigate the critical shortage of donor organs via in vitro fabrication of functional biological structures. Tissue engineering is one of the most prominent examples of interdisciplinary fields, where scientists with different backgrounds work together to boost the quality of life by addressing critical health issues. Many different fields, such as developmental and molecular biology, as well as technologies, such as micro- and nanotechnologies and additive manufacturing, have been integral for advancing the field of tissue engineering. Over the past 20 years, spectacular advancements have been achieved to harness nature's ability to cure diseased tissues and organs. Patients have received laboratory-grown tissues and organs made out of their own cells, thus eliminating the risk of rejection. However, challenges remain when addressing more complex solid organs such as the heart, liver, and kidney. Herein, we review recent accomplishments as well as challenges that must be addressed in the field of tissue engineering and provide a perspective regarding strategies in further development.

INTRODUCTION

Tissue engineering requires comprehensive efforts to combine engineering and physical sciences with life sciences for the purpose of restoring, replacing, or improving the function of damaged tissues and organs (1, 2). Tissue and organ failure due to injury or disease is considered a major healthcare challenge (3). Until the second half of the twentieth century, there were no suitable remedies for patients with dysfunctional organs. In 1954, Joseph Murray (1990's Nobel Laureate in Medicine) performed the first successful organ implantation, transplanting a healthy kidney donated by Ronald Herrick into his identical twin brother, Richard (4–6). In that procedure, the risk of an adverse immune response was eliminated because the donor and recipient were genetically identical. Five years later, Murray performed the world's first successful organ transplant between genetically nonidentical individuals (7). Many people's lives have been saved with organ transplantation as a result of Murray's pioneering operations. Nevertheless, the increasing number of people on transplant waiting lists, the shortage of organ donors, and an aging population necessitate the development of novel methods to restore the function of damaged organs and tissues (8). According to US Department of Health and Human Services (<https://optn.transplant.hrsa.gov>), an individual is added to the National Transplant Waiting List in the United States every ten minutes, and 22 people die each day while waiting for a transplant. The goal of tissue engineering is to mitigate the critical shortage of donor organs via in vitro fabrication of functional biological structures.

In tissue engineering, basic design approaches include cell structures only, cells and scaffolds, and scaffolds only (9). Autografts, allografts, and xenografts are biological constructs built from a patient's own cells, from another genetically nonidentical individual, and from a nonhuman animal species, respectively (10). Scaffolds are made of natural (e.g., collagen, decellularized matrices) or synthetic materials and are intended to replicate the natural three-dimensional (3D) environment, i.e., the extracellular matrix (ECM), so that cells can proliferate and organize into tissues or organs that can maintain their specialized configurations and their morphologies (2). Scaffolds must be compatible with both the tissue-specific cell types and the desired local environment within the human body (11). Therefore, different engineered tissues or organs necessitate unique designs and materials. In addition, the synthetic scaffolds must be fabricated with specific properties, such as pore size, geometry, permeability, and spatial distribution (12). The bulk and surface characteristics of scaffold materials may also affect cellular behavior (12). Ultimately, the degradation of the scaffold must be compatible with the production of the ECM by the cells. Recent progress in scaffold fabrication has propelled the field of tissue engineering toward higher goals (12–15). Furthermore, a relatively new approach of scaffold-free tissue engineering has been introduced (16, 17) that allows cells to produce their own ECM and self-assemble to build 3D biological structures.

Tissue engineering is one component in the field of regenerative medicine (18–20). Stem cell science, gene therapy, soluble molecules, and reprogramming of cell and tissue types are each part of the interface between tissue engineering and regenerative medicine. Over the past 20 years, several achievements in the construction of functional tissues and organs have helped to improve the quality of life for many patients (21). Fabrication of tissues and organs can be categorized in four levels of complexity. The least complex level is flat tissues and organs such as skin; the next level includes tubular organ structures such as blood vessels and tracheas. Hollow nontubular organ structures such as the bladder are the second most complex organs to fabricate, and the most complex structures are solid organs, including the heart, kidney, and liver.

Although flat, tubular, and hollow nontubular organ structures have been successfully constructed in the laboratory, fabrications of functional solid organs remain too complex for our current knowledge and capabilities. Numerous researchers around the world, with a wide range of backgrounds and specialties, are working to overcome the current challenges in this field. Issues

such as cell source, tissue vascularization, and compatible scaffolds must be addressed. The first important challenge of engineering a tissue or an organ is finding appropriate cell sources and providing large cell quantities. It is possible to supply oxygen and nutrients and remove waste materials from cells that are $<200\text{ }\mu\text{m}$ from the capillaries (22). To engineer thicker tissues and (ultimately) organs, vascularization is a key prerequisite. Moreover, an organ's function is based on the performance of individual cells, separate and combined; cells with different properties and functions interact with the ECM to achieve the organ's functionality. Providing sophisticated biomaterials and scaffolds to allow all cell types in an organ to work together and build their own ECM is another major challenge in engineering tissues and organs (23).

COOPERATIVE FIELDS AND TECHNOLOGIES

Matching tissue engineering technologies with biological and medical needs requires integration of various scientific disciplines, such as physics, cell biology, and developmental biology (24). For example, understanding cellular differentiation and growth and the effect of ECM components on cell function, or how tissues and organs form and self-assemble in the developing embryo, will help tissue engineers to accomplish their goals (25–27).

Some researchers have focused on the principles of developmental biology in general, and embryonic development in particular, to understand the process of natural self-assembly for use in tissue engineering (28). These efforts are converging toward the development of tissue- and organ-building methods that are scaffold-free (29). Embryonic development is a highly predetermined and systematic sequence of events (28). This process is based on the complex interplay of genetic, molecular, biochemical, and physical pathways. In the course of development from a single cell to a multicellular phase with many specialized differentiated cells, and ultimately multiorgan configuration, the embryo utilizes numerous principles of morphogenetic self-assembly.

Cell adhesion is one of the most important phenomena that develops in the zygote as it forms a multicellular organism. The differential adhesion hypothesis states that different cell types show different strengths of adhesion to each other as inherent properties (30). Numerous groups have verified the differential adhesion hypothesis and its predictions in several experiments (30–40).

Tissue liquidity and tissue fusion are the corollaries of the differential adhesion hypothesis (28). The concept of tissue liquidity is based on the analogy between multicellular systems in contact and immiscible liquids (e.g., oil and water) (28). Tissue liquidity indicates, among other implications, that the minimization of interfacial and surface free energies drives a random mixture of cell types to reach a “phase-separated” final configuration (28). Cell sorting is a morphogenetic phenomenon that engenders compartmentalization in the embryo and separation between tissues and organs (41). In a cell aggregate that consists of two randomly mixed cell types, cell sorting gives rise to the phase-separated configuration by arranging one cell type into the center of the aggregate surrounded by the other cell type. A simple molecular explanation for this phenomenon is provided by the differential adhesion hypothesis, by which the more adherent cells sort to the interior of the aggregate by maximizing their mutual adhesion and minimizing the configurational energy of the assembly (28). Tissue fusion, another self-assembly process, is analogous to the fusion of liquid droplets (41). In this process, two or more distinct multicellular assemblies in contact (such as multicellular aggregates and tissue fragments), merge and coalesce (42). Tissue fusion is an essential morphogenetic process in the course of early development that leads from simple structures to more complex configurations (41).

These phenomena have been applied extensively toward the fabrication of human tissues and organs. For instance, a special automatic delivery machine was used to make biological structures with different geometries by deposition of spherical and cylindrical microtissues or

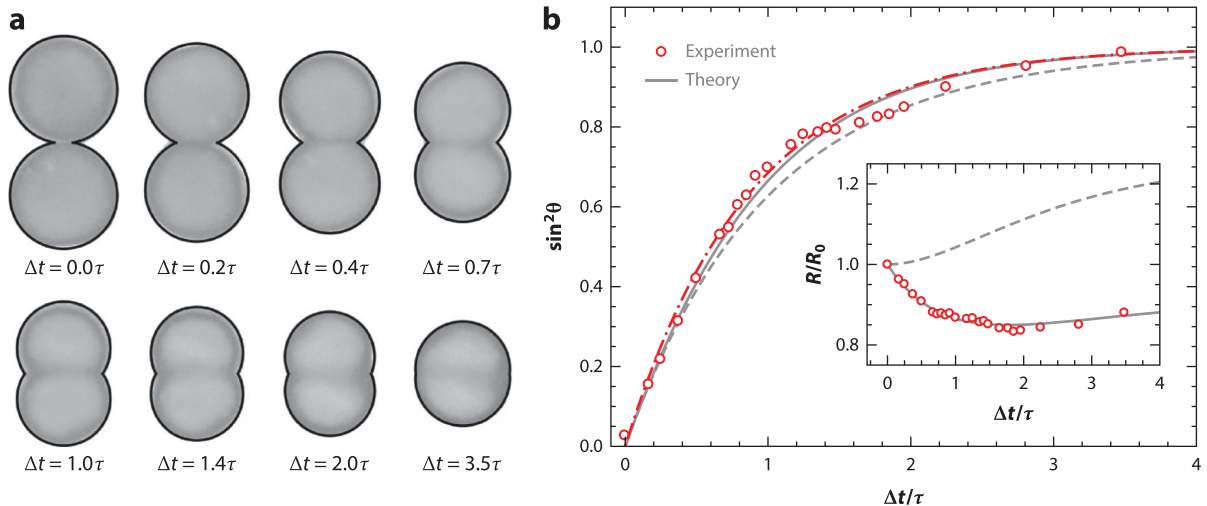


Figure 1

(a) Snapshots show the fusion of two spherical microtissues of human skin fibroblasts. The process was monitored over a week to determine the characteristic fusion time that was used to predict the shape evolution and maturation time quantitatively. Spherical or cylindrical microtissues can be used to build tubular biological constructs such as nerves or blood vessels. Fusion of microtissues is an important part of the fabrication in which self-assembly, cell rearrangements, and the maturation of the construct take place. (b) The characteristic fusion time is found theoretically and experimentally. Agreement between theoretical and experimental results as well as computational studies (not shown) were used to develop a predictive model for fusing multicellular systems. *Inset:* In this experiment, volume decrease of the microtissues was also observed and incorporated into the developed predictive formalism. It was assumed that decrease of the radii of the aggregates was governed by a time-dependent dimensionless quantity $a(t)$, such that $R_{i0}(t) = a(t)R_{i0}(0)$. It was shown that $a(t)$ could be well approximated by $a(t) = a_0 + (1 - a_0)\exp(-\lambda t)$, where a_0 was the volume change parameter and λ was the volume relaxation rate. Therefore, the solid and dashed curves were obtained by solving the theoretical integral numerically for non-volume-conserving and volume-conserving equations, respectively. Moreover, the dashed-dotted curve is for the non-conserved-volume situation obtained by a convenient simplifying function based on the volume change parameter and volume-conserving results. Reproduced from Reference 42 with permission from The Royal Society of Chemistry.

multicellular systems on supportive materials such as agarose. The multicellular systems underwent self-assembly, and tubular organ structures such as nerves and blood vessels were formed (43, 44). After printing, it takes time for the structure to mature via self-organization processes, in particular tissue fusion, and become fully functional. Post-printing shape evolution of multicellular systems was studied quantitatively (Figure 1), and a theoretical-computational-experimental formalism was devised to optimize the tissue engineering of tubular organ structures by bio-printing (42, 45). The formalism can be employed as a powerful technique to study biophysical aspects as well as dynamics of multicellular systems. The connection between simulation and experimental parts of the formalism was established through theory that had been independently developed based on continuum mechanics. Both computational and experimental results verified the theory. Subsequently, experimental results were used to calibrate some of the basic parameters for numerical simulation (time, energy, and length) necessary for prediction of time evolution of the multicellular system. Furthermore, experimental results were used to verify the successful prediction of the shape evolution of the multicellular systems by the formalism. These quantitative studies of evolution in complex biological systems are impossible without a deep understanding of the physics behind morphogenetic phenomena and biological events. Tissue engineering has been aided by advanced technologies such as additive manufacturing and micro- and nanotechnologies.

Bioprinting

Since the beginning of the twenty-first century, 2D and 3D printers have been used extensively in applications ranging from renewable energy (46, 47) to nanoelectronics (48, 49) to medicine and tissue engineering (42–45, 50). Their ability to precisely deposit different materials on various substrates rendered them an excellent choice for researchers in drug delivery and personalized medicine (51). 3D printing of sophisticated surgical planning models has enhanced surgical outcomes. Moreover, tissue engineering practitioners are using 3D printers to print cells, scaffolds, and cell-laden hydrogels to make 3D biological constructs. Several cell types are being used and different tissues are printed, as bioprinting has been accepted as a versatile fabrication technique. In cardiovascular research, for example, polyester urethane urea was used as a cardiac patch with human mesenchymal stem cells and human umbilical vein endothelial cells patterned onto it. Subsequently, the patches were implanted into rats with induced myocardial infarction, showing increased vessel formation and enhanced heart function (52). Printing has also been considered a reliable technique for heart valve fabrication. Methacrylated hyaluronic acid and methacrylated gelatin were used to develop a hybrid hydrogel to encapsulate valvular interstitial cells to print heart trileaflet valve conduits (53). Researchers also employ printing approaches for tissue vascularization (54, 55). Sacrificial elements, a sucrose/glucose/dextran combination, were used to cast a 3D vascular design, printed (55), and encapsulated in ECM and cells. Using culture medium, the sacrificial elements were dissolved, leaving an imprint throughout the tissue model that was lined with endothelial cells and perfused with blood.

Micro- and Nanotechnologies

Richard Feynman, who was awarded the Nobel Prize in Physics in 1965, sparked the idea of nanotechnologies. He opened up a new approach to human life in his famous speech entitled “There’s Plenty of Room at the Bottom.” Since then, physicists and engineers have accomplished many innovations and discoveries by using the idea of manipulating materials and manufacturing devices at nanoscale levels (one-billionth of a meter) (56, 57). Human health in general, and tissue engineering in particular, have benefited immensely from nano- and microtechnologies. For example, porous nanomaterials have led to highly efficient drug delivery systems because of their high affinity for target cells and tissues (58). Polyphenol-coated mesoporous silica nanoparticles, biodegraded by acidic pH and intracellular glutathione, enabled the system to release surrounded anticancer drugs. Nanofibers and nanopatterns are employed in tissue engineering applications because they provide cellular environments similar to those of native tissues (58–60). Scaffold-based tissue engineering requires scaffolds with specific properties for cells to maintain and regulate their functionality. Micro- and nanotechnologies facilitate the construction of sophisticated scaffolds with different properties, such as pore size, geometry, and distribution, with nanometer resolutions. Nanostructured scaffolds are gaining attention because of their ability to regulate the fate of cells by inducing specific cell signaling (59). Nanoengineered scaffolds with tailored biochemical, mechanical, and electrical properties have been employed to control tissue growth (60).

Micropatterning and nanopatterning can control the cellular microenvironment and govern cellular behavior in a desired direction. Surface topography also affects cell behavior at various levels, such as polarity, adhesion, migration, proliferation, and differentiation (59, 61). For instance, an *in vitro* culture platform with a specific spatial distribution of ECM protein was fabricated for adult neural stem cells (62). Investigation of the cells’ responses showed that the alignment of neurons and astrocytes could be controlled by changing the spacing of ECM stripes.

Cell shape, cell–matrix interactions, and cell–cell interactions have been successfully controlled using nano- and micropatterned substrates to make cell microarrays (12). Moreover,

micropatterned cell substrates have been combined into microfluidic channels for high-throughput measurements of cell behavior (63). These technologies allow fabrication of systems that can control tissues using the geometry of the attached cells as well as their biochemical interactions. Novel microfabrication technologies such as dip-pen nanolithography provide biochemical cues for cells by adjusting surface chemistry pattern geometry, density, and bioactive molecule specificity (64). Substrates with anisotropic micro- and nanotopographies have been made, using microtechnologies to study the behavior of fibroblasts (65). Effects of the local density of various micro- and nanotopographic patterns on cellular responses, such as cell migration, orientation, and shape, were defined. Cell adhesion and elongation in the denser pattern areas were much stronger, confirming dependence on substrate patterning.

TISSUE AND ORGAN FABRICATION

Tissue engineering has shown clinical feasibility in flat organs, such as skin and cartilage. Skin normally protects interior organs and tissues from outside microorganisms and bacteria. In traumatic and thermal injuries, an important priority is to protect the body from infection at sites of open wounds. Recently, an innovative antimicrobial skin tissue has been manufactured using dermal fibroblasts and epidermis derived from normal immortal keratinocytes (the NIKS cell line) (66, 67). The system was tested on several patients, and the performance was compared to cadaveric allograft by measuring in vitro lymphocyte proliferation, chromium release, and antibody production. Results showed the same levels of T and B lymphocytes and Langerhans cells in both systems. In addition, patients with the engineered coverage did not produce antibodies targeted to the NIKS cells.

In cartilage defects, autologous chondrocyte implantation has been used over the past several years and resulted in pain reduction and enhancement of life activities for many patients (68). In one study, cartilage was created using chondrocyte-derived progenitor cells for patients with substantial knee cartilage injuries (6–13 cm²) (69). These progenitor cells possess chondrogenic potential higher than that of mesenchymal stromal/stem cells while producing the same phenotype.

Tubular organ structures such as tracheas and blood vessels have been fabricated and used in human patients. For example, the fabrication and transplantation of a trachea into a pediatric patient has been reported with four years of follow-up. The trachea is performing well (70). A trachea from a deceased donor was decellularized and seeded with the recipient's respiratory epithelium and mesenchymal stromal cells. Computed tomography images of the neck and chest were acquired at different time points after transplantation. A differentiated respiratory layer was observed, and abnormal immune activity was not detected by histocytology. In this study, computational fluid dynamics was used for the first time to measure tracheal airflow. This could provide thorough information on how geometrical features correlate with airflow distribution. In another attempt, 3D bioprinters have been used to fabricate a trachea splint that was implanted into a newborn (71).

Both autologous and allogeneic human tissue-engineered vascular grafts have been fabricated and transplanted into patients with end-stage renal disease for hemodialysis (72–75). These patients had been receiving hemodialysis via an access graft, a procedure that carries a high risk of failure. A tissue engineering graft was constructed from fibroblasts taken from autologous skin and superficial vein. The fibroblasts were allowed to proliferate and assembled into sheets that were wrapped around a stainless steel mandrel. The cellular sheets fused to each other over the course of the maturation process for ten weeks in culture. One week before the surgery, the endothelial cells were seeded into the lumen of the vessels. Patients with autologous blood vessels were monitored for 6–20 months; these blood vessels showed mean burst pressure of 3,512 mm Hg and were effective

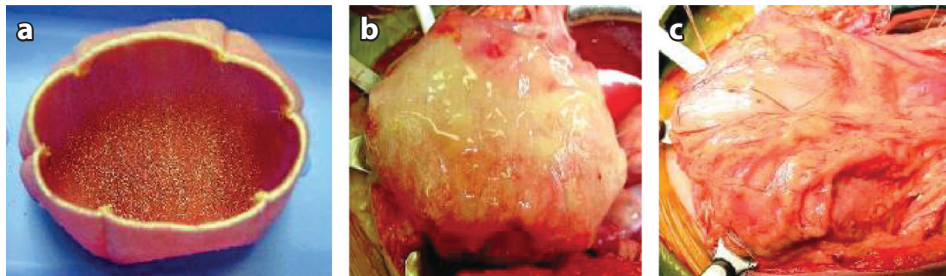


Figure 2

Engineering an autologous bladder. (a) Scaffold is seeded with cells; (b) scaffold with cells is then anastomosed to native bladder with 4–0 polyglycolic sutures. (c) Transplanted bladder with omentum and fibrin glue.

for hemodialysis. Nonetheless, the long production time of autologous vascular grafts (6–9 months with a mean of 7.5 months) is not favorable for patients who desperately need transplantation. Therefore, allogeneic human tissue-engineered vascular grafts were fabricated. They were found to maintain their mechanical strength when stored for several months. The vascular grafts did not show any degradation or immunosensitization in any of the patients, confirming the feasibility of using tissue-engineered human vascular grafts.

In 2011, autologous urethras were made and transplanted into five boys, age 10–14 years, with urethral defects (76). A tissue biopsy was taken from each patient, and the muscle and epithelial cells were cultured and seeded on tubularized polyglycolic acid:poly(lactide-co-glycolide acid) scaffolds. The constructs were incubated for a week in Dulbecco's Modified Eagle's Medium. Generally, neo-urethras took 4–7 weeks to form. After successful implantation, follow-up was continued for a median 71 months. Histological and functional characteristics of the engineered urethras are similar to native urethras and all five boys are continent.

Hollow nontubular organ structures have been engineered with positive results. In one major effort to produce human autologous organs, bladder biopsies were obtained from seven patients, age 4–19 years, who had poorly compliant bladders due to myelomeningocele (77). Subsequently, autologous bladders were fabricated *in vitro*. Biodegradable bladder-shaped scaffolds were created from either collagen or a composite of collagen and polyglycolic acid, which performed better in the long term according to previous reports (78, 79). Autologous bladder cells were cultured and seeded into the scaffolds (**Figure 2**). A few weeks later, the bladders were implanted into the patients. After a mean of 46 months, the organs were functioning normally (77). Four girls age 13–18 with vaginal aplasia successfully received tissue-engineered autologous vaginas (80). The engineered vaginal organs have been functioning normally up to eight years as of the report date. All patients have functional variables in the normal range for arousal, lubrication, orgasm, satisfaction, and painless intercourse.

Solid organs are the most complex organs to fabricate. Although tissue-engineered flat, tubular, and hollow nontubular organs are being used clinically, solid organs have not been constructed with current technology. Nonetheless, advancements in engineering small tissues and organoids are promising. Researchers are attempting to engineer cells and tissues to treat some heart disorders, such as myocardial infarction. Different tissue engineering techniques have been applied to combine cardiomyocytes with biodegradable and nondegradable biomaterials to cure diseased or dysfunctional heart muscles (81). Scaffold-free cell sheets, cultured on temperature-sensitive dishes, have been used to partially repair injuries to the myocardium. In one attempt, scaffold-free induced adipocyte cell sheets were implanted into rats with experimental autoimmune myocarditis (82).

Results revealed higher adiponectin and hepatocyte growth factor in the myocardium of rats with implants than in sham-operate controls, suggesting that this method has potential for treating myocarditis clinically. Cardiac patches have led to significant functional recovery after myocardial infarction in animal models. For example, notable reduction of infarct size was achieved using a fibrin-based heart patch containing neonatal rat heart cells (83). Cells encapsulated in hydrogels can be used in myocardial tissue engineering. Encapsulation within the matrix-supplemented hydrogel capsules has been used to increase cardiac stem cell engraftment and survival after myocardial injection (84); this system showed cardiac stem cell viability under hypoxic stress conditions. In an *in vivo* model of myocardial infarction, the system increased acute engraftment and improved cardiac function. Decellularized ECMs are also used in cardiovascular tissue engineering. Cell-laden constructs of heart tissues have been made using printers and decellularized ECM (85).

There is a desperate need for engineering human kidneys to permit the perfusion, filtration, secretion, absorption, and drainage of urine. In one attempt, researchers decellularized rat kidneys (86), creating scaffolds that were seeded with epithelial and endothelial cells. These structures were then perfused in a bioreactor. After transplantation, the bioengineered kidney was able to produce urine and clearance of metabolites *in vivo*.

There are several different cell types in the liver: sinusoid endothelial, Kupffer, biliary epithelial, and stellate cells, as well as hepatocytes. Among these, hepatocytes are the most important; most clinically measurable organ functions, such as metabolic homeostasis, protein synthesis, and detoxification, are related to hepatocytes. Therefore, providing sufficient amounts of functional hepatocytes is imperative in liver tissue engineering after vascularization (87). Researchers have obtained naturally derived vascular tree scaffolds by decellularizing the liver using a detergent that removes only its cellular components (88). In this procedure, the ECM and the vascular network remained intact and were seeded with new human fetal liver and endothelial cells. This system reproduced liver-like tissue behavior *in vitro*. Most attempts to fabricate liver tissues are for drug development purposes.

TISSUE ENGINEERING FOR DRUG DEVELOPMENT

Traditionally, cell lines have been used for drug screening and toxicology. First, the target agents are identified *in vitro*, and preclinical studies in animal models are then conducted. However, cell lines are not equivalent to normal human cells *in vivo* and do not mimic multiorgan responses (89). Moreover, animal reactions to drugs may not be indicative of human responses. Therefore, using human tissues for drug discovery may eliminate many irrelevant studies and save money and time. Accordingly, 3D human tissues and microtissues with multiple cell types are currently being developed for drug screening applications. The liver is the most important organ in pharmaceutical studies owing to its critical role in drug metabolism and toxicity. Combinations of HepG2 cells and endothelial cells have been printed into constructs; for example, liver cells printed and sandwiched between layers of human umbilical vein endothelial cells (90). This system showed hepatocellular function and cell–cell interaction comparable to actual diseased liver tissues, suggesting that such a system could be employed for drug screening. Engineered liver tissue can be combined with microfluidic devices to assess the responses of multicellular systems to external stimuli (91, 92).

CONCLUSION

Tissue engineering aims to cure diseased or dysfunctional tissues and organs. Different scientific fields such as cellular and developmental biology, combined with advanced technologies such as bioprinting, have been used to advance tissue engineering. Flat, tubular, and hollow nontubular

organ structures have been fabricated in laboratories and successful implantations in humans subsequently reported. However, if we are to fabricate laboratory-grown solid organs such as hearts or livers, major challenges must be overcome. Each tissue and organ requires vascularization to deliver oxygen and nutrients to the cells, and a large number of cells is needed to produce an organ. Appropriate sources of cells for tissue engineering efforts must be identified. Finding suitable scaffolds for ECMs is another challenge. Optimizing the production time and cost of lab-grown organs is the ultimate challenge to initiate a market for engineered human parts. Nevertheless, the remarkable discoveries and achievements over the past decade, in addition to current worldwide efforts, signal continued progress in both the short and long terms. Short-term goals of the field include tissue fabrication for drug evolution and discovery, as well as curing minor tissue disorders and diseases. The long-term goal will remain the fabrication of human solid organs.

DISCLOSURE STATEMENT

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