TISSUE ENGINEERING: ADVANCES IN ORGAN REPLACEMENT

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Keywords: Tissue engineering, skin, liver, nerve, cornea, articular cartilage, kidney, bladder, skeletal muscle, bone, blood vessels, organ replacement

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Summary

The human body has an amazing ability to undergo self-repair. However, this natural regeneration process is limited, and in those patients with infection or disease, the body's repair process may be compromised. To improve, maintain, or restore function to damaged or diseased tissues, scientists are looking to engineer tissue equivalents either as temporary substitutes while the body heals or as permanent replacements.

Tissue engineering is a multidisciplinary field, combining the principles of cell biology with those of engineering to recreate tissues in the laboratory. The aim of this article is to review strategies and advances in the field of tissue engineering, with reference to the major tissues under investigation.

1. Introduction

Much research has focused on possible ways of either encouraging the body to heal itself more rapidly or of developing some form of bioartificial device capable of either replacing the damaged organ *in vivo* or working *ex vivo* to supplement its impaired function. This led to the development of the field of tissue engineering. Tissue engineering combines the principles of engineering and cell biology to develop

biological substitutes able to maintain, restore, or improve tissue function. Much of this work has been stimulated by the observation that the body naturally has at least some capacity for regeneration (for example wound healing), and if the biological mechanisms of these processes can be better understood, they may be corrected when they go wrong, enhanced, or manipulated to allow the *ex vivo* production of fully functioning tissues or bioartificial devices.

Langer suggested in 1993 several strategies to achieve this goal:

1. Replacement of malfunctioning/dead cells with isolated cells or cell substitutes.
2. Administration of growth factors or cytokines to encourage the malfunctioning organ to regenerate itself.
3. Culture of the appropriate cell type on a support matrix to produce either an extracorporeal device capable of replacing organ function or for implantation.

Whichever strategy is used, the ideal solution will be one that returns organ function permanently without the need for repeated treatment and that is entirely biocompatible. This article reviews the strategies and advances in the tissue engineering of the major tissues under investigation.

2. The Skin

The largest organ in the human body, skin, has been one of the first true success stories in tissue engineering. The field has progressed rapidly, and there are more than six skin substitute products commonly used in clinical practice (Table 1).

Product	**Description**
Dermagraft®	Human fibroblasts seeded on a bioabsorbable scaffold
Epicel ®	Autologous epidermal sheets
Integra®	Acellular dermal matrix based on bovine tendon collagen
Apligraf®	Human fibroblast dermal layer and keratinocyte epidermis
Alloderm®	Freeze-dried acellular human dermal matrix

Table 1: Examples of skin substitute products

Prior to the development of these products, the main treatment for patients requiring skin because of severe extensive burn wounds or the excision of large areas of skin were autologous or allogenic skin grafts. This involves the removal of some of the patient's own skin or that of a donor, usually from the legs or buttocks, which is transplanted onto the area required. Both of these transplant methods are often limited by the amount of donor tissue available, the risk of infection, and graft rejection. The process is a

painful one and often results in permanent scarring. It is these problems that prompted the search for alternative therapies.

Skin is comprised of two main layers. The outer layer or stratified epidermis is formed from keratinocytes and acts as a physical barrier to moisture loss, trauma, and pathogens. Separated by a basement membrane from the epidermis is the dermis, which provides support and nourishment. This tissue consists mainly of connective tissues and collagen synthesized by fibroblasts that are present throughout this layer (Figure 1).

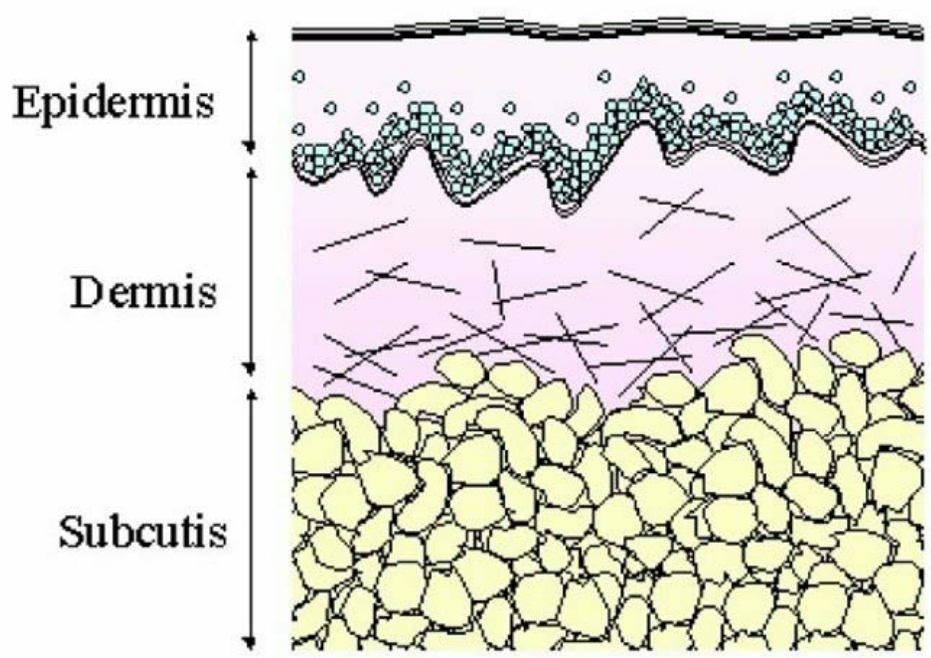

Figure 1. Diagram of the anatomy of skin

In the mid 1970s Rheinwald and Green pioneered the tissue engineering of skin in the serial culture of keratinocytes, allowing the expansion of the population and the first laboratory-produced skin tissue. The theory behind this method allowed the grafting of these cells onto burn injuries or chronic wounds. A small biopsy of the patients' own skin can be partially broken down by digestive enzymes, allowing the separation of the dermis and epidermis. Keratinocytes from the epidermis are then cultured *in vitro* with growth factors and their population expanded until a sheet of skin is produced that will adequately cover the wound. Unfortunately, the time needed for the propagation of the host's cells can be 3 weeks or 4 weeks to cover an extensive burn wound and so to have a ready supply of skin, allogenic keratinocytes are now used. Cells isolated from neonatal foreskin samples can be cultured and the resulting sheets of cells seeded on to a gauze support scaffold prior to transplantation. During the serial culture of keratinocytes, langerhans' cells, which are responsible for the activation of the immune system, are lost. The resulting tissue could therefore be transplanted without the risk of rejection.

Tissue-engineered skin formed via this method is extremely fragile and therefore difficult to handle. It has also been found to contract during the healing process and has poor acceptance efficiency.

In the early 1980s Bell and his coworkers perfected the technique by culturing epithelial cells on a collagen matrix containing dermal fibroblasts to produce the first skin equivalent with full thickness. Feeding this culture at the air/liquid interface resulted in skin equivalents with complete morphological differentiation. This model more accurately simulated *in vivo* skin, possessing an epidermal and dermal layer, and this led to the production of the clinical product Apligraf®. Many research groups have shown the importance of the dermis in epidermal maintenance, and in minimization of scar formation and wound contraction. Numerous dermal substitutes have been investigated including collagen-based lattices, fibrin glue, and synthetic polymers. All of these scaffolds rely on the ability of the fibroblasts seeded within them to excrete extracellular matrix molecules to produce the connective tissue of the dermis. This layer then encourages the proliferation of the fibroblasts.

Culture conditions vary from laboratory to laboratory but essentially involve medium modified with growth factors. Epidermal growth factor (EGF) plays a key role in the regulation of keratinocyte proliferation. Research by Werner noted that keratinocyte growth factor (KGF) is upregulated more than 100-fold by dermal fibroblasts 24 h after initial skin injury. Keratinocytes have also been found to excrete interleukins 1, 3, 6, and 8; transforming growth factor (TGF) α and β granulocyte colony stimulating factor (GCSF), fibroblast growth factor, platelet-derived growth factor, tumor necrosis factor (TNF) α, and many others. These cytokines and growth factors have shown limited effects when applied directly to wounds alone. However it is thought that multiple factors act synergistically to promote wound healing. Tissue-engineered skin replacement products, which incorporate cells, growth factors, and matrix, allow vitally important cell-cell signalling to occur at the initial stages after transplantation because of to the three-dimensional orientation of the cells. Long-term studies have revealed that donor cells are slowly replaced by the host's own cells over time, suggesting that the graft acts as a temporary biological dressing. The graft is able to provide the cells with the necessary growth factors and cytokines required for tissue repair, and in the appropriate chronological sequence.

Many skin substitutes have been developed, mainly comprised of a mixture of cells and matrices. Advances have been made with respect to tissue-possessing hair follicles, capillary networks, and sebaceous glands. The natural repair process of skin *in vivo* is not that of regeneration but healing via the formation of scar tissue. This tissue does not possess the elastic or flexible properties of the dermis. Future products may therefore not only be of use in patients requiring tissue to cover large wounds but may also become incorporated into commonly used small wound dressings.

3. The Liver

Despite advances in liver transplantation, such as the division of donor livers into several segments (which may then be transplanted into a number of recipients), there is still considerable demand for donor livers. Part of the problem is the short life span of

donor organs. The hope is that if a liver could be engineered, it could be available for transplant more or less on demand. In addition, because of the tremendous regenerative capacity of the liver, the development of a liver support device may be able to either bridge the gap between liver failure and regeneration of the host organ or the finding of a suitable donor tissue.

Tissue engineers face a considerable challenge in constructing liver tissue because of its structural complexity. The liver receives blood via the hepatic artery and hepatic portal vein, which is then distributed throughout the hexagonal lobules of the hepatic parenchyma. Each lobule consists of portal triads (with branches of the portal vein, hepatic artery, and bile duct) arranged around the central vein. Blood passes from the portal triad through fenestrated sinusoids, which surround the hepatocyte parenchyma, and into the central vein. Kupffer cells (specialized macrophages) line the sinusoids and phagocytose worn-out red blood cells or invading bacteria. Toxins and nutrients are absorbed by the hepatocytes, metabolized, and the products of metabolism and numerous other substances produced by the liver cells, such as plasma proteins, are secreted back into the blood. The central veins of each of the lobules eventually converge to form the central vein, which drains into the vena cava. Bile is secreted by the hepatocytes into the bile canaliculi, which run between the plates of hepatocytes, and flows toward the portal triad, to eventually drain into the bile duct and the gall bladder, where it is stored before expulsion into the digestive tract.

The liver is the largest metabolic organ in the body, and performs numerous vital functions including metabolism of carbohydrates, proteins, and lipids, detoxification of the waste products of metabolism, drugs, and toxins, the production of an array of plasma proteins (such as albumin and clotting factors), storage of various substances including glycogen, lipids, and a number of vitamins, the activation of Vitamin D, and the excretion of cholesterol and bilirubin. For a construct to be engineered suitable for implantation, ideally all of these functions should be retained. However, hepatocytes in culture have a particularly short life span, typically lasting no longer than a couple of weeks, and rapidly losing their differentiated function. For this reason much research has been focused on extending the life span of hepatocyte cultures, and maintaining their differentiation.

Culturing hepatocytes on extracellular matrix constituents has been shown by several investigators to improve hepatocyte adhesion, spreading, and maintenance of the hepatocyte phenotype for prolonged periods. Matrices that have been tried include individual matrix components such as fibronectin, laminin, and collagens type I and IV, along with complex mixtures of extracellular matrix such as liver biomatrix (from regenerating and normal livers) and that derived from the Engelbreth-Holm Swarm sarcoma. Each component has been shown to have differential effects upon the hepatocyte biochemistry. Efforts to mimic the polarity of hepatocytes *in vivo* by culturing them between two layers of matrix, e.g., collagen, have shown striking effects on the morphology and function of the liver cells including enhanced albumin secretion, improved cytochrome P450 induction responses, bile acid transport, and formation of gap junctions and bile canalicular networks. Long-term maintenance of the normal polygonal morphology and cellular distribution of actin filaments seen *in vivo* has also been observed. However many of these culture systems such as the EHS matrix, in

addition to enhancing hepatocyte differentiation, suppress proliferation. Mooney and his coworkers determined that the density of the extracellular matrix constituents is the critical factor determining whether or not hepatocytes proliferate or differentiate.
The differentiated function of hepatocytes may also be maintained by coculturing them with another cell type. Guguen-Guillouzo's group demonstrated this using liver epithelial cells as early as 1983, but it has since been shown to be an effect of several different cell types, even those from different species. This may not be surprising given the fact that the liver contains several different cell types *in vivo*. Cocultures have been shown to prolong albumin secretion, cytochrome P450 activity, gap junction formation, lipid synthesis, and glutathione-S-transferase activity as well as to improve cell viability and morphology. At least part of this effect may be due to matrix deposition by the cultured cells, although secreted factors and cell surface moieties may also play a role.
Proliferating a relatively small population of cells to a size suitable for production of a bioartificial construct is central to the concept of tissue engineering, particularly if autologous cell sources are to be used. This should also help prolong the life of the hepatocyte cultures. Several growth factors have now been identified, many through their involvement in the processes of liver regeneration and development. These include hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor-α (TGF-α), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). Some growth factors, for example HGF, also have morphogenic and motogenic effects upon hepatocytes (and on a variety of other cells types), and may therefore be important in establishment of normal liver structure. Michalopoulos and his research group showed in 1999 that hepatocytes cultured on collagen type I together with hepatic nonparenchymal cells, in the continuous presence of both EGF and HGF, not only proliferate but also rearrange into structures reminiscent of the liver acinus, including the formation of ducts and an extensive bile canalicular network. Use of either of the growth factors alone was not sufficient to produce these effects.

Hepatocytes are anchorage-dependent cells, however, when attachment to a surface is prohibited, hepatocytes aggregate together forming spheroids of cells that exhibit enhanced cell survival, and maintain many differentiated cell functions. It is thought this is due to the presence of a three-dimensional architecture, the laying down of extracellular matrix by the cells in the spheroid, and the establishment of important cell-cell contacts. It is apparent therefore that the cells themselves can carry out a certain degree of structural organization. However, the construction of large portions of physiological tissue remains difficult. To encourage this, attempts have been made to grow hepatocytes on polymer scaffolds. Several different types of scaffold have been tried including polymer films, porous polymer sponges, temperature responsive polymers, hydrogels, ligand-modified dendrimers, and microcarriers. Culture in bioreactors that subject the cells to a microgravity environment has also been tried, in an attempt to encourage the cells to grow in three dimensions. Khaoustov and his coworkers found in 1999 that hepatocytes grown in microgravity formed spheroidal aggregates up to 1cm long, and addition of 5 mm x 2 mm polyglycolic acid scaffolds created even larger aggregates, up to 3 cm long. Structural analysis of these aggregates showed cell arrangement around bile duct-like structures, and the hepatocytes continued to secrete albumin for the whole 60-day culture period. Considerable advances in maintaining hepatocyte function and viability have been made, and small three-dimensional aggregates of these cells can be formed. However, establishment of the full

liver architecture remains elusive at present. Increasing the complexity of the culture systems using growth factors, extracellular matrix constituents, appropriate scaffold materials, and coculture systems aims to resolve this issue.

4. Kidney

The kidney is responsible for the excretion of nitrogenous waste, principally urea, from the blood. The active units of the kidney are nephrons, which are located within the cortex and medulla. They are able to process the blood via ultrafiltration under pressure allowing the excretion of toxic metabolic waste products and the reabsorbtion of water, glucose, and electrolytes back into the blood. In addition to these important homeostatic controls, this organ has a critical role to play in the metabolism of growth factors and cytokines along with the formation and regulation of Vitamin D and erythropoetin.

Patients suffering from acute and chronic renal failure have only haemodialysis or haemofiltration as renal substitutes. These methods rely on the use of synthetic membranes that selectively filter out molecules of a specific size. This method of dialysis replaces the function of the glomerulus only, which means that this therapy is unable to offer the important transport, metabolic, and endocrinologic function of the kidney normally provided by the tubule. Although these systems are well established, they fail to provide equivalent function and continue to have high morbidity and mortality rates. For these reasons kidney dialysis is only seen as a temporary replacement for a kidney until a suitable donor can be found. As with most tissues, donor organs are in limited supply, and transplants are not without complications and graft failure.

The delicate and highly complex processes of the kidney present tissue engineers with some difficult challenges in attempting to recreate this tissue. A number of differing approaches have been investigated to optimize renal substitution therapy.

4.1. The Bioartificial Kidney

To provide the functions of fluid/electrolyte homeostasis, metabolism and hormone secretion researchers have looked at combining traditional therapy with a bioartificial renal tubule assist device (RAD). The first single-fiber device, developed by Mackay and colleagues in 1998, incorporated canine renal epithelial cells, which were cultured on the inner surface of a hollow nonbiodegradable polymer fiber that was permeable to water and solutes. The polymer (polysulphone) was first coated with laminin, an extracellular matrix protein that is the major component of the renal tubule basement membrane and encourages the growth and attachment of the cells. The renal cells were then flushed through the fiber and cultured to confluency to form a lumen (Figure 2).

Following this key research, tissue engineers in the same lab have managed to scale up this device to form a multifiber construct seeded with porcine renal tubule progenitor cells. This device was found to have differentiated reabsorption and secretory transport capabilities and the ability to provide both metabolic and endocrinologic functions in the form of ammonia excretion, glutathione reclamation, and 1,25-$(OH)_2$D3 production.

The use of these bioartificial renal tubule assist devices in series with an extracorpeal hemofilter, which perform the functions of the nephrons, can provide the full renal functions of the kidney. Further improvements of the system using human kidney proximal tubules allowed the phase I clinical trials of this system in patients to begin in March 2002

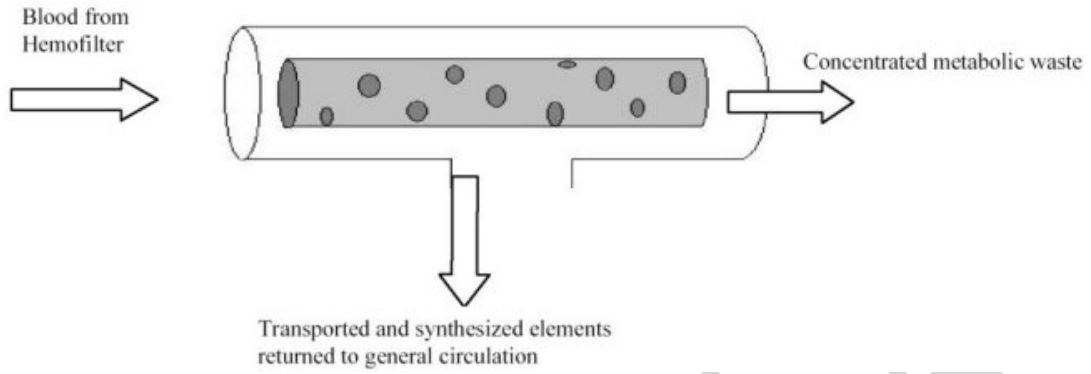

Figure 2. Single, hollow fiber bioartificial renal tubule. Renal tubule cells line porous polymer fibers, providing transport and metabolic function.

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The use of RAD in the clinical setting will have significant affect on the survival of patients with renal failure. This therapy, however, is still essentially an improvement of the current dialysis system and will continue to provide kidney function in a temporary setting. Long-term treatments require the permanent replacement of kidney functions, which is the aim of current research under way to engineer the whole organ.

4.2. Tissue Engineering a Kidney

Attempts to tissue engineer a complete kidney are not hindered by the retention of their function in culture, as isolated renal cells are able to retain their phenotype. It is the limited diffusion of nutrients and oxygen to large tissue volumes that presents the greatest challenge. In engineering a complete kidney organ, tissue engineers are using biodegradable scaffolds as a cell-support matrix. This porous matrix should encourage cell attachment and proliferation while allowing the free diffusion of nutrients. In 1996 both Yoo and Fung published papers demonstrating the seeding of polymer scaffolds with distal tubule, golmeruli, and proximal tubule cells and the ability of the cells to organize into nephron segments including reconstitution of proximal tubules, distal tubules, loop of Henle, collecting tubules, and collecting ducts. These constructs were found to excrete uric acid and creatinine in a yellow urine-like fluid. Further modifications of scaffolds to those from a naturally derived acellular collagen matrix have shown kidney cell proliferation and reconstitution of both renal tubular and glomerular-like structures. This natural matrix is identical to that found *in vivo* and therefore contains all necessary protein components specific for the kidney cell types.

The kidney was the first whole organ that a medical device was able to replace temporarily. Advances have improved renal substitution therapy immensely from the time of the first kidney dialysis more than 40 years ago. The growth of an entire organ *in vitro* is a challenging task for such a complex tissue with a vast array of functions.

While there has been progress, engineers agree it may be some time before the products of these findings are used in clinical practice and a cultured organ is ready for use in human transplants.

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Biographical Sketches

Lisa Riccalton-Banks graduated from Manchester University in 1998 with a B.Sc. (Honors) Pharmacy degree and is a qualified pharmacist with the Royal Pharmaceutical Society of Great Britain. Lisa joined the Tissue Engineering group at the Institute of Pharmaceutical Sciences, University of Nottingham, UK, to study for a Ph.D. in engineering liver tissue. Lisa is looking at the importance of cell-to-cell communication in the maintenance of hepatocyte function.

Andy Lewis graduated from Nottingham University with a B.Pharm. (Honors). He registered as a pharmacist in 1998 and worked as a pharmacist for a few months before going traveling around Southeast Asia, Australia, and New Zealand. While in Australia, he worked for three months in Professor Mike Roberts' group at the University of Queensland in Brisbane, studying hepatic fatty acid uptake. He returned to start work on his Ph.D. at Nottingham within the Tissue Engineering group. Working within the Liver Group, Andy's work focuses on ways of expanding a small population of liver cells to allow the engineering of liver tissue.

Kevin Shakesheff is Professor of Tissue Engineering and Drug Delivery at the University of Nottingham. His group is developing new methods of engineering liver, nerve, cartilage, muscle, and bone tissue for drug screening and medical applications. He has previously held fellowships at MIT (NATO) and Nottingham (EPSRC).