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Review Article

New Era in Health Care: Tissue Engineering

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Abstract

Tissue engineering is a rapidly expanding field, which applies the principles and methods of physical sciences, life sciences and engineering to understand physiological and pathological systems and to modify and create cells and tissues for therapeutic applications. It has emerged as a rapidly expanding 'interdisciplinary field' that is a significant potential alternative wherein tissue and organ failure is addressed by implanting natural, synthetic, or semi synthetic tissue or organ mimics that grow into the required functionality or that are fully functional from the start. This review presents in a comprehensive manner the various considerations for the reconstruction of various tissues and organs as well as the various applications of this young emerging field in different disciplines.

Key words; Tissue Engineering, Extra cellular Matrix, Scaffolds

1. Introduction

Tissue engineering is an emerging biotechnological area, which combines various aspects of medicine, cell and molecular biology, materials science and engineering for the purpose of regenerating, repairing or replacing diseased tissues.[1] The term 'tissue engineering' was officially coined by Fung in October 1987 at a National Science Foundation Workshop in Washington, D.C. and since the last decade, this field has moved from 'science fiction' to 'science fact' with the research-oriented acceptance of its potential to regulatory approvals allowing commercial products to be available for use in many countries.[2]

Tissue and organ failure, produced as a result of injury, or other type of damage is one of the most devastating and costly problems in medicine.[3,4] Transplantation and tissue reconstruction are among the most expensive therapies, costing billions of dollars a year. Donor shortages further aggravates the situation and additionally, transplantation recipients must follow lifelong immunosuppression regimes coupled with increased risk of infection, tumor development and unwanted side effects. The shortcomings of surgical reconstruction are the lack of available donor tissues and donor site morbidity. Furthermore, replacement with mechanical devices or artificial organs is limited by an increased risk of infection, thromboembolism and finite durability. [4]

Tissue engineering is emerging as a significant potential alternative or complementary solution to these problems, whereby tissue and organ failure is addressed by implanting natural, synthetic or semi synthetic tissue and organ mimics that are either fully functional or that grow into the required functionality.[3] The young but growing field of tissue engineering is truly a multidisciplinary one, where living cells are harvested, grown in the laboratory (on appropriate scaffold) and stimulated to form specific tissues that mimic the complex structures and physiological behavior of natural tissues. Ultimately these 'engineered spare parts' can be put into a patient, either by injection or by implantation of intact tissues or an entire organ.

Thus, tissue engineering has the potential to revolutionize methods in health care treatment to improve the quality of life for many and in the future it will provide a cost-effective and long-term solution to many age related conditions.[5] Furthermore, engineered tissues could reduce the need for organ replacement and could greatly accelerate the development of new drugs that may cure patients, thus eliminating the need for organ transplants.[6]

2. Scaffold

Considerable effort has been made to develop biocompatible scaffolds for tissue engineering. Scaffolds are porous, degradable structures fabricated from either natural materials (collagen, fibrin) or synthetic polymers (polylactide, polyglycolide or co-polymer of polylactide and glycolide). The structures can vary from sponge like sheets and fabrics to gels or highly complex structures with intricate pores and channels fabricated using new materials-processing technology. The spatial and compositional properties of the scaffold are key, with the porosity of the scaffold and interconnectivity of the pores being capable of enabling cell penetration into the structure as well as the transport of nutrients and waste products. The principle for the design of tissue-engineering scaffolds remains clear. The scaffold should mimic the structure and biological function of native extra cellular matrix (ECM) as much as possible, both in terms of chemical composition and physical structure. Native ECM does far more than just provide a physical support for cells. It also provides a substrate with specific ligands for cell adhesion and migration, and regulates cellular proliferation and function by providing various growth factors. It is reasonable to expect that an ECM-mimicking tissue-engineered scaffold will play a similar role to promote tissue regeneration in vitro as native ECM does in vivo.

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The main aim of tissue engineering is the regeneration of tissues or organs that can be put to use should the need arise. Tissue and organ reconstruction is a tricky process that needs to be handled with utmost care for the best possible results. The assembly of cells into tissues is a highly orchestrated set of events that requires time scales ranging from seconds to several weeks. To coax growing cells into a three-dimensional tissue/organ is relatively difficult, as it requires the correct combination of all factors affecting cell growth.

a. Factors affecting cell growth: Proper 3-D growth of cells is a vital part of tissue engineering. The in vitro conditions should be able to mimic the in vivo conditions for the proper growth and differentiation of cells. Producing a dynamic in vitro microenvironment for tissues is an important aspect in guiding the formation of tissues with certain structural and functional characteristics.[7] In the body, the extra cellular matrix (ECM) provides tissues with the appropriate architecture as well as signaling pathways. This also influences key cell functions such as migration, proliferation and differentiation which affects cell growth and which should be provided in appropriate concentrations to enable the cells to adjust to in vitro conditions. In addition, the developing tissues also need proper quantities of shear and mechanical forces that promotes their proper development. Moreover, a critical challenge in tissue engineering is feeding every cell as growing more than few millimeters require blood vessels grown into them to supply nutrients.

b. Desirable characteristics of scaffolds: Scaffolds are porous, degradable structures fabricated from either natural materials (collagen, fibrin) or synthetic polymers (polyglycolide, polylactide, poly lactide coglycolide).[6] In tissue engineering, the cell populations are usually expanded by culturing and seeding onto a scaffold that accommodates and guides the growth and proliferation of new cells in three dimensions.[8,9] The optimization of scaffolds onto which cells are seeded is the key to the uniform formation of tissues as it provides the necessary support for cells to proliferate and maintain their differentiated function. The scaffold architecture defines the ultimate shape of the new tissue,[6,9,10] and the porosity of the scaffold regulates the transport of nutrients and waste products.[2] Thus, the desirable physical characteristics of biomaterial scaffold for tissue engineering applications include high porosity, large surface area, large pore size, uniform distributed interconnected porous structure throughout the matrix and the ability to degrade in response to matrix remodeling enzymes released by the cells as tissue repair progresses. [11~13]

c. Biomaterials for Scaffolds/composition: A crucial factor in tissue engineering is the biomaterial from which scaffolds are fashioned. An ideal biomaterial for a scaffold would selectively interact with the specific adhesion and growth factor receptors expressed by the target cells in surrounding tissues required for the repair of damaged tissues.[8,14] It is also of prime importance that the target cells are provided with the appropriate in vivo environment together with the extra cellular matrix (ECM) in order to facilitate their proper growth and proliferation. In the body, cells are surrounded by the ECM that is a highly hydrated network comprising of insoluble hydrated macromolecules (collagen, elastin, laminin, fibronectin and hydrophilic proteoglycans), soluble macromolecules (growth factors, chemokines and cytokines) and proteins on the surface of neighboring cells.[15] It is the ECM that confers physical, mechanical and functional properties on tissues and organs i.e. strength of bone, elasticity of skin etc.[9,16]The ultimate decision of a cell to differentiate, proliferate, migrate, apoptose or perform other specific functions is a coordinated response to the molecular interactions with various ECM effectors.[5,15] Moreover, during development and wound repair, cells synthesize and remodel ECM and thus all cells spend, at least part of their time interacting with the ECM.[9,17] Thus the ECM

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plays a vital role in the proper development of the target tissues and organs.

In tissue engineering, exogenous ECMs are designed such that they mimic the functions of the ECM molecules naturally found in tissues. The synthetic engineered ECM should produce temporary mechanical support to withstand in vivo forces until the engineered tissue has sufficient mechanical integrity to support itself. The cells comprising the engineered tissues must express appropriate genes to maintain the tissue specific function of the engineered tissue and it should also maintain a potential space for tissue development.[8,18] The exogenous ECMs can be fabricated from two classes of biomaterials: naturally derived materials and synthetic materials. Naturally occurring materials are composed of polypeptides, polysaccharides, nucleic acids, hydroxyapatites etc. Though natural biomaterials have excellent physiological properties such as selective cell adhesion, mechanical properties similar to natural tissues and biodegrability, they are limited by risk of viral infection, antigenicity, unstable material supply and deterioration, in case of long-term implantation. They also offer limited versatility in designing an exogenous ECM with specific properties. On the other hand, synthetic materials such as polyglycolic acid (PGA), polylactic acid (PLA) and their copolymers (PLGA) can also be manufactured reproducibly on a large scale and can also be processed into an exogenous ECM in which the macrostructure, mechanical properties and degradation time can be controlled and manipulated.[8,18~20] However, the greatest disadvantage of the synthetic materials is the lack of cell-recognition signals.

d. Designing of scaffolds: A number of architectural characteristics including porosity, pore size and permeability play a significant role in biological delivery and tissue regeneration. Thus, along with the scaffold material, the porous architectural design also plays a significant role in tissue regeneration by preserving tissue volume, providing temporary mechanical function and delivering biofactors. [8,9,15]The ideal scaffold should be able to balance mechanical function with biofactor delivery so as to enable balanced degeneration in which the regenerated tissue assumes function as the scaffold slowly degrades. For tissue engineering, it can be correctly said that the art of scaffolding is where to put the holes and the biofactors. The scaffold architecture must be built layer-by-layer in order to maintain the appropriate porosity as mass-transport requirements for cell nutrition, porous channels for cell migration and surface features for cell attachment necessitates a porous scaffold structure. Moreover, providing adequate mechanical support is a critical scaffold requirement.[5,8,21,22]Thus, the scaffold plays a vital role in the development of engineered tissues and organs and it should mimic the structure and biological function of native ECM as much as possible, both in terms of chemical composition and physical structure.[18,23]

3. Application of Nanotechnology in Biomaterial Scaffolds

'Size does matter in tissue engineering'. To recapitulate proper function and organization of native tissues in tissue engineering approaches, it is important to mimic tissue properties at the nanoscale. To ultimately engineer the functional units of the tissue, not only the supercellular and cellular scale structures, but also the sub cellular scale structures (0.1-10μm) and nanostructures (1-100nm) need to be constructed to control cellular environment, cell-molecular interactions and cell-cell interactions. This is due to the fact that proteins contained in the extra cellular matrices are nanostructured, thus, cells in our bodies are accustomed to interacting with nanophase materials. Despite this fact, current materials used in tissue engineering scaffolds possess conventional surface features only. Using nanotechnology, biomaterial scaffold can be manipulated at atomic, molecular and macromolecular levels and constructed into specific geometrical and topological structures at 1-100nm scales. Nanophase materials (materials with constituent dimension less than 100nm in at least one direction) have the ability to mimic the dimensions of constituent components of

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natural tissues like proteins.[24]As nanofibres provide a connection between the nanoscale world and the macroscale world, use of modified nanofibres in tissue restoration is expected to result in an efficient, compact organ and a rapid recovery process due to the large surface area offered by nanofibres.[25,26] Nanofabricated and micro fabricated tissue engineering scaffolds also have the potential to direct cell fate as well as to regulate processes such as angiogenesis and cell migration. Other advantages of using nanotechnology for scaffold fabrication includes enhanced biocompatibility, improved contact guidance, reduced friction, reduced need for revision surgery and tissue growth promotion around the implant. It has been found that nanosculpturing the surface of the scaffolds may stimulate cell growth. The cells rapidly follow the nanoscopic-etched tracks, resulting in a faster filling of the matrix with the required cells or tissues.[27,28] Thus, using nanotechnology, tissue engineered products with highly predictable biological and physical properties may be obtained.

4. Source of Cells for Tissue Engineering

Cells for tissue engineering are obtained from a small piece of donor tissue, which is dissociated into individual cells. These cells are either implanted directly or are expanded in culture, attached to a support matrix, and then are implanted into the host after expansion. Cells used in tissue engineering can be derived from numerous sources, including primary tissues and cell lines.[4,7]Cells may be (a) autologous (self) (b) allogenic (nonself, same species) or (c) xenogenic (animal, other species). Autologous cells, besides being easy to isolate and expand in vitro, offers the advantage of manipulation with minimum risk of adverse host response and tissue manipulation. Allogenic cells offer the advantage of banking prior to need, but is more likely to be complicated by the presence of disease transmitting viruses. Moreover, both allogenic and xenogenic cells are more likely to generate an adverse response from the host.[29,30] Ideally, the cells should be nonimmunogenic, highly proliferative, easy to harvest and have the ability to differentiate into a variety of cell types with specialized functions.[7]

5. Stem Cells and Tissue Engineering

Current strategies for tissue engineering depend on autologous cells from diseased organs of the host. However, in extreme circumstances such as extensive end-stage organ failure, a tissue biopsy may not yield enough normal cells for expansion and transplantation. Thus, stem cells hold great promise as an alternative source of cells for treating damaged tissue where the source of cells for repair is extremely limited or not readily accessible.[31~33] Embryonic stem cells (ESC) are attractive because of their remarkable properties. They have the ability to proliferate in an undifferentiated, but pluripotent state (self-renewal) and the ability to differentiate into many specialized cell types. Human ESCs have been shown to differentiate in vitro from all three embryonic germ layers. However, though ESC has the highest potential to differentiate into different tissues, harvesting human ESC requires destruction of the human embryos that raises significant human and ethical concerns.[34~37]

Therapeutic cloning may be an alternative for generating a viable source of ESC that avoids the ethical and political controversies. Therapeutic cloning is used to generate cloned embryos that are explanted in culture and that can give rise to embryonic stem cell lines whose genetic material is identical to that of its source. These autologous stem cells would be useful in tissue engineering and tissue replacement applications as these have the potential to become almost any type of cell in the adult body.[38~40] Thus, theoretically, therapeutic cloning provides an alternative source of cells that may be limitless.

Adult bone marrow stem cells can also be collected from circulation after mobilization with cytokines. These can then be used clinically to treat a range of blood disorders. It has been reported that marrow-derived stem cells can give rise to hepatocytes, cardiac muscle cells and lung tissues. This suggest that efficient recruitment of bone marrow stem

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cells to sites of injury or their injection into the target sites may provide a source of cells for tissue repair. [41,42] Thus, the use of programmable stem cells is an emerging approach to tissue engineering based therapies that includes embryonic stem cells, progenitor cells from adult tissues and mesenchymal stem cells derived from bone marrow and peripheral blood.[35,41~43]

6. Bioreactors

Cells and tissues once harvested are grown into an appropriate scaffold to facilitate the faster growth and proliferation and moreover it provides the required three dimensional architecture. Apart from this, in order to lure the cells to grow into the desired tissues/organs, they must be provided similar in vivo growth environment. The in vivo growth conditions can somewhat be mimicked by the use of bioreactors. Bioreactors provide researches with a system capable of controlling environmental factors such as pH, temperature, oxygen tension and mechanical forces. The use of these dynamic in vitro culture systems also results in the maintenance of sterility and reduction in labor as these bioreactors when utilized in a closed manufacturing system allow for the seeding of cells as well as the growth, freezing and storage of the tissue engineered products all within the same container.[2,44,45] Future challenges in tissue engineering will include upgrading the bioreactor that puts shear and mechanical forces on developing tissues and that are competent in handling multiple cell types.

7. Applications of Tissue Engineering

a. Tissue Engineering in Orthopaedics: Musculoskeletal disorders have become one of the major health concerns because of an ageing population and increased occurrence of sports related injuries. Many orthopaedic disorders leave millions of people crippled, due to inefficient therapeutic methods. The conventional methods of treatment of orthopaedic disorders solves some, but not all the problems. In addition, several factors limit the use of transplanted grafts, including donor site morbidity, limited source, possible transmission of pathogens and problems associated with tissue storage. The use of synthetic nondegradable polymer prostheses yields unsatisfactory long term results. Prostheses produced from degradable polymers generally have inadequate mechanical strength and may degrade too rapidly, thereby limiting their near term clinical applications. Moreover, the half life of the implants may be a few years which necessitate the need of repetitive surgeries.[46]

Tissue engineering is an emerging alternative for improving existing treatments for bone disorders and for skeletal reconstruction. Among other things, engineering of bone, cartilage and ligament has been the prime focus of tissue engineers. Bone damage, due to pathologies or traumas, is a very common occurrence and represents a major problem in orthopaedics. The ability to generate new bones for skeletal use is a major clinical need. Autogenous grafts or allogenic bone, have been used for many years, but various problems such as failure of complete resorption of autologous bone, difficulties in shaping the bone grafts to fill the defects and lack of sufficient parent material greatly limit their potential as bone substitutes. Tissue engineering may be a way to circumvent the limitations of existing therapies.[47,48] Recently this task has become relatively easier with the isolation of growth factors such as transforming growth factor-β and its analogues, such as the bone morphogenic proteins (BMPs) BMP-2 and BMP-7 (OP-1). BMPs stimulate osteogenic precursor mesenchymal stem cells (MSCs) to form bone and these are being used clinically to enhance and accelerate bone repair and also to replace bone in association with tridimensional scaffolds.[49~51] The chemical composition of the scaffold is crucial for the osteoconductive properties and the resorbability of the material. Moreover, scaffolds should have an internal structure permissive for vascular invasion. Various scaffolds such as porous bioceramics (hydroxyapatites and tricalcium phosphate) are particularly advantageous as they are osteoconductive and they induce neither an

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immune nor an inflammatory response in the implanted host. Carbon nanotubes and nanofibres may also be of value in the development of novel devices for bone reconstruction.[52,53]

Apart from bone, cartilage, ligament and tendon injuries are also very frequent. The primary function of ligaments and tendons is to transmit mechanical forces Injuries and defects of the anterior cruciate ligament (ACL) and medial collateral ligament (MCL) frequently results in disabilities that can be permanent and disabling. This condition is further aggravated by its poor intrinsic healing capacity as it is enveloped by synovial fluid and lacks significant vascularity.[54] Many strategies have been used by tissue engineers to design the required orthopaedic tissues/organs that mimic the natural ones. The scaffolds designed for tissue engineered ligaments and tendons must possess appropriate mechanical properties to sustain the mechanical stresses experienced by normal ligaments and tendons. Collagen is a natural scaffold material for ligament and tendon replacements and cell cultures in collagen gels produce extracellular matrix and mimic cell alignment of ligaments in vivo.[55] Various combinations of scaffold biomaterials can also be tried (e.g. fibres of collagen or degradable polymers of PGA and PLA can be crosslinked/woven/braided) for the best possible mechanical strength of the resulting tissue engineered products.[56~61] Recently, because of their interesting mechanical properties, biocompatibility and biodegradability, silk-protein based matrices have been investigated for ACL tissue engineering.[62,63] Fibroblast-seeded collagen scaffolds have also been investigated due to their ability of cell attachment, proliferation and differentiation.[64] Autologous transplantation of MSC derived cells are recent cell-based approaches for the enhancement of ligament and tendon healing. The tendon-bone healing process has also been accelerated by growth factor delivery such as BMP-2 and BMP-12. Incorporation of these growth factors also improves biomechanical properties of the interface.[65]

Cartilage defects result from ageing, joint injury and developmental disorders cause join pain and loss of mobility. These injuries are also difficult to treat as cartilage generally has a limited capacity for self-repair.[66~68] Tissue engineering approaches can be used to repair articular cartilage defects and to restore joint functions. Tissue engineering may have the most important impact in the area of cartilage regeneration as cartilage does not have the ability for self-repair and it does not require vasculature for maintenance. For cartilage regeneration, either chondrocytes expanded in vitro or chondrocytes grown on three-dimensional scaffolds can be used. Chondrocytes and MSCs are most commonly used for cartilage regeneration.[26,47,69] Moreover, low oxygen environment also induces faster cartilage regeneration as cartilage has low oxygen requirements.[70,71] Growth factors (epidermal growth factor, transforming growth factor-β, insulin like growth factor etc.) have also been shown to promote cartilage growth and differentiation.[72,73]

A different approach was suggested by Green that seeding chondrocytes into synthetic biomaterials may yield new, viable cartilage.[67] Polymer scaffolds are primarily used for the delivery and retention of chondrocytes cells in cartilage tissue engineering. The materials of choice are biodegradable polymers of lactic acid and glycolic acid and their copolymers. The polymers are fabricated into three-dimensional, highly porous structures to allow cellular growth. PCL nanofibrous scaffolds also represent promising structures for tissue engineering applications as they are structurally similar to the ECM. The rationale for using nanofibres is that cells attach and organize well around fibres with diameters smaller than the diameter of the cells. Moreover, the nanofibrous scaffolds can be readily fabricated in any shape and size as needed and it also provides sound mechanical stability to provide a carrier for MSC transplantation in tissue engineered cartilage repair. Other products under investigation include epiphyseal growth plate, cranial

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sutures etc.[26,47]Thus, tissue engineering is a novel concept that may revolutionize the way orthopaedic disorders are treated as it incorporates aspects of engineering, biology and chemistry in designing the treatment methods.

b. Tissue Engineering: Advances in the Skin Trade: Skin is the body's largest organ and the body's first defense against disease causing organisms. It prevents dehydration, holds extensive capillary networks and sweat glands and maintains body temperature. Skin accommodates vitamin D synthesis, which is essential for normal bone and tooth structure. It also houses the nerves that receive stimuli of touch, pressure, heat, cold and pain, and relay them to the central nervous system.[75,76] Though skin cells can regenerate and repair themselves, the capacity for regeneration is very limited in the case of deep second degree and third degree burns. Chronic wounds also represent a different kind of challenge for wound healing. The most common chronic wounds include pressure ulcers and leg ulcers. Pressure ulcers are common among patients in long-term care settings and are characterized by tissue ischemia and necrosis. Among leg ulcers, venous ulcers are the most common, resulting from dysfunction of valves in veins of the lower leg that normally prevents the backflow of venous blood. Arterial insufficiency and diabetes also contributes to the development of leg ulcers which may not heal and in extreme cases, the limbs must be amputed. [77~79]

Human cadaver skin has been commonly used as temporary covering but insufficient supply, epidermal sloughing (requiring painful and costly removal and reapplication), immune rejection and disease transmission are the major limitations in this case. Alternatively, autologous grafts is also quite popular but though it provides timely wound coverage, it may lead to painful donor sites which are slow to heal and may be unsuccessful due to underlying deficiencies in wound healing. Moreover, autologous skin may not always be available in sufficient quantities. Tissue engineering is expected to have a great impact on wound repair. Skin is a difficult organ to transplant because of its strong immune defense system. Nevertheless, it has a relatively simple structure, making it a good testing ground for the talents of tissue engineers.[77~79]

The ideal tissue engineered skin should be bilayered and shear resistant. The skin product must be nontoxic and it should be minimally antigenic to reduce the risk of rejection. Production and application of the product must be achieved in a cost effective manner to ensure success, tissue engineered products must be designed to promote rapid revascularization by stimulating angiogenesis. Skin with both dermal and epidermal components is grown in the lab using a combination of cells and various polymer carriers and engineered skin products were the first tissue engineered products the FDA approved for clinical use.[80,81]Scaffolds for bioengineered skin include polyglycolic acid, polylactic acid and their copolymers. Hyaluronic acid is also slowly being recognized as potential scaffold biomaterial. By nature of its propensity to form highly hydrated and viscous matrices, it imparts stiffness, resilience and lubricious faculty to various tissues. Moreover it is a major constituent of the ECM, where it has a profound influence on a variety of cellular events includind cell migration and proliferation. Wound dressing with electrospun nanofibrous membrane can also be useful as it meets the requirement such as higher gas permeation and protection of wounds from infection and dehydration. Polycaprolactone nanofibres also support fibroblast-cell cultures. The medical profession is using artificial skin technology to pioneer organ reconstruction and significant advances have been made in the area of tissue engineered skin and a variety of living and nonliving substitutes are already available.[75,76,82,83]

c. Cardiovascular Tissue Engineering: The prevalence of atherosclerotic arterial disease is increasing in an ageing society.[84] Cardiac and peripheral vascular diseases remain a

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significant cause of morbidity and mortality in the Western world. Successful treatment has been limited in many situations by the poor performance of synthetic materials used for tissue replacement. Current surgical therapy for diseased vessels less than 6 mm in diameter involves bypass grafting with autologous arteries or veins.[85] Vascular grafting is a common surgical practice but it has significant limitations and complications. Arterial conduits have restricted dimensions and are limited in supply while venous conduits may have various degenerative alterations that can lead to aneurysm formation during high pressure arterial circulation. Moreover, allografts are problematic because of a high rate of rejection. Synthetic materials based on expanded polytetrafluoroethylene (ePTFE) and polyethylene terephthalate (Dacron) though used for the construction of heart valves, blood vessels etc. may carry some risk of rejection and thromboembolic complications.[86,87]

Some other problems associated with synthetic vascular grafts include platelet adhesion and activation and a decreased compliance compared with the adjacent arterial tissues. In addition, a significant portion of the affected patient population is children with congenital defects. Therefore, a severe limitation of all of the treatment modalities available is the inability to grow and remodel with the surrounding tissue. Treatment of heart ailments is also difficult because heart lacks the ability to regenerate, as it lacks a population of proliferating cells.[88,89] There are also no stem cells and injury and injury to the myocardium, such as myocardial infarction, results in an irreversible loss of cells and replacement by fibroblast. 88 Because of these problems, significant efforts are being made in myocardial tissue engineering. Cardiovascular tissue engineering is focusing on the development of blood vessels, heart valves and myocardium. Tissue engineering techniques may also be used to improve the function of the native tissues, such as congestive heart failure.

An ideal substitute should be non-obstructive, non-thrombogenic, infection resistance, chemically inert and non-hemolytic, durable and easily and permanently inserted.[90] It should have mechanical properties closely matching those of normal vessel to withstand the pressures associated with blood flow. There are various approaches to cardiovascular tissue engineering. Cells can be transplanted directly to the desired site or alternatively cells can be seeded onto a biodegradable scaffold which is gradually moulded into desired shape. The biodegradable polymers such as PGA and PLA are well suited for the delivery of a large number of cells. It also facilitates vascularization and structural integration of the new tissues with surrounding native tissues after implantation due to its high porosity and surface area. Strategies are now being developed to encourage the formation of endothelial or smooth muscle cells from undifferentiated stem cell precursors.[91~93] This provides purified cells capable of very high cell-doubling capacity for long periods of time. These progenitors are also capable of differentiating into more than one lineage: haematopoetic, mesenchymal and endothelial and mesenchymal stem cells have been shown to differentiate into cardiomyocytes and vascular like structure when stimulate in vitro.[94,95] Furthermore, these can differentiate into cardiomyocytes and endothelial cells when transplanted into various in vivo models.[96,97] Thus, cardiovascular tissue engineering will certainly be central to the development of a central heart and cardiovascular tissue engineering is focusing on the development of blood vessels, heart valves and myocardium. Current work on tissue-engineered blood vessels will one day create an ideal blood vessel substitute. Research on alternative heart valves is focusing on the development of a functional identical copy of a healthy heart valve in order to reduce the risk of infection and the need for life-long anticoagulation drugs. Stem cell technologies and gene therapy will also likely contribute significantly to the field of cardiovascular tissue engineering

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d. Dental Tissue Engineering: Damaged or missing teeth are a large and significant problem both from the aesthetic and practical point of view. Periodontal disease is one of the most widespread disorders of mankind and dental caries remain one of the most prevalent young adult and childhood diseases.[98] Current treatment modalities include dentures, bridges or implants. Dental implants are synthetic tooth replacements, usually consisting of titanium post implanted in the jaw bone, supporting a ceramic/porcelain crown. These synthetic implants though available are not the 'real thing'. New advances in stem cell biology and tissue engineering are leading to the development of cutting edge approaches to dentistry, both in the repair and replacement of teeth. Through dental tissue engineering, the hopes are to regenerate dentoalveolar tissues including alveolar bone, periodontal ligament, dentin and enamel and perhaps to grow whole new teeth.[98,99] BMPs have proved to be an important tool in this respect. BMPs have the capacity to stimulate bone formation in different bones, including jawbones. They also stimulate alveolar bone formation around teeth and induce the regeneration of periodontal attachment.[100,101] Evidence suggest that even if the odontoblast (cells that produce dentin) are lost due to caries, it may be possible to induce formation of new cells from pulp tissue using certain BMPs.[102,104]

Recent isolation of stem cells (odontoblast stem cells) from the human periodontal ligament represents potential therapeutically viable tissue-forming cells for its regeneration.[105] It appears, therefore, that BMPs are growth and differentiation factors which can stimulate the differentiation of pulpal stem cells into odontoblast as both bone and dentin matrices (contains BMPs) stimulate dentin formation when implanted into the dental pulp and this effect can be mimicked by recombinant BMPs.[104]Tissue engineering of dental pulp may also be possible using cultured fibroblast and synthetic polymer matrices. 106 Growth factors can also be incorporated into inert or biodegradable materials for promoting periodontal tissue engineering.[100,107~110] Regeneration of enamel also presents significant challenges to tissue engineers as regeneration of enamel is more problematic than that of dentin and dental pulp. In addition, as there are practically no stem cells for the enamel-production in adult tissues, enamel does not regenerate after traumatic injury. Nevertheless, a ray of hope is evident from the work of Harada et al. who have identified epithelial stem cells in the cervical loop of mouse incisors. [111] Tissue engineers are also investigating various prospects to grow new teeth.[112,113] Though regeneration of whole new organs like teeth is certainly more demanding but by understanding the genetic control of the key processes that form teeth in the embryo, the development of a tooth could be recreated in the mouth of an adult patient.

e. Corneal Tissue Engineering: The cornea is a transparent convex and avascular tissue, which comprises one-sixth of the anterior surface of the eye. It serves as the gateway of the external images into the eye and it accounts for more than two thirds of the total refractive power of the eye. Blindness caused by damage to or destruction of the cornea, either by accident or disease, is accompanied by reduced quality of life, disability and social isolation. Corneal transplantation is the conventional method for treating these conditions. Moreover, corneal transplantation represents the most successful transplants due to the relative inaccessibility of the avascular cornea to the cells of the immune system and corneal grafts require only local immune suppression. With improved techniques, immunosuppression etc. it is expected that the number of candidates for corneal transplantation and thus the need for corneal tissue will increase. Though, corneal transplantation is a viable option, it too has its limitations which necessitate the development of various new technologies. First and foremost is the availability of sufficient donor tissue for transplantation. Corneas are harvested and stored in specially designed media, and range in quality depending upon medical conditions before death, length of storage and surgical technique. Furthermore, an ageing population, as well as vision correction procedures such as laser eye

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surgery makes the collection of viable, healthy donor corneas for transplantation difficult. Finally, with over six million people blinded worldwide by infectious diseases of the cornea, there is a tremendous unmet need for corneal tissue due to lack of donor tissue. Thus, there is an urgent need to develop viable alternatives to the use of donor tissue.

Tissue engineering of the cornea emerges as a challenging field to the researchers' world wide as it can serve as a new modality of treatment of the corneal disease.[114,115] Tissue engineering of the cornea represents a paradigm shift in medical treatment to overcome the present disadvantages of corneal transplantation, primarily immune rejection and the shortage of donor corneas. Transplantation of cultivated corneal epithelial cells expanded ex vivo from corneal epithelial stem cells has been developed and has already entered the clinical realm. However, there remain many hurdles to be overcome. It will be a real breakthrough, allowing diseased or damaged corneas to be replaced by tissue-engineered human corneal equivalence that resemble in all respects their natural counterparts. Tissue engineering can help tackle an international shortage in cornea donors caused by modern corrective surgery. The development of tissue engineered human corneas will also provide a non-animal alternative, which will therefore alleviate animal suffering. An added advantage is the availability of tissues for toxicity testing closer to the natural human tissues.[116,117] This will also reduce the risk of passing on transmissible diseases through surgery.

f. Applications of Tissue Engineering in the Genitourinary Tract: Tissue engineering may also play a vital role for alleviating the problems and disorders associated of the genitourinary tract. The genitourinary tract is susceptible to both congenital abnormalities as well as to acquired disorders, such as cancer, trauma, infection, inflammation and other conditions. All these may lead to organ damage or loss of tissue and eventual reconstruction. Apart from these, other problems associated with the genitourinary tract is urinary incontinence and problems with the proper functioning of the bladder, ureter, urethra etc. Though organ reconstruction is possible, but shortages of patients own tissues may be a limitation. Moreover, there is a significant degree of morbidity associated with the harvest procedure.[118] In addition, these approaches rarely replace the entire function of the original organ. Many other problems also limit its use, as for instance gastrointestinal segments are commonly used as tissues foe bladder replacement or repair. However, when gastrointestinal tissue is in contact with the urinary tract, many complications may emerge e.g. infection, metabolic disturbances, urolithiasis, perforation, increased mucus production and malignancy.[119~122] These problems have aggravated the intervention of tissue engineers to find a solution to the ever increasing problems of the genitourinary tract. The success of using tissue engineering strategies for various reconstruction purposes depends on the ability to use donor tissue efficiently and to provide the right conditions for long-term survival, differentiation and growth. Many tissue engineering strategies have been recently applied clinically. These include the use of cells as bulking agents for the treatment of vesicouretral reflux and incontinence, urethral replacement and bladder reconstruction[32,118,123~125]. Thus, engineered urologic tissues may have wide clinical applications in the future.

8. Challenges and Possibilities

The promise of tissue engineering is great, but there exist major challenges that must be met for this new field to reach its potential application. A critical challenge in tissue engineering is to regenerate tissues that grow and/or remodel in concert with the changing needs of the human body. Another principle requirement for tissues engineered in vitro is the sufficient supply of oxygen and nutrients and the removal of carbon dioxide and waste as they do not have their own blood supply. Thus, a well established vascular network is essential. A solution to this may be the use of engineered scaffolds to slowly release growth factors such as vascular endothelial growth

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factor (VEGF) or fibroblast growth factor (FGF) that induce or speed up angiogenesis.[6] Furthermore, the need for preformed vascular beds or rapid angiogenesis can be avoided by the use of stem and other progenitor cells as they are resistant to low oxygen conditions.[6,98,126] Apart from this, the tissue engineered products should be subjected to appropriate stress and mechanical forces similar to that of the in vivo environment. Future challenges in tissue engineering will include the upscaling of bioreactors that put shear and mechanical forces on the developing tissues and the production of final products involving multiple cell types. The successful large-scale production of engineered tissues requires an adequate source of healthy expandable cells, the optimization of scaffolds and the creation of bioreactors which mimic the environment of the body and that is amenable to scale-up.[2,6] Moreover, cells must be expanded and introduced back into their three-dimensional architecture without being genetically altered or contaminated. Understanding the mechanisms of interactions among cells, growth factors and biomaterials in tissue engineering will undoubtedly advance the end goal of developing off-the-shelf tissue engineered products. Advances in cell sourcing, particularly using stem cell precursors and a better understanding of extracellular matrices and their interactions is also crucial.[127] Regulatory issues also present a major challenge to the development of the tissue engineering industry. Every newly developed, tissue engineered product must be successfully undergo expensive clinical trials and the approval of the regulatory authority before they are released to the market.[128,129] Thus, the cost of these tissue engineered products should also be taken into consideration. Additional challenges include the preservation of the products so that it has a long shelf-life and the successful use of various approaches to prevent tissue rejection.

9. Conclusion

Despite the myriad of challenges, the field of tissue engineering will definitely pave a way towards a better health care system for mankind. It may be possible, in the years to come, to harbor organs and tissues in advance, should the need arise in future. Tissue engineering will definitely leap forward from 'Science Fiction' to 'Science Fact' and this technology will certainly have a major impact in future health care practice.

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