

Tissue Engineering in Medicine

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TRANSPLANTATION NEEDS

Transplantation of whole organs and complex tissues has progressed significantly over recent history.¹ Much of this progress has been due to technical advances in immunosuppression and organ and tissue preservation. Unfortunately, many of these advances have already reached their limit, while the disease burden of organ failure and tissue damage continues to grow.

Allogeneic transplantation from donors remains the definitive method; however, the need for organ viability and lack of donors results in a disproportionate demand. According to the Canadian Organ Replacement Register 2014 Annual Report,² this supply/demand discrepancy persists across all types of transplantation. Data for patients requiring organ transplants in 2012 is summarized in Table 1. These data demonstrate that despite the large number of transplants performed, many patients will end up on a waiting list and pass away while awaiting a donor. Moreover, patients who receive solid organ transplants must live with life-long immunosuppression and the risk of transplant rejection.

Furthermore, many other tissues are transplanted regularly and are subject to the same problems with shortages and immunosuppression. Additionally, there are no methods to restore function of deceased tissue such as after myocardial infarction. Taken together, these issues highlight the need for new methods of performing organ or complex tissue transplants and regeneration.

TISSUE ENGINEERING

A subfield of regenerative medicine, tissue engineering is concerned with the use of cells, matrix scaffolds, and associated biochemical and biophysical factors to create organ or tissue substitutes.³ Tissue engineering has the potential to address organ shortages for transplantation patients, as well as provide complex tissue substitutes for a wide array of medical issues. This review serves to introduce readers to the field of tissue engineering, its methods for tissue construction, and current experimental progress in the field. Readers will learn of technologies that show clinical promise as well as those that have already been surgically implanted in patients.

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Tissue engineering is multi-disciplinary field, drawing on aspects of cellular biology, biomaterials, chemical engineering, and surgery. Fundamentally, an organ or tissue consists of its component cells, the extracellular matrix, and surrounding biochemical factors. Tissue engineering uses these variables to induce differentiation of cells along a defined developmental pathway. An extracellular matrix scaffold is obtained either by manufacture or decellularization (removal of cells) of an allogeneic or xenogeneic organ or tissue. This scaffold is then recellularized with autogeneic cells or stem cells. Maturation of the developing tissue is completed in a bioreactor under finely tuned conditions. This approach has the hypothetical advantage of bypassing immunosuppression through the use of autologous cells. Interestingly, extracellular matrix molecules such as collagens and laminins are highly conserved among several species; xenograft scaffold transplantations do not result in severe immunogenic reactions.⁴ A conceptual overview of the tissue engineering process is depicted in Figure 1.

Although the importance of the biochemical milieu has long been known to impact cellular differentiation and function, the importance of biophysical factors imparted by the extracellular matrix and tissue microarchitecture on gene expression and phenotype is a more recent development. These biophysical factors include such things as matrix rigidity⁵ and diffusion dynamics of paracrine signals.⁶ Scaffolds capturing some of these properties can be generated through manufacturing techniques like electrospinning, which is the use of an electric charge to design fine materials at the micrometer or nanometer scale.⁷ Nevertheless, extracellular matrix scaffolds are complex and manufacturing methods cannot yet replicate the 3D complexity of higher level organs such as the heart. For this reason, the use of decellularized scaffolds has gained popularity.⁸ This technique involves obtaining a donor tissue and clearing it of cells and other molecules through the use of detergent. Manufacturing of scaffolds is constantly being improved, and patterning technologies such as micro-printing, 3D-printing, and soft lithography allow generation of artificial micro-architectures for the creation of “mini-tissues.”⁹ Although not yet allowing full organ recreation, these technologies have resulted in tissue substitutes that have applications in fields such as toxicology testing.¹⁰ In the future, generation of extracellular scaffolds may rely on a combination of decellularization and patterning technologies.

Examples of successful tissue engineering experiments

are provided below. Although this review is by no means comprehensive, studies highlighting major successes are described. Much of the clinical success using tissue-engineered structures has been accomplished using simpler structures, although work on whole organs is pending. Patients who have had surgical implantation of these technologies will require unique medical management and continuous monitoring. By understanding how these technologies are designed, and how they differ from traditional tissue grafts and organ transplants, clinicians will be better equipped to deal with them in practice.

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EXAMPLES OF TISSUE ENGINEERED ORGANS AND TISSUES

Cardiac Tissue

An experiment using decellularized extracellular matrix to regrow rat hearts validated the use of an allograft scaffold for tissue engineering.⁸ Coronary perfusion of detergent in hearts resulted in viable allograft scaffolds that were then repopulated with autologous cardiac or endothelial cells. Constructs were incubated in bioreactors simulating cardiac physiology, and eventually showed contractions that could generate two per cent of adult pumping function. The decellularization approach has also been extended to the study of heart valve regeneration.¹¹

Engineered substrates also have the potential to restore function to infarcted cardiac tissue.¹⁴ Other studies using smaller constructs such as cardiac cells embedded into micro-templated substrates and mesenchymal stem cell sheets have demonstrated successful integration of grafts *in vivo*, as well as partial restoration of function after infarction.^{12,13} Clinically, the CARDiosphere-Derived aUtologous stem CELls to reverse ventricUlar dySfunction (CADUCEUS) trial demonstrated that cultured autologous stem cells can be successfully infused into patients after myocardial infarction.¹⁴ Although this trial did not demonstrate improvement in left ventricular function after infarction, it showed that engineered cell therapy approaches are safe in patients and should be explored in further trials.

Blood Vessels

Tissue-engineered vessels using autologous cells have been

validated in a human adult arterial model.¹⁵ The vessels, which displayed physiological biomechanical properties, were implanted in hemodialysis patients whose original arteriovenous shunts were failing. The authors showed that these vessels could withstand physiological pressures and remain viable 24 months after surgery. Additionally, an older clinical report demonstrated successful implantation of a tissue-engineered pulmonary artery.¹⁶

Cornea

Nishida *et al.* demonstrated the use of autologous mucosal epithelial cells to create tissue-engineered sheets for corneal transplantation.¹⁷ These sheets were used to reconstruct corneal surfaces in four patients with bilateral total stem cell deficiencies. There were no complications, grafts remained stable after 13 months, and vision improved in all patients. Bilateral disease prevents the harvest of autologous corneal stem cells for graft creation; this new approach bypasses this problem through the use of mucosal cells.

Urethra

Raya-Rivera *et al.* demonstrated the results of implantation of tissue-engineered urethras in five boys who required urethral reconstruction.¹⁸ Tissue biopsies taken from each patient provided the source cells, which were then seeded onto tubularized polyglycolic acid:poly (lactide-co-glycolide acid) scaffolds. Patients underwent reconstructive surgery and were followed with a median follow-up time of 71 months. All five boys were continent at their last follow-up. Urethral biopsies showed that the engineered grafts developed an architecture that appeared normal by three months after surgery. Adequate outflow was maintained for up to six years.

Bladder

Cystoplasty for end-stage bladder disease uses gastrointestinal segments, although these may be associated with complications. Atala *et al.* demonstrated the use of autologous engineered bladders in seven patients (aged 4-19 years) with myelomeningocele with high pressure or poorly compliant bladders.¹⁹ Urothelial and muscle cells obtained from biopsy were grown on collagen or collagen/polyglycolic acid scaffolds. After transplantation, patients were followed up for a mean of 46 months. Patients did not demonstrate metabolic consequences or urinary calculi, and renal function was preserved. Bladder capacity of patients was less than what it was prior to surgery; mean bladder leak point pressure was decreased. Although the bladder is structurally relatively simple compared to other organs, this study demonstrates the feasibility and clinical possibility of whole organ autologous engineering.

Kidney

The kidney is a complex organ; thus, no tissue-engineered substitute has yet been developed for clinical use. However, basic research efforts in kidney engineering are plentiful. Of particular note, decellularization techniques to obtain rat, porcine, and human kidney scaffolds have been successful.²⁰ Recellularization and incubation of the rat scaffolds yielded bioartificial kidneys with very basic urinary generation function *in vitro*. When

orthotopically transplanted in rat, grafts became perfused through recipient circulation and produced urine *in vivo*.

Lung

Work from the same laboratory that generated the bioartificial kidney used the same approach to generate a bioartificial lung.²¹ After decellularization of rat lungs, scaffolds were seeded with epithelial and endothelial cells. Incubation in a bioreactor simulating the environment of a developing lung yielded constructs that could gas exchange *in vitro* at levels comparable to native lungs. Orthotopic transplantation yielded *in vivo* gas exchange for up to six hours after extubation. Similar to kidneys, no tissue engineered lung constructs have yet been transplanted into humans.

Trachea

Tissue engineering of trachea has yielded clinical successes. Macchiarini *et al.* demonstrated that transplantation of a tissue-engineered trachea into a 30-year-old woman suffering from end stage bronchomalacia immediately restored respiratory function and improved her quality of life.²² A human donor trachea was decellularized and seeded with autologous epithelial and mesenchymal stem cell derived chondrocytes. It was then used to replace the patient’s left main bronchus. A five-year follow-up of the patient showed that the engineered trachea had remained open, well vascularized, completely and appropriately cellularized, and had normal ciliary and mucus clearance functions.²³

Another study demonstrated the transplantation of a bone marrow mesenchymal stem cell seeded graft into a 12-year-old boy with long segment congenital tracheal stenosis and a pulmonary sling.²⁴ Prior to surgery, the patient’s airway had been maintained by metal stents. The graft was vascularized within one week of surgery, but restoration of epithelium was not evident until one year later. Additionally, the graft did not have sufficient biomechanical strength until 18 months. However, at two years follow up, the patient had a functional airway and required no further interventions.

CONCLUSION

This article provided a brief introduction to current concepts in tissue engineering, as well as some examples of successful experimental and clinical proof of concepts. Fuelled by advances in stem cell biology, biomaterials, and manufacturing technologies, tissue engineering and regenerative medicine will lead to novel methods of restoring tissue function for a spectrum of medical conditions. Although the field is in its infancy, the clinical successes thus far have been impressive. Nevertheless, all relevant advances should be validated through rigorous clinical trials. In the future, physicians in many fields will need to be made aware of these technologies as they become prominent in health care delivery. 

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