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Cell Therapy and Tissue Engineering: An Overview

By Gary C. du Moulin

Conventional medical technologies to address tissue and organ dysfunction have resulted in a host of approaches, largely device-based. Examples include maintenance dialysis for renal dysfunction, use of pacemakers, stents, oxygenators, and valves to neutralize the effects of cardiovascular dysfunction, and replacement of large joints with mechanical substitutes. Advances in transplantation science have led to increasing success in replacing diseased kidneys, livers, hearts, pancreata, and lungs. There are, however, significant and severe limitations to these conventional therapies, most notably the demand by a growing and aging population. There is a well-recognized limitation in the supply of tissues and organs. In the year 2000, for example, 77,000 people were awaiting organ transplants, while only 23,000 were performed. High tech medicine is costly; U.S. healthcare expenditures as a percent of gross domestic product are expected to reach 16.7% by 2007. With allogeneic transplants, lifelong immunosuppression is necessary, and in most cases, the effectiveness of transplanted cadaveric tissues and organs is limited. Implanted mechanical devices eventually fail because they are subject to wear and do not integrate with surrounding tissues. Lastly, most of these conventional approaches significantly reduce the quality of life. Because of these continuing unmet medical needs, the drive to develop tissue repair and tissue engineering approaches for organ repair or replacement, including new therapeutic avenues such as xenotransplantation, and therapies based on stem cell biology, has rapidly accelerated. These technologies may usher in an era of regenerative medicine in which permanent repair of a structural or mechanical dysfunction can be fashioned or a diseased metabolic condition ameliorated. While these approaches will remain in development for the foreseeable future, the promise of cell therapy and tissue engineering has already begun to demonstrate a robust proof of principle.[1] And, with the initiation of clinical trials, several technologies are gradually working toward meeting FDA standards for licensure.

A proposed working definition for tissue engineering is, "The application of principles and methods of engineering and life sciences toward fundamental understanding of structure and function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function."[2] A basic knowledge of mammalian cell culture techniques has emerged and procedures for cell and tissue manipulation have been developed. However, the application of this technology for the successful development of products and services has by no means been optimized. *Ex vivo* manipulation of cells and tissues from human and animal sources and their concomitant interaction with media, growth promoting factors, and various atmospheric conditions, as well as contact with a host of biomaterials has led to a wide array of potential applications in tissue regeneration. There exists, however, only a handful of tissue engineered products and cellular therapies that have undergone sufficient development, clinical validation, and regulatory review for FDA approval as devices or biologics.

Scientific and regulatory components of the federal government have recognized the potential of tissue engineering. FDA has been collaborative in providing guidance and regulatory algorithms for developers of these technologies. Additionally, organizations such as the United States Pharmacopoeia are developing standards of practice specific for this emerging industry.[3] There has also been a significant increase in the number of professional journals devoted to tissue engineering and biomaterials, in which the efforts of hundreds of multidisciplinary research groups have appeared. The number of national and international scientific conferences dedicated to the area continues to increase.

Cell therapy and tissue engineered product characteristics

Part of the problem in developing these products is the biological com-

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plexity and inherent variability that will undoubtedly be the subject of intense scrutiny in the evaluation of the chemistry, manufacturing, and control section of regulatory submissions. Product characteristics may be comprised of one or more of the following components: isolated cells, scaffolds, and signal molecules.[4] Each component requires controls to ensure consistency during manufacture. Cells may have an autologous, allogeneic, or xenogeneic origin and may be procured from embryonic as well as adult tissues. A basic physiological aspect of using cells to construct these products is their innate ability to form an extracellular matrix.[5] Extracellular matrix is a complex mixture of molecules synthesized by normal cells and is essential for cell survival. Extracellular matrix is a mixture of proteins and carbohydrates and can include proteoglycans, cell adhesion molecules, fibronectins, hyaluronans, collagens, laminins, or other biomaterials. Once implanted, these cells respond to the physiological environment by synthesizing extracellular matrix which integrates with surrounding native tissues. Demonstration of these natural phenomena in animal models can be problematic.

Scaffolds are comprised of biomaterials that may be either biodegradable or non-biodegradable. A scaffold's primary function is to facilitate the localization and delivery of cells in the body and to provide sites for cell anchorage.[6] The scaffold provides three-dimensional structural guidance and mechanical stability. Scaffolds can be designed to degrade at a rate that matches cells' regeneration rate. Scaffold materials can include polyglycolic acid, polyethylene glycol hydrogels, polyvinyl alcohols, or natural substances such as alginates, fibrins, collagens, chitosans, or gelatins.[7]

A third necessary component are signaling factors (hormones, cytokines, or growth factors.) These natural or synthesized materials provide additional and essential stimuli that guide cells toward a desired configuration.[5,8] They may be expressed by cells interacting with the extracellular matrix or they may be isolated or synthesized and attached to an extracellular matrix before contact with cells.

Formats

Tissue engineered or cell therapy products have been categorized into three basic product types: structural or surrogate, metabolic or immunoisolatory, and cell therapy or biomolecule formats.[9] Structural or surrogate products are manufactured outside the body for later implantation. They may take the form of devices that induce regeneration of functional human tissues. Metabolic or immunoisolatory tissue engineered products are extracorporeal or implantable devices containing human or animal tissues designed to replace the function of diseased internal tissues. Lastly, a third form utilizes living cells themselves as therapeutic or diagnostic reagents. For example, the use of autologous activated T lymphocytes as a therapeutic modality in the treatment of cancer. [10]

Some examples of cell therapy and tissue engineered products

Autologous Cultured Chondrocytes (Carticel)

Carticel® is an autologous chondrocyte implant product that replaces damaged articular cartilage in the knee.[11] The process begins by collecting a cartilage biopsy. After it is sent to a central manufacturing facility, the cells are expanded to the requisite number of chondrocytes, packaged in a vial, and then sent to an orthopedic surgeon who implants the cells into the patient's knee under a periosteal patch. After a period of rehabilitation the defect is filled with hyaline cartilage, eventually affecting a permanent repair. Carticel is the first cell therapy approved by FDA and, to date, autologous cultured chondrocytes have been implanted into 8,000 patients. Ninety-one percent of femoral chondyle patients improve from baseline examination with improvement seen at one year and sustained at five years.

Cultured Epidermal Autografts (Epicel)

Epicel® is a technology indicated for permanent skin replacement in the treatment of life threatening injuries due to burns.[12-14] Keratinocyte grafts are formed from an autologous skin biopsy. The biopsy is enzymatically treated to release keratinocytes that are cocultured with irradiated murine cells. Keratinocyte cultures are expanded to meet the dimensions of the injury. Epicel is provided in 50-cm^2 grafts that are placed on a prepared wound surface. This product protects the patient and accelerates the healing process. Figure 1 depicts the application of cultured epidermal autografts during surgery. Figure 2 shows the therapeutic outcome on a patient after application of the autografts.

Autologous Cultured Myoblasts

In a process termed cellular cardiomyoplasty, autologous skeletal myoblasts are procurred from a leg muscle biopsy, expanded in culture, and implanted into damaged myocardium to slow or prevent the progression of congestive heart failure.[15] Once implanted, the myoblasts populate the scar tissue, grow, and gradually take on the characteristics of heart tissue — resulting in improvement in cardiac function.

Product development challenges

Validation

Process validation is one of many challenges that remain.[16] The principles, procedures, and practices for understanding and validating promising technologies are only now being applied at a medically relevant scale. The sole guideline for process validation and aseptic processing was issued by FDA in 1987 and pertained to conventional biologics. Although a new guideline is being prepared, elucidating the complexity of cell- and tissue-scaffold relationships will take some time. Process validation of tissue engineered products is arduous and the cost of scaling up manufacturing to meet the medical needs of thousands of patients is substantial.

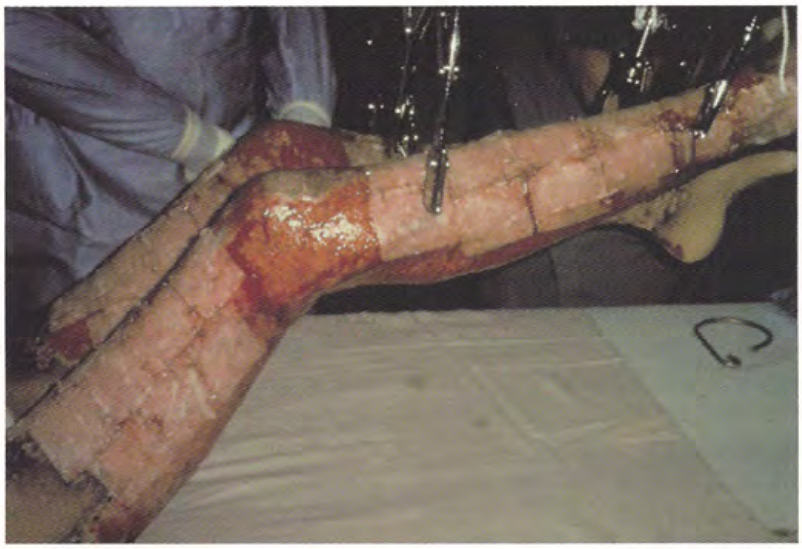

Figure 1: Surgical application of Epicel (cultured epidermal autografts)

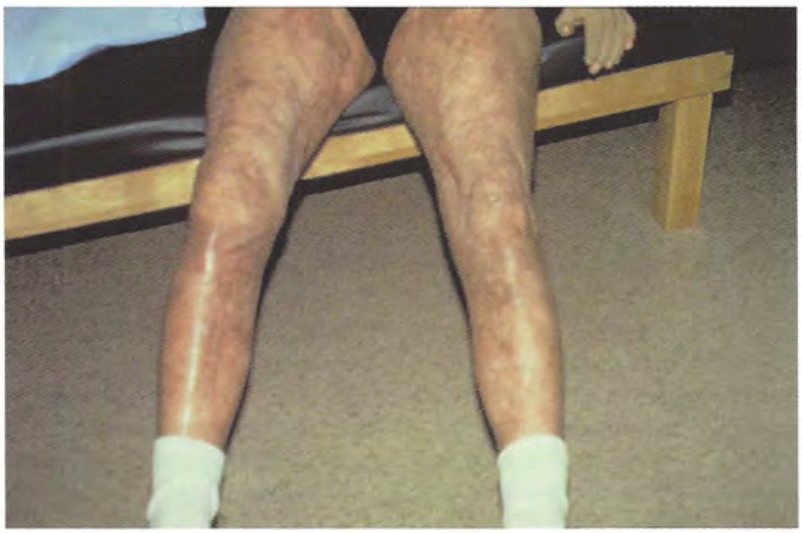

Figure 2: Results of application of Epicel after approximately one year

Manufacturing infrastructure to support tissue engineering is typically built around highly controlled cleanroom environments. Validated quality control and quality assurance programs will be necessary to control biologic variability and ensure product reproducibility for every lot of therapeutic material produced.[17-19] Packaging and shipping procedures will have to become more robust to ensure that short shelf life products comprised of living cells and tissues arrive at the clinical site with their therapeutic potency intact. Validation of rapid assays is also needed for product characterization as well as lot release. It is essential in the development of products of this complexity that formalized design control procedures be rigorously followed. This assumes that all design inputs and specifications are identified at the earliest possible point and incorporated into product development programs prior to Phase I or Phase II clinical trials.

Human resources

The ultimate success of this field will demand the mobilization of a host of scientific disciplines. Materials science, cell biology, microbiology, genomics, chemical engineering, molecular biology, robotics, computational biology, computer assisted design, immunology, and microscopic imaging are but a few of the scientific areas needed to help develop this field. This multidisciplinary approach requires a common scientific language and a mutual appreciation and understanding of others' areas of expertise. Expertise is also required to comprehend the dynamics of cell growth on two- or three-dimensional structures and the infinite complexities of optimizing cell growth, reconciling phenotypic drift, understanding mechanisms of differentiation and dedifferentiation, and identifying the nutritional and metabolic requirements to ensure product reproducibility.

Raw materials

Understanding optimal conditions for mammalian cell culture is also in need of continuing refinement. Historically, classical media formulations were all that was available to cell biologists. These formulations were sufficient to support the well-understood fibroblastic cell lines traditionally used for research. Today, however, these media are inadequate for the highly differentiated, functionally complex requirements of the specialized cells needed for tissue regeneration applications. Cell culture media should hormonally include serum-free formulations and defined media important for harmonizing tissue engineered products with the corresponding physiologic environments of the body. Additionally, manufacturers of cell culture media will have to retool their manufacturing capabilities into high quality manufacturing processes with greater GMP controls over media manufacture, similar to the parenteral pharmaceutical industry.

Critical Control and Regulation Parameters

A final challenge involves rapidly developing regulatory constructs to ensure safety and efficacy. In the early days of cell therapy, the *ex vivo*

manipulation of mammalian cells was largely a hospital-based research activity. FDA became concerned after published reports demonstrated a significant potential for patient risk. The agency began to view cell-based therapies and tissue engineered products as manufacturing processes requiring GMPs, not the practice of medicine where the concepts of rigorous manufacturing controls are not well understood. In the U.S., the public health and regulatory concerns associated with cellular and tissue-based products have been articulated by a series of questions that are now asked and discussed with every product sponsor:

1. How can the transmission of diseases be prevented?
2. What processing controls are necessary?
3. How can clinical safety and effectiveness be demonstrated?
4. How can FDA best monitor and communicate with the practitoner, patients, and the cell and tissue industry?

FDA has constructed tiered regulations based on the potential for risk from each production process. Products require greater regulatory oversight if they are more than minimally manipulated, or if cells are used for purposes other than for homologous function. They require even greater oversight if cells are combined with non-cell or non-tissue components, or if the intended product use is to provide a metabolic function. Currently, each potential product is reviewed on a case-by-case basis. Many regulatory parameters fall outside established guidelines and, in some cases, protracted discussions are required to achieve consensus between a product sponsor and FDA.

Good manufacturing practices

Control in the development and manufacture of engineered tissue is essential. The level of control is largely dependent upon the source of the tissue (autologous, allogeneic, or xenogeneic.) Nearly all forms of tissue engineered products will benefit from the requirements of GMP control.

In short, these practices incorporate controls over the manufacturing process, the facility and environment in which the manufacturing occurs, the raw materials and components, and the equipment needed for manufacture.[20] Because finished products may exhibit a limited shelf life or must be administered immediately, it is essential that controls be placed far upstream in the product manufacturing cycle. Any failing or deficiency in an element of GMP control can result in flawed manufacturing — producing products that are neither safe nor efficacious. GMP control offers the best chance for the successful manufacture of tissue-engineered products. Some people believe that products and services can be similarly processed within the framework of hospital environments. However, there is little evidence to suggest that, without extensive compliance efforts, hospital-based tissue engineered manufacturing programs will have adequate manufacturing controls to insure safety and efficacy and will be challenged trying to meet FDA's exacting standards.

FDA has produced a series of publications that provide guidance to developers of these products. Because there are no unified international regulations, products with unproven safety and efficacy profiles could significantly damage or impair the evolution of this industry. However, FDA has a close relationship with our industry; it has resulted in, for example, an effective cell-based therapy for articular damage to cartilage. FDA's newly expanded Office of Cellular, Tissue, and Gene Therapies maintains a commitment to bring safe and efficacious products through the regulatory pipeline.

The Future

Hundreds of research groups representing academia and industry are working to better understand the concepts that will lead to the next era of treatment advances.[21,22] For example, an extracorporeal or implantable bioengineered liver is being developed. Bioengineered liver tissue needed to analyze the metabolism and cytotoxicity of novel products is also under development. Artificial ureters, bladders, and kidneys are being assessed. Therapies that deliver substances to the central nervous system are under development to treat amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and chronic pain. Repair strategies have been developed (and tested in animal models) that promote regrowth of damaged nerves. Regenerating an injured spinal cord is within the realm of possibility.[23] Corneal epithelial cells seeded on polyvinyl alcohol hydrogels will aid in restoring corneal damage. Esophageal and bowel segment replacement is in the laboratory development phase. Dental pulp tissues are being studied as a first step toward the creation of teeth. Growth factors stimulate periodontal attachment structures such as alveolar bone, periodontal ligaments, and tooth root cementum. Precursors of mature adipocytes can be cultured and used in reconstructive surgery of congenital malformations, past traumatic malformations, or defects caused by malignancies. Finally, small diameter blood vessels comprised of smooth muscle cells, endothelial cells, and fibroblasts have been successfully seeded on biodegradeable polymers in tubular shapes — yielding complete vessels.

Conclusion

The bioengineers Mooney and Mikos stated, "Ten millennia ago the development of agriculture freed humanity from a reliance on whatever sustenance nature was kind enough to provide. The development of tissue engineering should provide an analogous freedom from the limitations of the human body."[4] As cell therapy and tissue engineering continue to evolve and mature, cooperative endeavors between academic, medical research, biotechnology, and government entities will come to fruition. Together these multidisciplinary teams will develop the applications, technology transfer paradigms, process validation

procedures, and sound regulatory constructs to insure safe and efficacious cell therapies and tissue engineered products. If the cell and tissue regeneration industry is committed to quality, excellence, and continuous improvement, the virtually unlimited potential of this exciting area of biological therapy can be fully realized.

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