Review Article

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Tissue engineering: Present concepts and strategies

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INTRODUCTION

Medical or surgical procedures that have attempted to partially replace lost tissue, account for a large part of health care resources which exceed over $400 billion per year.[1] Replace like tissue with like tissue is a basic principle of surgery. However, certain congenital, post-traumatic, postablative, or even operated multiple times deformities may be so severe that autologous tissue does not exist in sufficient quantity, or is difficult to obtain in order to achieve a favorable result. Current therapeutic modalities to treat tissue or organ loss include total artificial or mechanical devices, biological processed tissues, transplanting organs from one individual into another, surgical reconstruction, autologous transfer of tissue to the diseased-site or supplementing metabolic products of the lost tissue. Although these traditional forms of tissue replacement have rescued millions of patients and have improved countless lives, they suffer from problems of scarce supply, increased susceptibility to infection, immunologic rejection, as well as uncertain long-term interactions with the patients; and however still remain imperfect solutions. The problems are much more severe in the pediatric age group, since implantable mechanical devices do not grow as the child grows.

Tissue engineering is an interdisciplinary field which applies the principles and methods of engineering and the life sciences towards the fundamental understanding of structural and functional relationships in normal and pathological tissue and the development of biological substitutes to restore, maintain, or improve function.[2] Tissues that are engineered using the patients own cells, or immunologically inactive allogenic or xenogenic cells have the potential to overcome current problems of replacing lost tissue function and offer new therapeutic options for diseases where currently no options are available. Tissue engineering technology can play a vital role in the future management of pediatric patients.[3] Tissues or organs absent at the time of birth, in congenital anomalies such as esophageal atresia, bladder exstrophy and congenital diaphragmatic hernia to name a few, pose a serious challenge in surgical repair. The advances in prenatal diagnostics, that allow earlier detection of such anomalies, could give sufficient time to engineer the missing tissue or organs and have them fabricated for surgical replacement at the time of birth.

GENERAL STRATEGIES TO REPLACE TISSUE LOSS

The primary intention of all approaches in tissue engineering is the functional or structural restoration of tissue through the delivery of living elements which become integrated into the patient. Most of the techniques of guided tissue restoration developed during the last two decades have mainly been only cell based or only matrices based. However investigators in the rapidly emerging field of tissue engineering presently use a combination of both to achieve new tissue formation.

Cell and cell based techniques

In cell based tissue engineering methods, isolated and disseminated cells are injected into the blood stream or a specific organ of the recipient. The cells transplanted using such injection methods will utilize the blood supply for nutrients and the ground substance provided by the host tissue as a matrix bed for attachment, reorganization and desired growth.[4] The cell injection approach avoids the complications of surgery, allows substitution of only those cells that supply the needed function, and permits manipulation of cells before infusion. This method might find applications in replacing lost metabolic functions. However, its application in replacing functions of structural tissues is severely limited.

Cell encapsulation techniques

In this concept for cell transplantation, the cells are cultured and encapsulated in a semipermeable membrane that isolates the cells from the body. However, at the same

time the semipermeable membrane, which allows the diffusion of nutrients and wastes, prevents macromolecules such as antibodies, complement factors and immune cells from accessing the transplant. This application of this system has been successfully demonstrated in the production of a bioartificial liver, using xenogenic hepatocytes, as an extracorporeal liver support system and was effective in clinical trials for the treatment of acute liver failure.[5]

Tissue engineering using open systems of cell transplantation

The primary goal of the open system of cell transplantation is to engineer new tissues by having the transplanted cells in direct contact with the host with the intention to provide a permanent natural solution to the replacement of lost tissue. Cells for transplantation using this technique are attached to matrices consisting of natural materials or synthetic polymers and are then implanted into the host. This cell-polymer construct then incorporates itself into the recipient's own tissue. In cell culture experiments it has been observed that dissociated mature cells tend to reform their original structures when given the appropriate environmental cues. This has been demonstrated by the formation of tubular structures by capillary endothelial cells in culture, as well as the formation of milk secreting acini by mammary epithelial cells *in vitro*.[6] Isolated cells also have the capability to reform their structure, however, only up to a limited degree when placed as a suspension into the host. This is mainly due to the absence of an intrinsic tissue ground substance framework. Implantation of tissue in larger volumes is also severely restricted because diffusion limitations restrict interaction with the host environment for nutrition, gas exchange and waste elimination. Based on these observations, approaches to engineer tissue by attaching isolated cells to porous polymeric templates has been developed. (Figure 1) The application and introduction of cell-polymer construct concept in the engineering of new tissue was to provide a structural foundation to support tissues undergoing constant remodeling, integration and restructuring. Over the past two decades, a lot of research work has been done to design, develop and experimentally demonstrate the increasing advantages of polymer matrices based open cell system transplantation in the manufacturing of new tissues.

BIOCOMPATIBLE POLYMER MATRICES

Polymer matrices used in tissue-engineered devices need to be biocompatible and have been designed to meet the nutritional and biological needs of the cell populations involved in the formation of new tissue. The next important feature is that the materials should also be resorbable so that they leave a completely natural tissue replacement. Furthermore, polymer matrices should be reproducibly processable into desired structures and shapes and be able to retain their shapes after implantation in the host. If desired, the material should promote the growth of cells to increase the mass of the tissue. Finally, the surface of the material should interact with the transplanted cells to allow retention of differentiated cell function. Materials on the other hand that do not possess the above properties or are nonresorbable carry a permanent risk of infection.[7] The most commonly used materials as substrates or encapsulating materials in the field of tissue engineering are either synthetic polymers such as lactic-glycolic acid or polyacrylonitrile polyvinyl chloride, or natural materials such as collagen, hydroxyapatite, or alginates.

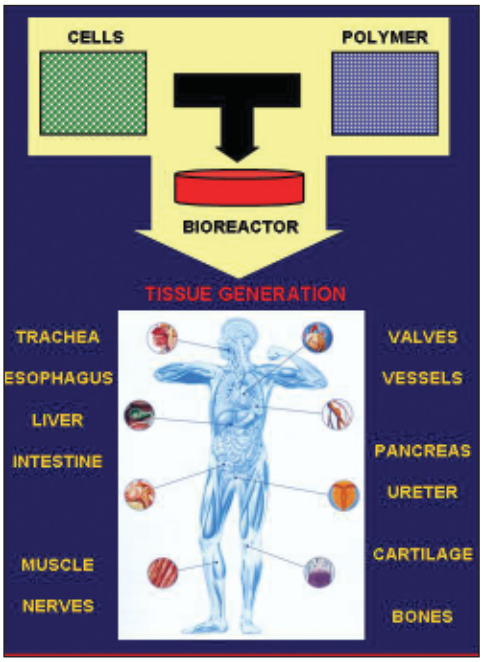

Figure 1: Schematic representation of the process of tissue-engineering

CELL SOURCING IN TISSUE ENGINEERING

Scientists have investigated virtually every tissue type in the human body in terms of tissue engineering. For clinical applications at present, cells have been derived from the patients themselves, from family members or close relatives, or other individuals. However, although this may be the most ideal source of cells, availability and accessibility may often be quite difficult. One approach to overcome the cell-source difficulty could be isolation of human stem cells. Human stem cells can be proliferated through multiple generations and made to differentiate into the appropriate cell type. Recent studies have shown

that stem cells derived from human embryonic blastocytes possessed these characteristics.[8] These stem cells identified, can be signaled to turn into different morphological cell types by changing the culturing conditions of the cells prior to implantation. Although there are a number of technical hurdles such as the need for pure stem-cell preparations, methods to reduce adhesion of cells during cell culture and processes to generate large number of cells to create tissue; work on stem cells involved in the creation of muscle, bone, and cartilage has demonstrated promising results in tissue engineering applications.

IN-VITRO CULTURE SYSTEMS

Static Cell Cultures

Early work in the field of tissue engineering was based on the use of standard static cell-culture conditions for the *in vitro* fabrication of tissues before implantation. However, there have been certain limitations to the exchange of nutrients and gases. These limitations are being overcome by advanced application of mechanical engineering expertise in the field of biotechnology by designing and producing dynamic cell culture systems, better known as *bioreactors*.

Bioreactors

Bioreactors are dynamic cell culture systems that allow more control to generate larger volumes of cells when compared to conventional static-culture techniques. (Figure 2) The flow of tissue culture medium and their mixing within bioreactors can be controlled to enhance mass transfer of nutrients, gases, and metabolites in order to regulate the size and structure of the tissue being generated.

PRESENT STATUS OF TISSUE ENGINEERING

Seeding of cells on 3-D biodegradable polymers in cell culture or flow bioreactor gave the opportunity to study tissue development, tissue regeneration, and tissue repair *in vitro*. Investigators have attempted to study the properties of almost all mammalian cell types under *in vitro* conditions. Implantation of the cell polymer constructs allows further study *in vivo*. Knowledge obtained from the different disciplines participating in active tissue engineering research has promoted the evolution of this new field from primary animal research to clinical trials. The present status of different structural and functional tissue has been described below:

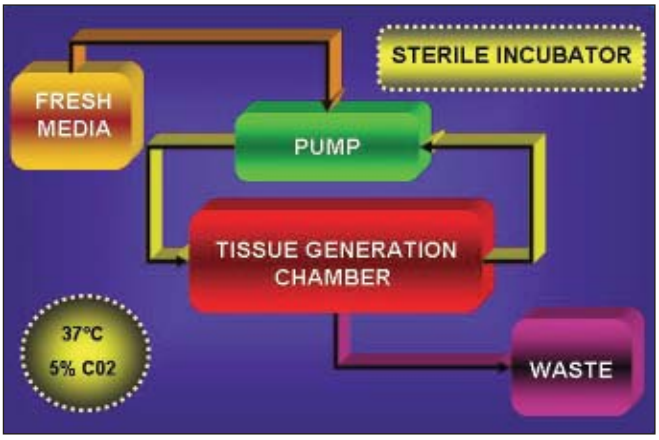

Figure 2: Dynamic cell culture or *bioreactor* concept for tissue generation using 3-D polymers

Nervous System

Progress has also been reported in attempts to regenerate the peripheral nerve. Synthetic nerve guides, fabricated in the shape of tubes, have been found to aid the regeneration of nerves that have been injured and separated by gaps that are too wide for healing. The tubes help the regenerating nerve from scar infiltration and also direct the new regenerating axons towards their target. It has been also demonstrated in several animal models that synthetic guides composed of natural polymers and synthetic polymers have enhanced axonal regeneration *in vivo*.[9,10] The process of nerve regeneration has been further promoted by lining polymer membranes with Schwann cells.[11] Additionally, polymers have been also designed to slowly release growth factors that aid in the regeneration of the damaged nerve over longer distances.

Skin

Burn and burn related injuries are known to be associated with severe morbidity and mortality. In preclinical models, acellular dermis has been populated with keratinocytes and fibroblasts and has been tested as skin substitute.[12] Biosynthetic analogs of skin, on the other hand have combined cultured skin cells with polylactic/polyglycolic fabric, collagen gels, and collagen-glycosaminoglycan (GAG) sponges to provide skin replacements. This approach involves the *in vitro* culture of keratinocytes obtained from small skin biopsies (1 cm^2) of burn patients. The rapid expansion of the keratinocyte population is achieved by cultivating keratinocytes on a feeder layer of irradiated fibroblasts (NIH 3T3) in association with certain media components. The advantage of this method is that it allows the graft to cover extremely large wounds; a disadvantage, however, is that 3-4 weeks are required for cell expansion. It is possible that cryopreserved allografts may help to address this problem.[13]

Another approach to fabricate skin, utilizes human neonatal dermal fibroblasts grown on degradable polyglycolic acid polymers. In this method selective cultures of dermal fibroblasts are inoculated into the porous

reticulations of the substrate. In case of severe burn injuries involving all the skin layers, the graft is placed directly on the wound bed and a skin graft (cultured epidermal autograft) is placed above. This graft then organizes and vascularizes to form organized dermis like tissue. Furthermore, the addition of a dermal matrix to epithelial tissue engineered replacements adds the theoretical advantage of a thicker, more durable graft that more closely resembles the "gold standard" of split-thickness skin grafts.[14]

Liver

Donor shortfall for liver transplantations presents a severe challenge to clinicians worldwide. After tedious research it has been found out that a number of critical steps determine the success of hepatocyte transplantation. Simple manipulations such as sandwiching the cells between hydrated collagen gels have prolonged the secretion of albumin, transferrin, fibrinogen, bile acids and urea from cultured hepatocytes.[15] Furthermore, in order to maintain their differentiated function, hepatocytes must be attached to the polymer substrates, and also be implanted in vascular areas of the body so that a constant supply of hepatotrophic factors can be maintained.[16] These factors taken in to account, have demonstrated that tissue structures can be formed when hepatocytes have been placed on appropriate polymers. The hepatocytes have been found to maintain a distribution of filament network similar to the *in vivo* state and the hepatic tissue generated has also shown evidence of bile ducts and bilirubin removal.[17] Vast spectrum of investigations are presently being undertaken to enable larger transplantation of hepatocytes, as well as to prolong the cell viability, using various polymers.[18]

Pancreas

Diabetes mellitus affects a major part of the adult population worldwide. Transplantation of grafts of isolated pancreas islets has been performed to tackle this problem, however despite immune alterations of the transplants no success has been reported in animal models. To overcome the problem of immunorejection and autoimmune rejection, the concept of immunoisolation has been advocated. This is achieved by enclosing the pancreatic islets by semipermeable and biocompatible membrane (bioartificial pancreas). The membrane is impermeable to high molecular weight antibodies, but permeable to oxygen, glucose and internally generated hormones (insulin, glucagons, somatostatin, pancreatic polypeptides and other islet proteins).[19]

Urinary tubular structures

Replacement of the ureter, bladder, and urethra using parts of other organs for reconstruction, often leads to reflux, infection and dilatation of the upper urinary tract. Nonbiological or metal implants have been used to replace ureters but have largely failed due to poor biocompatibility, lack of peristaltic activity and deposition of salt on their surfaces. Since the urothelium has a good regenerative capability, the fabrication of urothelium-polymer constructs as implants for replacement therapy have been explored. Successful fabrication of the ureter and segmental ureteral replacement has been achieved using urothelial cells seeded onto polyglycolic acid polymer tubes.[20]

Esophagus

The application of the concepts of using tubular polymers has broadened from ureteric replacements to the possible replacements of other tubular structures such as the trachea, esophagus and intestines. Co-polymer tubes of lactic-glycolic acid used in a dog model as an esophageal replacement have shown encouraging results. The tubes were over time covered by connective and epithelial tissue and the dogs were able to drink freely and eat semisolid food.[21] Further research using better designed copolymer tubes and cell seeding techniques could provide breakthrough for esophageal replacement and the management of malformations such as long-gap esophageal atresia.[22]

Small Intestine

In a similar approach, fetal intestinal cells have been seeded onto polymer tubes and have shown promising results in new intestinal tissue generation. The new intestinal epithelium engineered has demonstrated the presence of differentiated intestinal epithelial cells lining the tubes.[23] Histology has revealed the development of crypt-villus structures in the neointestine cells which have the functional capability to secrete mucous. Promising results emerging after the anastomosis of tissue-engineered neointestine to native small bowel, and its successful structural and functional integration, gives hope for the management of patients with short bowel syndromes.[24]

Cartilage

Research has been aimed at creating new cartilage based on collagen-glycosaminoglycan templates, isolated chondrocytes and chondrocytes attached to natural or synthetic polymers. Articular cartilage is composed of a highly organized extracellular matrix, which consists mainly of type-II collagen and proteoglycan (chondroitin and keratan sulfate glycosaminoglycans) and a relatively sparse cell population of articular chondrocytes. The mechanical performance of articular cartilages is dependant on the architecture of its extracellular matrix and the

ability of the constituent chondrocytes to maintain the matrix. The use of well-stirred bioreactors for cultivating chondrocytes on polymer scaffolds *in vitro* may enable nutrients to penetrate the center of this nonvascularized tissue, leading to strong and thick implants.[25] Cartilages for replacement of various tissue such as nasoseptum, human ear cartilage as well as trachea replacement have been engineered. In the case of joint replacements, tissue engineered cartilages have been shown to induce the same lubricity, produce the same coefficient of friction, as well as absorb and dampen the joint forces settings of a normal articular cartilage

Bone

Culture of seeded osteoblastic cells in 3-D osteoconductive scaffolds *in vitro* is a promising approach to produce an osteoinductive matter for the repair of bone defects.[26] Small phalanges and joints resembling the shape and composition of human phalanges have been successfully fabricated using periosteum, chondrocytes, and tenocytes seeded differentially onto biodegradable polymer scaffolds. To achieve this, the gross form of a composite tissue structure was constituted *in vitro* by assembling the parts and suturing them to create models of the phalanges. The sutured composite tissues were then implanted subcutaneously and resulted in the formation of new tissue after 20 weeks.[27]

Muscle

Skeletal muscle comprises approximately 48% of the body mass and is responsible for voluntary control and active movement of the body. Application of tissue engineering techniques and successful fabrication of skeletal muscle mass holds now a promising future for the restoration of 3-D contour as well as the loss of function for the affected part of the body.[28,29] In order to generate skeletal muscle tissue, myoblasts which are skeletal muscle tissue precursors, have been employed. Myoblasts have been seeded onto polyglycolic acid porous polymers with successful generation of vascularized new skeletal muscle *in vivo*.[30]

Under cell culture conditions, myoblast cultures have exhibited twitching movement on stimulation.[31] This exhibition of movement by myoblasts in culture is very important for the fabrication of new skeletal muscle which is not only a structural but more an important functional tissue.[32] Research is presently being carried out in improving the vascular infrastructure of the muscle to enable generation of larger muscle mass.[33] Gel polymers have also been successfully used for the transplantation of myoblasts.[34]

Heart valves and blood vessels

Tissue engineering of heart valve was demonstrated using autologous myofibroblasts and endothelial cells harvested from the femoral artery of a lamb. The cells were expanded in culture and cell separation was performed using a fluorescent activated cell sorter. The myofibers were first seeded onto a polymer scaffold composed of polyglactin polymer mesh sandwiched between two polyglycolic acid meshes. The endolthelial cells were seeded onto the polyglycolic acid meshes after a time lag of 7 days. The construct was trimmed to size and was implanted in the same lamb to replace the right pulmonary leaflet. The polymer disappeared after 6 weeks of implantation leaving behind a leaflet histologically resembling the native pulmonary valvular architecture. The mechanical properties of the tissue engineered leaflet approached the natural parameters over time.[35] Grafts are also being designed from relatively inert materials (with heparin coatings) or with materials that interact with blood cells in a desirable way.

FUTURE ASPECTS AND RESEARCH

For the clinical success of tissue engineering numerous areas of research are critical. Precise understanding of cell biology with emphasis on cellular differentiation, cell to cell interaction and extracellular matrix formation will be of paramount importance. Cell sourcing, cell preservation and cryopreservation of cells, or establishment of cell banks, which are only possible for certain tissues at present must be made possible for other tissues in the future. Large-scale cell culture systems, capable of resolving the nutrient transport issue and allowing the proliferation of larger number of required cell types *in vitro*, must be designed. The continuous work performed in designing better polymers is another major area of study. Tissue engineering is a technology with profound benefits and an enormous potential that offers future promise in the treatment of loss of tissue or organ function as well as for genetic disorders with metabolic disorders.

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