SPECIAL ARTICLE

Tissue Engineering

Toward New Solutions for Transplantation and Reconstructive Surgery

Satoshi Kaihara, MD; Joseph P. Vacanti, MD

Great advances in the field of transplantation have been made in the last half of this century. However, the severe scarcity of donor organs, especially in the pediatric population, has become a major limitation. A new field, tissue engineering, applies the principles of biology and engineering toward the development of biological substitutes that restore, maintain, or improve tissue function. This article discusses the groundwork and challenges of this interdisciplinary field and its attempts to provide solutions to create new tissue for transplantation and other fields of reconstructive surgery. *Arch Surg. 1999;134:1184-1188*

Millions of people in the United States are affected by organ and tissue loss every year from accidents, birth defects, and diseases. In the last half of this century, innovative drugs, surgical procedures, and medical devices have greatly improved the care of these patients. There have been great advances made in the past 40 years, especially in the field of transplantation, and a wide variety of organs are clinically available. Major challenges, however, limit the applicability of organ transplantation; these include the critical shortage of donor organs, the high cost and technical difficulty of the procedures, and the intensive postoperative care.[1,2] For example, the supply of donor organs for liver transplantation has increased only slightly during the past 5 years in the United States, while the number of patients on the waiting list and the number who die each year still waiting for a donor organ have continued to grow at a disproportionate rate.[3] Even when organs are available, the cost of the whole organ transplantation is high.[3] Great progress has been made in preventing the rejection of allografts; however, there remain difficulties such as increased risk of adverse effects, including infection and new tumor formation, associated with lifelong immunosuppression.

From the Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston (Drs Kaihara and Vacanti); and the Department of Transplantation Immunology, Faculty of Medicine, Kyoto University, Kyoto, Japan (Dr Kaihara).

These shortcomings have stimulated investigation into selective cell transplantation instead of entire organ transplantation because the approach has many potential advantages.[1,4,5] If functional tissue could be reconstructed in vitro using cell transplantation, it would alleviate the donor organ shortage by using cells from a small amount of donor tissue and expanding them in vitro to create a potentially limitless supply. The risk and expense associated with major surgical procedures and protracted hospitalization would be decreased as well. In some applications, it may also be possible to avoid the need for immunosuppression with autologous cells for transplantation. Cells isolated from a patient and expanded in vitro may be modified by gene therapy to replace a defective gene and reimplanted.[6]

During the past 10 years, our laboratory has been investigating the fabrication of functional tissue, or tissue engineering. *Tissue engineering* is defined as an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function.[4,7] We have been using synthetic biodegradable polymer scaffolds as delivery vehicles for cell transplantation. This approach is based on the behavior of tissue and cells: (1) every tissue undergoes remodeling; (2) isolated cells tend to reform the appropriate tis-

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sue structure under appropriate conditions; (3) although isolated cells have the capacity to form the appropriate tissue structure, they do so only to a limited degree when placed as a suspension without intrinsic organization or a template to guide structure formation; and (4) tissue cannot be implanted in large volumes because of diffusion limitations.[7] These polymer scaffolds allow cells to be delivered and immobilized in a given location, serve as a template for tissue development before and after cell transplantation, and provide a space for cells to reorganize into higher-order structures. The polymer scaffolds eventually resorb, avoiding a long-term foreign body response. Synthetic polymers can also be synthesized reproducibly in varying sizes and shapes. Using these devices, we have investigated many tissues and organs.[8-16] Although there are differences in the process of yielding functional new tissue with the different tissue types, the basic concept is similar. In the subsequent sections, we discuss the engineering of liver and small intestine. Based on the promising results, clinical applications have been formulated and are undergoing rigorous investigation in an effort to achieve permanent replacement of lost organ function.

LIVER

Despite the major advances in the fields of immunology and transplantation, 26 000 people die of end-stage liver disease each year in the United States, with an estimated annual cost of $9 billion.[17] Unlike patients with kidney or other organ failure, liver transplantation is the only established successful treatment for end-stage liver disease, and more than 3000 liver transplantations have been performed annually in the United States; however, organ donor shortage has been a major limitation and has stimulated investigation into selective cell transplantation.[3]

Various approaches have been used in the past to transplant hepatocytes. Nearly every organ system has been chosen for the transplantation site, including the liver, portal venous system, spleen, peritoneal cavity, small-bowel mesentery, omentum, pancreas, nephric capsule, lung, and subcutaneous tissue, in various animals.[8,13,18-35] These approaches also include injection of cells, encapsulated cells, or cells attached with microcarrier beads.[8,36-41] However, these approaches have met with limited success.

There are many important properties that should be incorporated into the development of hepatocyte implantation, according to established characteristics that hepatocytes should fulfill after implantation. Primarily, hepatocytes are anchorage-dependent cells and require an insoluble extracellular matrix for survival, reorganization, proliferation, and function. Second, hepatocytes are highly metabolic cells and require rapid access to oxygen and nutrient supply. Finally, hepatocytes have a tremendous regenerative capacity with hepatotrophic stimulation in vivo.[42,43] Based on these characteristics, we have been investigating hepatocyte transplantation using synthetic, highly porous, biodegradable polymer scaffolds as a novel approach to the treatment of end-stage liver disease. The biodegradable polymer scaffolds serve as a template to guide cell organization and growth. The microporous structure allows diffusion of oxygen and nutrients to and removal of waste from the implanted cells. This structure also provides a space for hepatocyte reorganization and neovascularization from surrounding tissue. As the cell-polymer constructs become incorporated into the recipient, the polymer scaffolds resorb, leaving behind only the new natural tissue. In early studies[8,13,42-45] using highly porous biodegradable disks, we demonstrated the survival of hepatocytes transplanted in a peripheral site and in small-intestine mesentery, the improvement in survival of transplanted hepatocytes with hepatotrophic stimulation after portacaval shunt and partial hepatectomy, and a prevascularization method to improve hepatocyte engraftment and survival. The implanted hepatocytes also exhibited partial correction of single-enzyme liver defects.[46]

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Figure 1. *A, Polymer tube made with polyglycolic acid fiber. The scale is in centimeters. B, Scanning electron micrograph of the polymer (original magnification ×100). Reprinted with permission from Lippincott Williams & Wilkins, Phialdelphia, Pa.*

One of the major problems of this approach was the insufficient engraftment and survival of an adequate mass of transplanted cells to replace defects in liver function. The critical limitation of oxygen and nutrient diffusion during the initial period after implantation and until the development of neovascularization has effects on the engraftment and survival of the implanted hepatocytes. To overcome this problem, we have been investigating 2 different approaches. One approach is the development of polymer devices that can be implanted directly into the bloodstream. For this purpose, we have been using polymer tubes constructed of nonwoven fiber meshes of polyglycolic acid (**Figure 1**). These polymer scaffolds are microporous, which allows hepatocytes direct access to blood and induces optimal nutrient-waste exchange. They also have a large surface area, allowing greater hepatocyte engraftment than the previous 2-dimensional polymer disk. The isolated hepatocytes were initially attached on polymer devices and continued to

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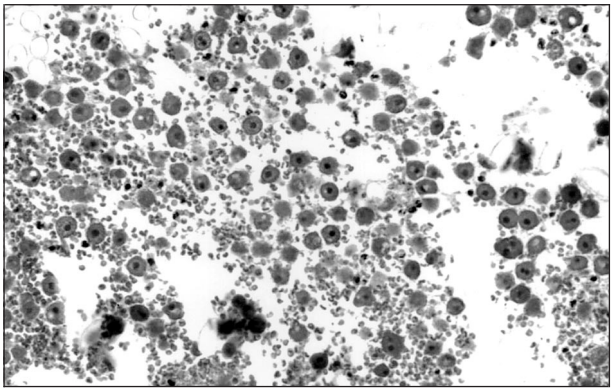

Figure 2. *Hepatocytes on polymer scaffolds 2 days after implantation (original magnification ×400).*

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Figure 3. *Scanning electron micrograph of the hepatocytes attached to the 3-dimensional printing biodegradable polymer scaffolds (original magnification ×300). Reprinted with permission from Lippincott Williams & Wilkins, Philadelphia, Pa.*

express liver-specific function in vitro.[47] Since portal blood contains many hepatotrophic factors, these cell-polymer constructs were successfully implanted into a vascular conduit perfused with portal blood (**Figure 2**).[48] This project is ongoing, and in preliminary experiments, hepatocytes survived in portal blood 2 days after implantation.[48]

The other approach is the development of a polymer device with an integrated vascular network to provide immediate access to the blood supply after implantation. Using a 3-dimensional printing (3DP) fabrication technique that was developed by investigators at the Massachusetts Institute of Technology, Cambridge, we have been able to design and fabricate complex, 3-dimensional, synthetic, biodegradable polymer scaffolds. The polymer scaffolds have an intrinsic network of interconnected vascular channels, and this technique allows the fabrication of polymer scaffolds in any shape or size with a high degree of macroarchitectural and microarchitectural complexity (**Figure 3**).[49-51] In initial experiments using scaffolds seeded with hepatocytes, the implanted cells attached and survived under dynamic culture conditions in vitro, and albumin synthesis by the hepatocytes was demonstrated.[14] The cells also reformed histiotypical structures in the channels of the polymer devices.[52] These results suggest great potential not only for providing immediate access of the implanted hepatocytes to the blood supply but also for the capacity to fabricate devices for a large mass of cells within a highly structured environment.

INTESTINE

Short-bowel syndrome is a clinical condition characterized by malabsorption and malnutrition after massive small-bowel resection. With the development of total parenteral nutrition, many patients may survive for an extended period; however, total parenteral nutrition is accompanied by various complications, such as hepatic dysfunction, progressive nephric insufficiency, bone demineralization, and catheter sepsis. The annual mortality is estimated to be 2% to 5%.[53-56] On the other hand, numerous attempts at bowel lengthening or slowing intestinal transit to increase the absorptive time have been made for patients with short-bowel syndrome; however, none have been considered routinely successful.[57,58] Recently, small-bowel transplantation has been undertaken as a therapy for patients with short-bowel syndrome. However, it has been limited because of the difficulties in controlling rejection, immunosuppression-related complications, and donor supply.

Using the principle of tissue engineering, our laboratory has investigated the transplantation of intestinal cells using synthetic biodegradable polymer scaffolds to generate new intestinal tissue as an alternative approach to the treatment of short-bowel syndrome. In our initial studies,[8] fetal intestinal cells seeded on polymer tubes formed vascularized cysts with a well-differentiated intestinal epithelium lining with mucous secretion. Subsequently, crypt stem cells isolated from adult rats were transplanted onto biodegradable polymer scaffolds to generate stratified epithelium, reminiscent of embryonic gut development; however, the neomucosa was not well differentiated compared with native small intestine.[59,60] Recently, we have been using crypt cells as an epithelial-mesenchymal unit called the intestinal epithelial organoid unit. This unit consists of a villous structure with an overlying epithelium and core of stromal cells and preserves the epithelial-mesenchymal interaction thought to be important for normal organ development. A tube created of nonwoven polyglycolic acid fibers was chosen as the polymer template because the openness of the devices would allow nutrient-waste exchange between implanted cells and surrounding tissue. The organoid units seeded on polymer scaffolds survived, vascularized, proliferated, and formed cystlike structures after implantation.[15] The inner lumen was lined with a well-developed neomucosal layer characterized by crypt-villous structures, and it was surrounded by smooth muscle.[15] The neomucosa expressed brush border enzymes, basement membrane proteins, and electrophysiologic properties similar to normal small intestine.[16] The morphogenesis and differentiation of the tissue-engineered neointestine were stimulated with massive small resection and, to a lesser extent, portacaval shunting.[61] We also demonstrated that the anastomosis between tissue-engineered neointestine and native small bowel was successful and that the anastomosis had a positive effect on the development of the neointestine (**Figure 4**).[62-64] We are now focusing on the functions, such as absorption, wall motility, and neural innervation, of the tissue-engineered neointestine.

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Figure 4. *Tissue-engineered neointestine with anastomosis to native small bowel 10 weeks after implantation. A, Outer surface. B, Inner lumen. C, Histological characteristics of the neointestine and anastomosis. The arrow indicates the anastomotic site; to the left of the arrow is tissue-engineered intestine and to the right is native small bowel (original magnification ×100). Reprinted with permission from Lippincott Williams & Wilkins, Philadelphia, Pa.*

HEART VALVE AND CARTILAGE

Valvular heart disease is a major cause of morbidity and mortality in the United States. One solution to this problem has been valve replacement. However, while effective, prosthetic valves have limitations, and no ideal exists for the pediatric population. To overcome these problems, we have been investigating the fabrication of tissue-engineered heart valve leaflets. Some potential advantages of using a tissue-engineered heart valve created from autologous cells include the capacity for normal repair and growth, greater durability provided by a living structure, and biocompatibility of the tissue with minimal risk of infection and thromboembolism. In previous studies,[9,10] component cells of the normal heart valve were harvested and seeded onto a highly porous biodegradable polymer mesh in the shape of a valve leaflet. After in vitro culture, the leaflet was implanted back into the lamb from which the cells were harvested, replacing one of the pulmonary valve leaflets. These studies[27,28] demonstrated appropriate function of the tissue-engineered leaflet, as determined by echocardiography, up to 11 weeks. Future investigations will be directed toward evaluating the long-term durability of the leaflets in vivo and tissue engineering an entire heart valve that can be used for replacement for diseased heart valves.

The fabrication of cartilaginous tissue is one of the most successful areas of tissue engineering. Previous studies[65-69] in our laboratory have investigated the fabrication of cartilaginous tissue in the shape of a human ear, a temporomandibular joint disk, nasoseptal implants, meniscal tissue, and tracheal replacement tissue.

SUMMARY AND FUTURE DIRECTIONS

With the continued critical scarcity of donor organs, tissue engineering offers tremendous potential for alleviating the limitations of current therapy. Various cell types have been transplanted using biodegradable polymer devices, and appropriate tissue structures formed following cell reorganization. Although there are differences between each tissue, many common elements exist regardless of the cell type. In all cases, implanted cells receive signals to guide their appropriate development from the polymer devices, the surrounding tissue, and the cells themselves. However, tissue engineering of the visceral organs such as liver, small intestine, and kidney is more challenging than tissue engineering of other tissues such as bone, cartilage, bladder, and skin because of their complicated structures and many functions. Further advances in the area of biomaterials and chemical engineering may provide better polymers that can direct cell growth, maintain differentiated function, and develop higher-ordered tissue structure from originally disorganized cells. Regardless of the tissue type, the cell source and cell expansion may be other important issues in tissue engineering. For example, the challenge of maintaining in vitro function and survival in hepatocytes makes them the most difficult cell type. It is important to develop the appropriate cell source and culture conditions for the success of tissue-engineered liver. A great effort is under way to isolate and identify the characteristics of stem cell populations for various tissues.[70-72] The use of stem cells may supply an almost limitless supply of cells for transplantation; however, it will first be necessary to establish the isolation and culture methods and confirm the direction of cell differentiation in various culture conditions.

While there are still many important issues to be solved, tissue engineering has been rapidly making progress using a multidisciplinary approach including biology, surgery, and chemical engineering. The success of this approach in animal models will lead to the clinical application of this technology and may ultimately be able to replace lost tissue function.

Reprints: Joseph P. Vacanti, MD, Department of Surgery, Massachusetts General Hospital, Warren 11, 55 Fruit St, Boston, MA 02114 (e-mail: jvacanti@partners.org).

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