Bioreactors for Tissue Engineering— A New Role for Perfusionists?

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Abstract: Tissue engineering is an exciting new area of medicine with rapid growth and expansion over the last decade. It has the potential to have a profound impact on the practice of medicine and influence the economic development in the industry of biotechnology. In almost every specialty of medicine, the ability to generate replacement cells and develop tissues will change the focus from artificial organs and transplantation to growing replacement organs from the patient’s own stem cells. Once these organs are at a size that requires perfusion to maintain oxygen and nutrient delivery, then automated perfusion systems termed “bioreactors” will be necessary to sustain the organ until harvesting. The design of these “bioreactors” will have a crucial role in the maintenance of cellular function throughout the growth period. The perfusion schemes necessary to determine the optimal conditions have not been well elucidated and will undergo extensive research over the next decade. The key to progress in this endeavor will development of long-term perfusion techniques and identifying the ideal pressures, flow rates, type of flow (pulsatile/nonpulsatile), and perfusate solution. Perfusionists are considered experts in the field of whole body perfusion, and it is possible that they can participate in the development and operation of these “bioreactors.” Additional education of perfusionists in the area of tissue engineering is necessary in order for them to become integral parts of this exciting new area of medicine. Keywords: tissue engineering, bioreactors, organ preservation, transplantation. JECT 2003;35:200–202

Tissue engineering is an exciting new area of medicine that has seen rapid growth and development since the mid-1990s. Tissue engineering will have a profound impact on the future of medical practice and will have a positive impact on economic development in biotechnology industry (1). There are potential applications for tissue engineering in almost every specialty of medicine. Early research has focused on the generation of replacement cells. As these cells are formed into tissue structures, eventually organs will be completed. This will change the surgical focus from the use of artificial organs and transplantation, to growing replacement organs.

Tissue engineering can be defined as the “application of the principles of life sciences and engineering in developing biological substitutes for the maintenance, modification, improvement, restoration, or replacement of organ function.” Traditional surgical treatment either enhances the supply of oxygen and nutrients, removes dead tissue, repairs with synthetics, or replaces with transplantation. In the future with tissue engineering, living cells will be replaced to allow the organ to rebuild and function sooner. Using living cells will result in a higher level of tissue function than possible with growth factors or hormones.

Using biologic components imitates the normal tissue building process that occurs during embryological development. Understanding these processes are the key to advances in tissue engineering.

Tissue engineering may have applications in all of the following areas: blood vessels, heart valves, cornea, pancreas, parathyroid gland, GI tract, liver, nerve regeneration, spinal cord injuries, dental implants, skin (wound repair), red blood cell substitutes, kidney, bone regeneration, cartilage, tendons and ligaments, hearing, vision, and artificial womb.

There is a triad of major components identified as essential for tissue engineering. These are cell sources, signaling, and scaffolds.

CELL SOURCES

Sources of stem cells include: adult stem cells and embryonic stem cells. The stem cells can be either autogenic or allogenic. Immature stem cells are able to multiply faster than fully differentiated cells and can differentiate as a function of the environment in which they are placed because of signaling from the extracellular matrix (EM).

A new source of stem cells is fat tissue (2). Because of this readily available source of stem cells, it may be possible to develop tissues and organs from the patient’s own cells, eliminating the current requirements for immunosu-
pression following solid organ transplantation. Another source for cells is bone marrow. Hematopoietic stem cells (HSC) from adult mouse bone marrow can produce cardiac tissue under conditions similar to that identified for embryonic mesoderm to undergo cardiogenesis (3).

**SIGNALLING**

Important signals for differentiation from the EM include: TGFβ, FGF, Homeobox, Hedgehog, and WNT. Mechanical signals also play a role in cell differentiation. Cardiac morphogenesis is controlled by morphogens, which signal the transcription of regulatory proteins. These proteins direct downstream transcriptional events that lead to the synthesis of contractile proteins that eventually become heart muscle cells. Morphogens trigger the same response in stem cells. Adult and embryonic stem cells have identical tissue potential to differentiate. HSC comprise 0.5% of the cells within the bone marrow. Within 10 days of culture, there is a 1000X increase in HSC. Of these cells, 10–20% exhibit a cardiac phenotype. After transplantation into adult mouse hearts, they were found to be grafted into the ventricular tissue and expressed the cardiac-specific marker ventricular myosin light chain-2 (MLC2v) (4).

**SUPPORTS**

The scaffold is the backbone of the tissue construct and determines its architecture. Types of scaffolds include: gels, foams fibers, and membranes. There are a number of different ways to organize the cells. One method is “rolling a monolayer.” Using this technique confluent cultures of smooth muscle can be transformed into a completely biological vascular graft. Another method is seeding on a synthetic or natural scaffold. The scaffolding is used to organize the cells into a matrix that resembles the organ or tissue that it is replacing. Common scaffolding materials are collagen gel, polyglycolic acid (PGA), and aligned collagen substrate. Collagen gel is used to form a tissue construct with biomechanical properties. A polyglycolic acid tubular scaffold (PGA) is made of the same substance as the biodegradable polymers already approved by the FDA as suture materials. Aligned collagen substrate has been used because it enhances cell differentiation.

Another method for cell alignment is microcontact printing and photolithography. Using this technology, three-dimensional structures can be formed as cells can be transferred onto a gel resulting in a patterned surface.

**MICROGRAVITY**

Microgravity adds a new dimension to tissue engineering. This has already been accomplished on four space Shuttle missions. A monolayer of aligned myocytes growing on aligned collagen substrates was used as a template for the fabrication of a multilayered aligned myocardial tissue engineered construct. The purpose for using microgravity was to induce cell-cell interaction to align newly differentiated cells to the template layer, thereby producing a multilayer system of fibroblasts and myocytes with an in vivo-like phenotype.

**CARDIOVASCULAR TISSUE ENGINEERING**

Dr. Michael Sefton, at the University of Toronto, is coordinating a major research effort in cardiovascular tissue engineering called the LIFE Initiative. LIFE is an acronym for Living Implants from Engineering. This is a collaboration of researchers working together to create an unlimited supply of hearts, livers, and kidneys for transplantation. The ultimate goal is the development of a generic heart replacement within two decades.

There are many steps along the way. The first important step is the development of a tissue-engineered contractile myocardial tube that is lined with endothelium and has the potential to become vascularized.

**BIOREACTORS**

Automated perfusion systems termed “bioreactors” will be necessary to maintain the tissues until harvesting. The bioreactor will allow the tissue to grow during the assembly of the myocardial tube constructs. The components of the bioreactor include: a video system to record tissue growth, a computer control module, media transport systems to pump fluid, affect gas transfer, and a temperature control system. Bioreactors are designed to allow for mechanical stretch simulation during culture. Biomechanical training of the constructs will be performed to ensure that critical mechanical parameters are achieved.

The design of these bioreactors will have a crucial role in the maintenance of cellular function throughout the growth period. The perfusion schemes necessary to determine the optimal conditions have not been well elucidated and will undergo extensive research over the next decade. The key to progress in this endeavor will be development of long-term perfusion techniques and identifying the ideal pressures, flow rates, type of flow (pulsatile/nonpulsatile), and ideal perfusion solutions.

**CONCLUSIONS**

Curriculum changes are necessary in perfusion education programs to be an integral part of this exciting new area of medicine. A course in tissue engineering, expanding on many of the subjects outlined in this paper will be required. In addition, a course in biotransport processes,
including the mass transfer of gases, temperature, and nutrients will be needed. Another area of importance is medical infomatics, which covers the acquisition and processing of biological signals. Computer control of these bioreactor perfusion systems is crucial to their economic success.

One of the future benefits of this technology may be pediatric perfusion. With the low level of investment by manufacturers in pediatric perfusion devices, technology in the field has almost come to a standstill. The development of miniaturized perfusion systems may be the answer to matching the size of the perfusion circuit to the size of the neonatal patient, something that has rarely been achieved.

Perfusionists are experts in the field of whole body perfusion and should become actively involved in local tissue engineering programs and participate in the development and operation of bioreactors in order for them to become integral parts of this exciting new area of medicine.

REFERENCES