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Tissue Engineering

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INTRODUCTION

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain, or improve tissue function. The potential impact of tissue engineering from both a therapeutic and an economic standpoint is enormous. The total U.S. health care costs for patients suffering from tissue loss or organ failure exceed $400 billion annually. Approximately 8 million surgical procedures are performed annually in the United States to treat these disorders and 40–90 million hospital days are required (1). It has been estimated that the total market for tissue-engineered products in United States is $80 billion annually (2).

One of the earliest demonstrations that engineering a tissue may be possible was by Bisceglie in the 1930s, in which he encased mouse tumor cells in a polymer membrane and inserted them into a pig's abdominal cavity (3). These studies showed that cells could survive and not be destroyed by the immune system. This approach was an early example of cell encapsulation, which allows nutrients and wastes to diffuse through membranes, yet prevents immune cells or large molecules such as antibodies (which might destroy the encapsulated cells) from entering. Therefore, the cells can survive. This approach was later used in the 1970s by Chick and co-workers to create semipermeable membranes in which islet cells were encapsulated to aid in glucose control for diabetes in animal models (4).

In the late 1970s and 1980s, cells on sheets of collagen, or collagen–glycosaminoglycan composites, were used in tissue regeneration in an attempt to create new skin (5, 6). These sheets were two-dimensional systems, and the materials composing them were naturally occurring, i.e., isolated from animal or fish sources.

The next critical step was creating three-dimensional structures that enabled large numbers of cells to be housed, required for creating tissues in three dimensions. In addition, scaffolds composed of chemically produced synthetic polymers were introduced and provided significant advantages in tailoring such properties as strength, degradation rates, and incorporation of cell-recognition properties. Liver cells were used in early studies of this approach (7), but more than 20 tissue types have been studied subsequently, some of which are discussed below.

The strategy of tissue engineering generally involves the following steps:

(i) An appropriate cell source must be identified, isolated, and produced in sufficient numbers.

(ii) An appropriate biocompatible material that can be used as a cell substrate (open system) or cell-encapsulation material (closed system) must be isolated or synthesized and manufactured into the desired shape and dimensions.

(iii) The cells must be uniformly seeded onto or into the material and grown in a bioreactor.

(iv) The engineered structure is placed into the appropriate *in vivo* site. Depending on the site and the structure, vascularization may be necessary.

TISSUE ENGINEERING: CURRENT STATUS

Scientists have attempted to engineer tissues or organs in nearly every part of the body. For example, in brain diseases where dopamine production is lost, e.g., Parkinson's, immortalized PC12 cells have been encapsulated in semipermeable polymer membranes and shown to release dopamine for more than 6 months in animal models of this disease (8).

Several approaches have been used to aid in nerve regeneration as well. For example, Schwann cells derived from sciatic nerves have been placed in Matrigel and seeded in polymer membranes (9). In another case, electrically conducting polymers such as polypyrole have been used to stimulate nerve outgrowth. When PC12 or Schwann cells are placed on such polymers and a small voltage (100 mV) is applied, neurite outgrowth is enhanced (10).

Another tissue under investigation is the cornea. In these studies, corneal epithelial cells were placed on polyvinyl alcohol hydrogels and transplanted into rabbit corneas. The gels adhered to the cornea and the cells proliferated for up to 2 weeks (11).

Critical issues in creating an implanted liver include enhancing cell survival, maintaining differentiated function, developing a significant cell mass, and achieving vascularization. To these ends, several different approaches have been followed. In one case, hepatocytes placed on polymer scaffolds and implanted into animals were shown to produce albumin and other liver function markers. They also cleared products of bilirubin and urea metabolism, similar to a normal liver (12). In a related approach, hepatocytes were sandwiched between two hydrated collagen layers. In addition to maintaining their morphology, the hepatocytes secreted functional markers at physiological levels for at least 6 weeks (13).

Tissue-engineered livers have been studied not only as implants but also as extracorporeal units. In one example, porcine hepatocytes are placed in hollow fiber membranes and connected to a patient. This approach, which is now in advanced clinical trials, has been used as a "bridge to transplant" on nearly 100 patients waiting for a new liver (14).

There have been several attempts to create an artificial pancreas. The most common approach has been encapsulating pancreatic islet cells inside a semipermeable

membrane such as alginate or polyacrylonitrile–polyvinyl chloride (PAN–PVC) (15). This has shown some success in animals and a few attempts have been made in humans. One critical issue is the biocompatibility of the membrane used, as fibrous encapsulation of the membrane can prevent reproducible insulin outflux. A second issue is islet sourcing on a sufficiently large scale.

Urinary structures have been created by several methods. In one of these, urinary epithelial cells have been placed on lactic–glycolic acid copolymer tubes to create new ureters as a potential treatment for hypospadius (16). In another, autologous chondrocytes have been delivered endoscopically to treat urinary reflux in children and urinary stress incontinence in women (2). Both of these studies are in advanced clinical trials in humans. One of the first attempts to create an actual whole urinary organ involved constructing polymer composites in which bladder cells are placed, an approach that has created new bladders in dogs (17).

Much effort has focused on creating tissue-engineered blood vessels. In one recent study, the bioreactor conditions under which the tissue-engineered blood vessels are grown were shown to be essential to this process. By placing vascular smooth muscle cells and, subsequently, endothelial cells on a polymer tube and growing them under pulsatile conditions (similar to what happens in the body when a heart beats), the cells make significantly more collagen and are physically stronger than blood vessels produced in static tissue culture. In addition, the pulsatile-engineered blood vessels possess pharmacological properties similar to normal vessels. When sutured into pigs, the vessels were shown to be patent for up to a month, the duration of the study (18).

Several studies have focused on cartilage, which has been produced by placing chondrocytes on lactic glycolic acid copolymer scaffolds and transplanting them into several different animal models (19, 20). The reactor conditions under which the cells are grown *in vitro* are critical to successful treatment. By mixing the cells appropriately during seeding, as well as during cultivation, the cells can be made to produce desirable amounts of glycosaminoglycans and collagen, molecules that are important to the eventual mechanical strength of the cartilage tissue (21).

Creating heart components is the aim of several different studies. Tissue-engineered heart valves have been created in which endothelial cells and fibroblasts were coseeded on polymer scaffolds that formed in the shape of a valve. When these engineered valves are placed into sheep they appear to be functional, as judged by ultrasound imaging, for up to 6 months (the duration of the study) (22). Heart muscle cells have also been placed on polymer scaffolds and grown under different reactor conditions with various growth media. The resultant engineered structures beat like a normal heart and demonstrate biochemical and pharmacological characteristics similar to normal heart muscle (23).

To create a tissue-engineered tendon, tenocytes were isolated by enzymatic digestion of tendons and grown on fibrous polymer pieces *in vitro* and then transplanted subcutaneously into mice. After 6 weeks, all experimental specimens resembled the normal calf tendons from which the cells had been isolated, as judged by gross examination. Histologic evaluation demonstrated organized collagen fibrils with polymer remnants. Ten weeks after implantation, histologic evaluation showed parallel linear organization of collagen bundles throughout the specimens, centrally and peripherally. Mechanical analysis of neotendon constructs after 8 weeks shows that they have tensile strength comparable to and mechanical characteristics similar to those of the normal tendon (24).

Major resection of the small bowel often leads to a state of malabsorption and malnutrition known as "short bowel syndrome." Parenteral nutrition improves survival, but prolonged hyperalimentation can often lead to infectious complications, loss of vascular access, and progressive liver disease. To examine whether tissue engineering could produce a new intestine, cell–polymer (polyglycolic acid) constructs were created using enterocytes isolated from crypt cell-enriched fractions of intestine which were allowed to attach to the polymer scaffolds *in vitro*. Morphological analysis by phase-contrast microscopy of *in vitro* constructs indicated that the cell populations were composed almost exclusively of round-, oval-, and columnal-shaped cells as well as distinctive goblet cells. Rare small round cells characteristic of lymphocytes were also observed. These cell–polymer constructs were subsequently implanted in animals and, after 14 days, enterocytes were observed organizing into a stratified epithelium overlying the polymer fibers and granulation tissue. Successful engraftment occurred 86% of the time (25).

More than one million operations annually involve bone repair. Conventionally, bone "ingrowth" is accelerated through the use of either autogenous bone grafts or allogenic bone. The first can be a successful procedure, but is often material-limited and can cause donor site morbidity and contour irregularities. The second can also be successful, but cell-mediated immune responses to transplantation alloantigens and pathogens can be problematic. To address this problem via tissue engineering, synthetic biodegradable polymers have been used as templates onto which cells (osteoblasts or osteocytes) are seeded prior to implantation. Specimens at 6 weeks are primarily composed of cartilage, with islands of osteoid tissue seen peripherally associated with blood vessel invasion. At 10 weeks, these implants show increasing amounts of new bone growth. Examination at 20 weeks reveals gross bone formation in all specimens based on osteoblast and osteocyte activity. Histological evaluation with hematoxylin and eosin stains of such specimens revealed that they were composed of trabeculated bone enveloping islands of residual cartilage. Representative of endochrondral bone formation, these basophilic areas of residual cartilage contained hypertrophied cells. It is also significant that specimens demonstrating organized bone formation at 20 weeks contained a hypocellular bone marrow. In addition, viewing these specimens under polarized light revealed the presence of lamellar bone (26).

Finally, tissue engineering has implications for gene

therapy. One critical issue for gene therapy is having a large enough number of cells expressing desired gene products. In one study the human growth hormone (hGH) gene was transfected into rat hepatocytes to see if it was possible to demonstrate *in vivo* expression of hGH following transplantation. To accomplish this, the optimal conditions for *in vitro* gene transfer were first defined in rat hepatocytes. Then, to study these cells *in vivo*, a portacaval shunt was performed on rats by ligation of the portal vein and creation of an end side to side anastomosis with the vena cava. Seven days later, polymer sponges were implanted into the subcutaneous tissue or mesentery. The sponges were vascularized over 5 days, and then the transfected hepatocytes were injected into the sponges (27).

Histological examination of tissue sections from the cell–polymer construct was performed at different time points following hepatocyte implantation. Organized plates of viable hepatocytes were seen as late as 15 days postimplantation filling the spaces of the prevascularized polymer device. In animals containing implants with the transfected cells, serum hGH levels, which were undetectable prior to cell injection, were found to be within the human physiological range on day 2 postimplantation, but fell thereafter. Although this early study demonstrates that tissue-engineering approaches may be useful in assisting in gene therapy, clearly more research is needed (29).

FUTURE CHALLENGES

Several significant challenges still face tissue engineering. One of these is identifying reliable cell sources. The recent development of human embryonic stem cells potentially provides one important approach to addressing this problem (28). Some of the critical issues in this area involve understanding how to control the differentiation of stem cell populations into the desired cell types, for example, liver, cartilage, or others. There are also a variety of technical challenges such as creating pure cells and processes to create large populations of such cells to make desired tissues.

Another major challenge is the creation of universal donor cells that would not be rejected by the body. One interesting approach involves using molecules that could mask the histocompatibility proteins on the surface of cells that normally cause the donor cells to be recognized as foreign. This type of approach is being studied using porcine cells for transplantation to patients with Parkinson's disease (27).

Another challenge is developing the appropriate bioreactors to affect desired mass transport or impart mechanical characteristics to the cells. One approach has involved designing bioreactors that have appropriate mixing to achieve uniform cell seeding. Mixing is also important to create engineered tissues because it aids in the transport of nutrients and wastes during tissue cultivation and growth (30).

Regulation of cell behavior represents another important challenge. For example, it has been shown that hepatocytes can produce different levels of particular proteins depending on the adhesion of the cells to the material on which the cells are grown (31). To develop tissue-engineered organs, it is important to understand how to grow the cells of that organ under conditions that maximize the ability of these cells to perform their physiological roles. Understanding remodeling is also important for creating tissues.

Another major area of study is the creation of materials for tissue engineering. Of particular value is the creation of synthetic materials that have appropriate strength, degradation times, microstructure, permeability, and the ability to contain cell recognition properties that can be used to regulate cell growth and adhesion. One way that this is being attempted is to synthesize polymers in such a way that desired regulatory molecules such as specific amino acid sequences that can regulate cell behavior, for example, R-G-D, can be grafted onto them (32).

Still another challenge is the ability to induce vascularization of a tissue. One approach being explored involves the use of controlled release of growth factors such as epidermal growth factor (33). By placing growth factors in polymer microcapsules or scaffolds, the growth factor can be steadily released for weeks, enhancing local vascularization.

In addition, the design of novel polymer structures through manufacturing techniques such as three-dimensional printing, which can build in complex vascular structures, is being explored (34). Another challenge involves cyropreservation so that tissues or organs can be kept frozen until needed. This is particularly important for more complex and larger tissues.

In summary, tissue engineering has enormous potential, yet many challenges remain. Nonetheless, several tissue-engineered skins have received FDA approval and many other tissues as discussed above are in advanced stages of clinical trials. It is hoped that as the years progress, tissue engineering will be able to be used not only to create new tissue structures but also as vehicles in which genes can be placed to deliver large numbers of cells to create desired gene products, thereby aiding gene therapy as well.

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