Review Article

Tissue Engineering

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ABSTRACT

The term "tissue engineering" was officially coined at a National Science Foundation workshop in 1988 to mean "the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function". Tissue engineering applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ.

Key words : Tissue Engineering

Introduction -

The term "tissue engineering" was officially coined at a National Science Foundation workshop in 1988 to mean "the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function"[1]. Tissue engineering applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ [2].

As medicine therapy advances, we can now realistically set goals for fully functional living replacement of nearly every diseased organ or tissue[3]. To date, there are over 100,000 Americans awaiting organ transplantation; however, the actual number of available organs will provide for only a fraction of those with end-stage organ failure. Tissue engineering holds the promise of creating replacement tissues and organs outside the human body[4]. About 3,000 individuals in the United States are awaiting a donor heart; worldwide, 22 million individuals are living with heart failure. A bioartificial heart is a theoretical alternative to transplantation or mechanical left ventricular support[5].

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Materials and Scaffolds for Tissue Engineering -

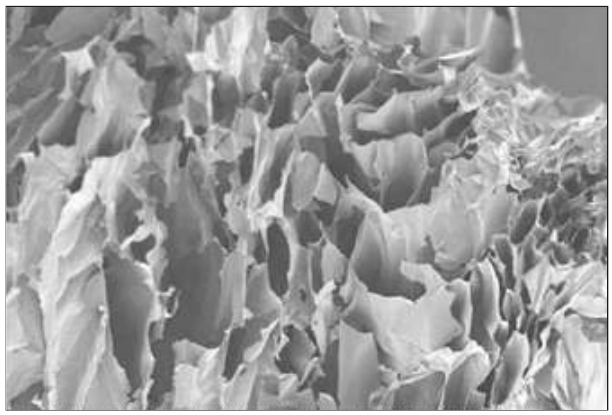
The principal mechanical supporting structure of any engineered tissue is the scaffold. These three-dimensional constructs are often composed of several materials and support the living cells required to generate a functional tissue. The mechanical properties of the scaffolds, such as strength and elasticity, must correspond to the mechanical properties of the target tissue. Moreover, cells respond to environmental cues such that the scaffold should mimic the target tissue to achieve the desired cell alignment and the three-dimensional arrangement of the cells. The ideal scaffold materials for engineered tissues are resorbable materials that break down over time. During resorption, the engineered tissue is remodeled by normal healing processes, leaving only living cellular tissue with natural supporting connective tissue. These technologies such as lung assist devices, liver assist devices, and even composite implants with resorbable and nonresorbable components for orthopedic and hernia repair represent an important step toward developing fully resorbable scaffolds for all tissues. Current research is focused on resorbable synthetic polymers (e.g., polyglycolic acid, resorbable polyurethanes, polyglycerol sebacic acid); naturally occurring polymers (e.g., collagens, fibrin); and minerals (e.g., calcium triphosphate). Scaffold materials can be

supplemented with growth factors or other cytokines to improve cellular incorporation and differentiation. Other surface modifications including surface texturing and protein or antibody coatings may improve cellular adhesion and migration. Decellularized connective tissue, or organs, represents a rapidly developing area of materials for engineered tissues. Decellularized hearts reseeded with myocardial cells have been demonstrated to function in vitro, and research is focused on expanding this approach to other organs such as the lung and liver as well as soft tissues using material such as decellularized dermis [3].

Biological scaffold materials derived from the extracellular matrix (ECM) of intact mammalian tissues have been successfully used in a variety of tissue engineering / regenerative medicine applications both in preclinical studies and in clinical applications[6].

Most of large-sized tissues and organs with distinct three-dimensional form will require support for their formation from cells. The support is called scaffold, template, or artificial extracellular matrix (ECM). The major function of scaffold is similar to that of the natural ECM that assists proliferation, differentiation, and biosynthesis of cells. In addition, a scaffold placed at the site of regeneration will prevent disturbing cells from invasion into the site of action. A variety of methods have been applied for fabrication of porous scaffolds. Among them are freeze-drying and porogen leaching[7]. In addition to the fixability and resorbability, scaffolds should meet other several requirements. Among them is resistance against stricture. This is necessary when a scaffold is used for regeneration of tubular tissues like blood vessels and esophagus. Reinforcement of porous scaffolds with fibre, mesh, or stent will be effective for these cases.

A novel scaffold material based on an alginate hydrogel which contained carbon nanotubes (CNTs) was prepared, and its mechanical property and biocompatibility evaluated. Soluble CNTs were prepared with acid treatment and dispersed in sodium alginate solution as a cross-linker. After which, the mechanical property (elastic deformation), saline sorption, histological reaction, and cell viability of the resultant nanocomposite gel (CNT-Alg gel) were evaluated. The CNT-Alg gel showed faster gelling and higher mechanical strength than the conventional alginate gel. Saline sorption amount of freezedried CNT-Alg gel was equal to that of the alginate gel. In terms of histological evaluation and cell viability assay, CNTAlg gel exhibited a mild inflammatory response and non-cytotoxicity. These results thus suggested that CNT-Alg gel could be useful as a scaffold material in tissue engineering with the sidewalls of CNTs acting as active sites for chemical functionalization[8].



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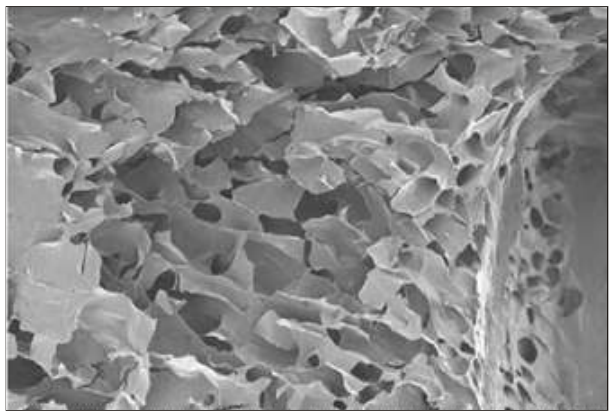

B

SEM microphotographs of the cross-section of freeze-dried Carbon Nano Tube-Alg-4 gel (A) and alginate gel (B : Alg-2, control). (Source : Preparation of Carbon Nanotube-alginate Nanocomposite Gel for Tissue Engineering. Minoru

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Properties of Scaffold[9] -

Many scaffolds for tissue engineering initially fill a space otherwise occupied by natural tissue, and then provide a framework by which that tissue may be regenerated. In this capacity, the physical properties of the material are inherent to the success of the scaffold. Specific physical properties include gel formation mechanisms and dynamics, mechanical characteristics, and degradation behavior. Once the scaffold is produced and placed, formation of tissues with desirable properties relies on scaffold material mechanical properties on both the macroscopic and the microscopic level. Macroscopically, the scaffold must bear loads to provide stability to the tissues as it forms and to fulfill its volume maintenance function. On the microscopic level, evidence suggests that cell growth and differentiation and ultimate tissue formation are dependent on mechanical input to the cells . Hydrolysis occurs at a constant rate in vivo and in vitro, the degradation rate of hydrolytically labile gels (e.g. PEG-PLA copolymer) can be manipulated by the composition of the material but not the environment . As discussed in the materials section above, collagen, HA, and chitosan are all degraded by enzymatic action. Synthetic linkages have also been introduced into PEO to render it susceptible to enzymatic degradation.

Materials used to form gels engineered to exist in the body must simultaneously promote desirable cellular functions for a specific application (i.e. adherence, proliferation, differentiation) and tissue development, while not eliciting a severe and chronic inflammatory response. Hydrogel forming polymers are generally designed to be nontoxic to the cells they are delivering and to the surrounding tissue.

Bioreactor -

A bioreactor may refer to any manufactured or engineered device or system that supports a biologically active environment.10 In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic.

NASA Tissue Cloning Bioreactor -

In bioreactors in which the goal is to grow cells or tissues for experimental or therapeutic purposes, the design is significantly different from industrial bioreactors. Many cells and tissues, especially mammalian ones, must have a surface or other structural support in order to grow, and agitated environments are often destructive to these cell types and tissues. Higher organisms, being auxotrophic, also require highly specialized growth media.

NASA has developed a new type of bioreactor that artificially grows tissue in cell cultures. NASA's tissue bioreactor can grow heart tissue, skeletal tissue, ligaments, cancer tissue for study, and other types of tissue[11].

By enabling reproducible and controlled changes of specific environmental factors, bioreactor systems provide both the technological means to reveal fundamental mechanisms of cell function in a 3D environment, and the potential to improve the quality of engineered tissues. In addition, by automating and standardizing tissue manufacture in controlled closed systems, bioreactors could reduce production costs, thus facilitating a wider use of engineered tissues[12].

Cells for Tissue Engineering -

The cellular components of engineered tissues must achieve organ functionality (e.g., hepatocytes), maintain organ structure (e.g., fibroblasts and stromal cells), and deliver blood to the tissue (e.g., endothelial cells and pericytes). Induced pluripotent stem cells and adult mesenchymal stem cells (e.g., bone marrow or adipose derived) are two promising autologous progenitor cells sources. Differentiation cues including soluble signals and incorporating genes can drive the progenitor cells into cell lineages and end-organ cells. Allogenic cells remain an

option for many tissues and have been the principal source of clinically successful engineered tissues to date. Embryonic stem cells continue to hold great promise as an allogenic cell option[3].

Most human tissues do not regenerate spontaneously; this is why cell therapies and tissue engineering are promising alternatives. The principle is simple : cells are collected in a patient and introduced in the damaged tissue or in a tridimentional porous support and harvested in a bioreactor in which the physico-chemical and mechanical parameters are controlled. Once the tissues (or the cells) are mature they may be implanted.Embryonic stem cells are potentially more interesting since they are totipotent, but they can only be obtained at the very early stages of the embryo. Finally, the properties of foetal stem cells (blood cells from the umbilical cord) are forerunners of the haematopoietic system but the ability of these cells to participate to the formation of other tissues is more problematic[13].

Cells used for tissue engineering are obtained from a small biopsy of tissue which is dissociated in the culture. The resulting cell population is expanded, seeded on to the matrix, and implanted back in to the host. The source of donor tissue can be allogenic (donor derived) or autologous (the host's cells), but autologous cells are preffered because they are not rejected by the immune system, and the use of immunosuppressant drugs is avoided. However, inherent difficulty of ex vivo expansion is a major limiting factor for use of some autologous cells. While autologous cells are recognized as the ideal transplantation resource, some patients with end-stage organ disease do not produce enough cells for transplantation. In this case, allogenic cells may be advantageous. Furthermore, some primary cells, whether autologous or allogenic, cannot be expanded from particular organs, such as the pancreas. In these situations, pluripotent stem cells are envisioned as an alternative source of the cells from which the desired tissue can be derived. Pluripotent stem cells represent an endless source of versatile cells that could lead to novel sources of replacement organs. Human embryonic stem cells (heS) are pluripotent (the ability to differentiate in to most tissues of the embryo) and retain the ability to self-renew[14].

Adult Stem Cells and Tissue Progenitor Cells -

Adult stem cells are, especially in the area of hematopoetic stem cells, better understood than any other aspect of stem cell biology. Ability to renew themselves as well as the ability to differentiate in to various cell types - these two characteristics are still used to define stem cells today. Adult stem cells tend to be tissue specific, self-renewing population of cells which can differentiate into cell types associated with the organ system in which they reside. A notable exception to the tissue specificity of adult stem cells is the mesenchymal stem cell, or what is more recently called the multipotent adult progenitor cells. This cell type is derived from bone marrow stroma. Such a cell has been shown to differentiate in vitro in to numerous tissue types and to also differentiate developmentally if injected in to a blastocyst. Multipotent adult progenitor cells develop into multiple tissues incliding neuronal, adipose, muscle, liver, lung, spleen, and gut, but notably, not bone marrow or gonads[14].

Embryonic Stem Cells -

Disease that might benefit from embryonic stem cell-based therapies included diabetes, heart disease, cerebrovascular disease, liver and renal failure, spinal cord injuries and Parkinson's disease. In 1981, pluripotent cells were found in the inner cell mass of the mouse embryo, and the term "embryonic stem cell" was coined[14].

In Vitro Maturation of Engineered Tissues -

Nearly all tissues have baseline mechanical requirements and many tissues such as heart valves, blood vessels, bone, and tendons must have adequate mechanical properties to achieve function. Mechanical forces are important to induce cell alignment and in the production of an extracellular matrix. Bioreactors have been developed to impart the needed mechanical forces to engineered tissues, including shear stress, pusatile flow, and pressure for valves and blood vessels, and axial tension and compression for bone, cartilage, and tendons[3].

Tissue engineering aims at replacing or regenerating tissues lost due to diseases or traumas[15]. However, mimicking *in vitro* the physiological complexity of vascularized tissue is a major obstacle, which possibly contributes to impaired healing *in vivo*. Ideally, the vascularization of in vitro-generated implants should follow a hierarchical network and include consecutively a vessel allowing microsurgical connection to the host vasculature (~1 mm diameter) linked to smaller branches mimicking arterioles or venules (80 to 100 im) and leading to a capillary tree (10 to 15 ìm) embedded in the tissue. These smaller structures (arterioles and capillaries) however are, until now, too small to be fabricated and need to be generated by self-assembly and self-organization of multicellular systems. Two strategies are developed to grow capillaries in an implant : (i) by promoting invasion of vessels from the host by combining the implant with tools including drug release, functionalized matrices or by surgical techniques or (ii) by forming, de novo, a vascular network in the construct before implantation. This is achieved by orchestrating the proper cascade of events in vitro. This field of investigation is very active to develop tissue constructs with engineered capillary networks, which promotes a more rapid perfusion and improved survival and differentiation of the associated tissue. There is, therefore, an important interest in further development of those self-assembled prevascularized tissues.

Microfluidic Network System for Oxygen Transport in Engineered Tissue -

Langmuir *et al* proposed a process to design a microfluidic network by combining an oxygen transport simulation with biomimetic principles governing biological vascular trees. Porous scaffolds containing an embedded microfluidic network were fabricated. The reliability of the procedure was demonstrated by experiments using the scaffolds. This approach established a practical basis for designing an effective microfluidic network in a cell-seeded scaffold[16].

Clinical Applications -

Engineered skin substitutes were the first true clinical success of the principles of tissue engineering. These clinically available products use autologous fibroblasts grown on a resorbable polymer scaffold for a single-layer product or fibroblasts covered with keratinocytes for a two-layer product. Recent products for promoting the healing of skin and dermal wounds also incorporate autologous fibroblasts delivered into the wound. Autologous chondrocytes are being used to heal damaged joints, and they have shown excellent results. Acellular collagen-based materials derived from human or animal dermis are being implanted for soft tissue reconstruction or hernia repair and become cellularized in vivo with autologous cells. Many engineered tissues are in clinical trials or under development, including those for bone, cartilage, nerve, skeletal muscle, small-diameter blood vessels, heart valves, and vital organs, including heart, liver, lung, and kidney[3].

Table Modified from Source : Tissue engineered Human Living Skin Substitute : Development and Clinical Application. Kwang Hoon Lee . Yonsei Medical Journal. Vol. 41, No. 6

Although very few engineered tissues have been approved by the US Food and Drug Administration (FDA), more than 70 companies have recently been developing new products[17].

Tissue Engineered Skin Substitutes

Product Name	Company	Material	
		Epidermis	Dermis
Epicel	Genzyme tissue repair	Living cultured Autologous keratinocytes	None
Alloderm	Life cell	None	Salt Processed, human cadaveric skin with acellular dermis
Integra	Integra life sciences	Silastic membrane	Bovine tendon collagen and shark Glycosaminoglycan
Dermagraft -TC	Advanced tissue sciences	Silicon polymer	Nylon mesh with non-viable cultured foreskin-derived dermal fibroblasts and their products
Dermagraft	Advanced tissue Sciences	None	Living human neonatal foreskin-derived dermal fibroblasts on a bioabsorbable polyglactin mesh without silastic layer
Apligraft	Organogenesis	Living human neonatal foreskin-derived keratinocytes	Living human neonatal foreskin-derived dermal fibroblasts and bovine tendon-derived collagen plus the fibroblast-produced matrix and growth factors

Challenges Ahead -

Thin tissues such as skin, cartilage, heart valves, and blood vessels are advantageous in that they can survive on diffusion of oxygen and nutrients, while new blood vessels sprout for permanent tissue formation. Thick tissues and solid organs depend on an elaborate network of blood vessels that course within 150200 micron mm of each cell in that tissue. The heart requires so much oxygen that often there are blood vessels on both sides of an individual cardiomyocyte. Development of blood vessel networks within tissues is the principal challenge for engineered tissues. Angiogenesis can be used to create capillary networks for musculoskeletal and soft tissues while engineered vascular networks utilizing microfluidic principles are being developed for solid organs such as the liver and lung. In vitro expansion and differentiation of progenitor cells to tissue-specific cells also remains an important milestone. This includes optimization of the different cell types and ratios for each tissue for long-term tissue function. Conditions necessary for in vitro culture and preimplant logistics such as delivery and storage are just beginning to be addressed for complex tissues[3].

All above denote the importance of collaboration with medical doctors, especially surgeons, who conduct implantation and evaluation of the scaffolds provided by biomaterials scientists. Without intimate collaboration among different fields, it would be unlikely for tissue engineering to successfully respond to the expectation of patients who have been suffering from lost or severely diseased tissues or organs. It seems probable that a major reason for delayed clinical trials of tissue engineering be ascribed to insufficient responses of biomaterials group to the requirements of medical groups, apart from recent excessive regulations and stringent assessment levels of review board on tissue engineered products[18].

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