SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOMEDICAL ENGINEERING

COURSE MATERIAL

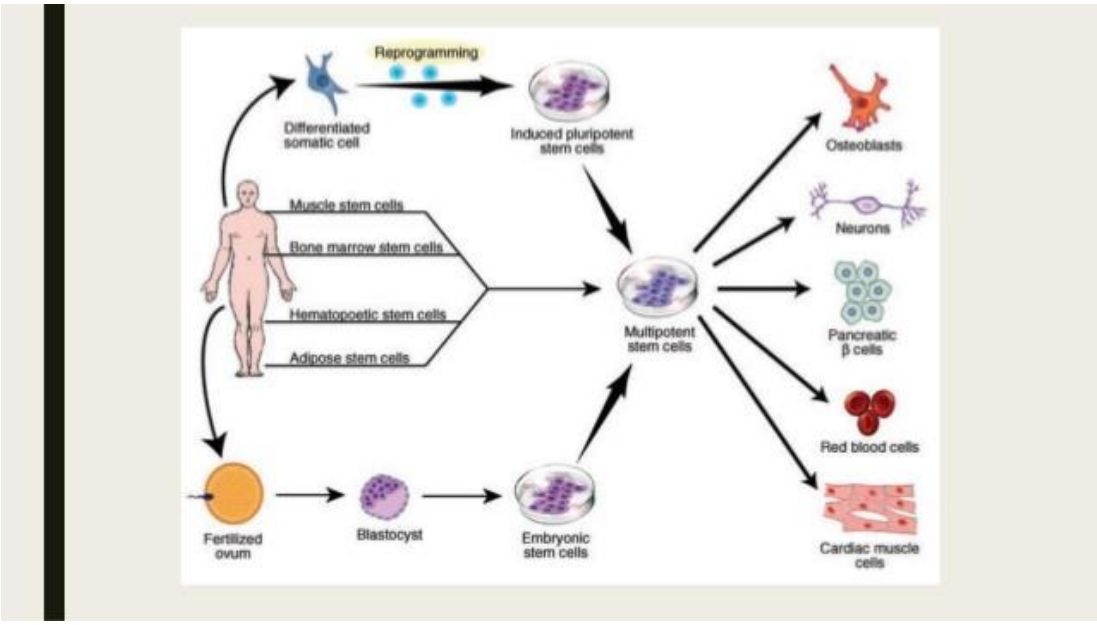
SUB.NAME: Artificial organs & Tissue Engineering UNIT V SUB. CODE: SBM1306

STEM CELLS – The Biology of Stem cells – Embryonic stem cells – adult stem cells – aging of stem cells – the importance of stromal cells - Tissue engineering of Bone marrow

STEM CELLS

Stem cells in tissue engineering. The concept of producing 'spare parts' of the body for replacement of damaged or lost organs lies at the core of the varied biotechnological practices referred to generally as tissue engineering.

Tissue engineering based on stem cells has gained interest recently as attempts are made to engineer scaffold environments mimicking the stem cell niche, which contains a reservoir of multipotent stem cells that can maintain normal tissue or restore unhealthy cell populations in response to mechanisms of quiescence, self-renewal, and differentiation of the stem cells. These cell behaviors are governed by soluble signals that are systemic or presented by local niche cells. In this review, current and emergent approaches based on stem cells in the field of tissue engineering are presented for specific applications of human tissues and organs. The combination of stem cells and tissue engineering opens new perspectives in tissue regeneration for stem cell therapy because of the potential to control stem cell behavior with the physical and chemical characteristics of the engineered scaffold environment.



Chronic limitations of traditional transplantation surgeries still exist due to the lack of appropriate donor tissues, risk of disease transmission, and potential for immune rejection. Tissue engineering, the multidisciplinary application of biology, chemistry, physics, engineering, and medical science, offers an alternative method to overcome these issues [1,2]. For therapeutic application of tissue engineering, engineered tissue is grown either within a patient or outside the patient and subsequently transplanted into the patient. Figure 1 provides a schematic representation of the process of tissue regeneration in tissue engineering. Human cells are harvested from a patient and after in vitro cell culture, cells are seeded onto scaffolds with medium containing chemical stimuli, such as growth factors and differentiation-inducing factors. Scaffolds are three-dimensional (3D) matrices that support cellular growth processes, such as cell adhesion, migration, proliferation, and differentiation, by which cells are colonized onto the scaffold. The cell-colonized scaffold is then implanted into the patient, to regenerate biocompatible, immunocompatible, and biofunctional tissues or organs inside the patient body. Cells and scaffolds are essential to regenerate new tissues with tissue engineering. Cells become the primary component of the engineered tissue and the scaffold provides cells with an appropriate physical and chemical environment where they

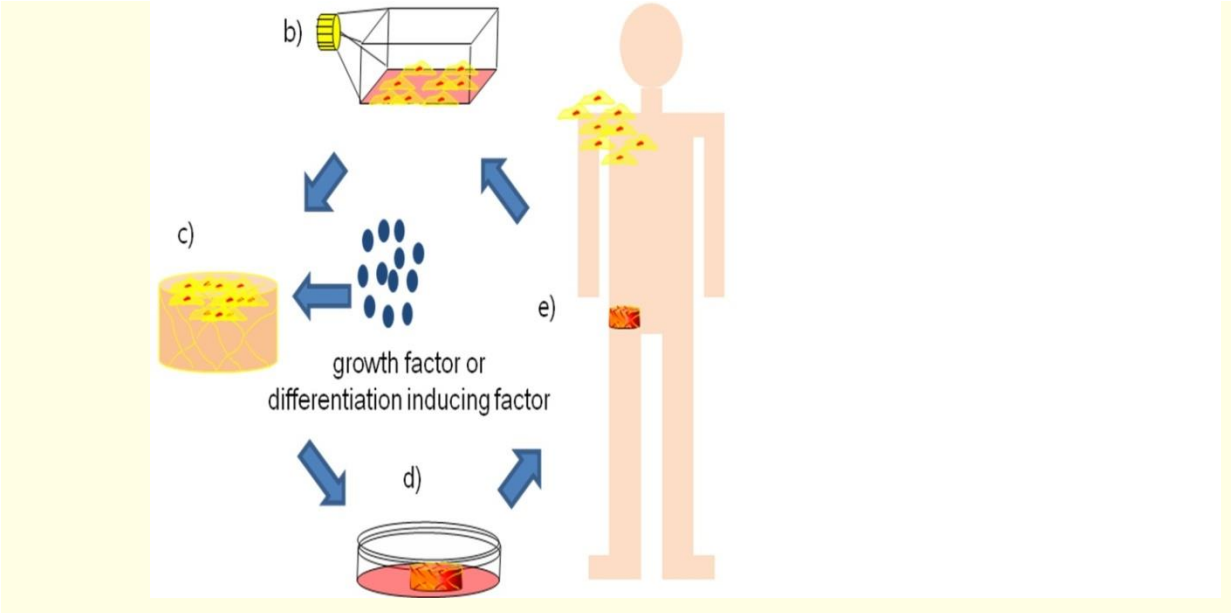


Figure 1. a) Tissue harvested from a patient's body to obtain cells b) in vitro cell culture c) a scaffold seeded with cells d) in vitro cell culture for cell colonization of the scaffold e) implantation of cell-colonized scaffold within the patient.

can attach to the surface of the scaffold, migrate through the scaffolds' pores, and then proliferate. In some instances, such as stem cell therapy, collaboration of cells and scaffolds with differentiation-inducing factors is essential for stem cells to differentiate into engineered cell lineages and to develop new tissues.

A scaffold is a 3D matrix that provides the framework and initial structural support for cells to attach, proliferate, and differentiate, facilitating the formation of an extracellular matrix (ECM) Characteristics of an ideal scaffold include: 1) contains a network of interconnecting pores so that cells can attach, proliferate, and migrate throughout the entire scaffold; 2) has channels through which oxygen and nutrients are provided to cells and waste products are carried out; 3) is biocompatible with a high affinity for cells to attach and proliferate; and 4) has appropriate mechanical properties Various processing techniques have been used for fabricating scaffolds which have biocompatibility and appropriate surface properties to support cellular attachment, proliferation and differentiation. Examples of scaffold fabrication methods include emulsion/freeze-drying, solvent casting/particular leaching, computer-aided design/computer-aided manufacturing, electrospinning, nanofiber self-assembly, and photolithography.

For cell based tissue engineering, cells are usually seeded onto scaffolds which are made of materials such as acellular tissue matrices, naturally derived materials (natural biomaterials), and synthetic polymers (synthetic biomaterials). Acellular tissue matrices may be animal or human-derived with all cells removed during manufacture and natural biomaterials extracted from animal sources, such as fibrin, collagen, gelatin, chitosan, alginate hyaluronic acid etc. Synthetic biomaterials fabricated from laboratories or factories, such as polycaprolactone (PCL) [40], polylactic acid (PLA) poly(glycolic acid) (PGA), poly(d,l-lactic-co-glycolic acid) (PLGA) polyvinyl alcohol (PVA) polyethyleneglycol (PEG) polyurethanes, carbon nanotubes (CNT), TiO_2 nanotubes, etc. are also widely used. Synthetic biomaterials have tunable mechanical properties, however, the biocompatibility of natural biomaterials is better than synthetic

materials, thus, hybrids of natural and synthetic materials are also used for scaffold fabrication. To support tissue regeneration for in vitro stem cell study, differentiation-inducing factors can be loaded into scaffolds to promote and to induce differentiation of stem cells, but these factors under specific circumstances remain indispensable. Achieving success in tissue engineering is attributed only to stem cells and scaffolds, suggesting that the effects of differentiation factors may be substituted with suitable scaffold structures.

2. STEM CELLS IN TISSUE ENGINEERING

Tissue engineering may be used for tissue regeneration such as bone, cartilage and neural tissues using degradable biomaterial scaffolds. For example, tubular collagen nerve guides (Neuragen from Integra Life Sciences) were used clinically to treat peripheral nerve injuries and the critical gap length treated by nerve guides was longer than 10 mm in primates and could be further increased by adding fibers or hydrogel with cells. In addition, tissue-engineered constructs for bone was osteoconductive to enhance bone cells to adhere, proliferate and migrate. For instance, PCL based scaffolds using fused deposition modeling was developed, approved by the FDA and used clinically as burr plugs and sheets for orbital floor reconstruction in more than 200 patients In addition, tissue engineering treatments for cartilage repair have been established clinically but are not widespread because of limitations in efficiency, consistency and applicability Furthermore, stem cells are used in in the field of various tissue engineering such as cardiac, neural, bone, liver tissue engineering, etc. and some examples of the use of scaffolds, biomaterials, and stem cells in tissue engineering were summarized in Table 1.

2.1. Cardiac Tissue Engineering

Congestive heart failure, resulting from myocardial infarction (MI) and ischemic loss of functional CMs, remains the leading cause of death in the United States. The complex events involved in ischemic myocardial cell loss, and the subsequent post-MI remodeling leading to heart failure are not efficiently addressed by existing therapies. Tissue engineering and stem cell therapy could be a promising approach for cardiac repair. Natural acellular scaffolds made of hydrogels have the mechanical structure to support the infarcted heart, reducing wall stress, compensating for contraction function, and inhibiting ventricle remodeling. In vivo study has shown that hydrogels alone can provide mechanical support to the infarcted heart by attenuating

wall stress, compensating for contraction function and preventing ventricle remodeling. Basic fibroblast growth factor (bFGF) plays an important role in angiogenesis and bFGF encapsulated in heparin-alginate microspheres, within a pig model of chronic MI, demonstrated significant enhancement in myocardial function in vivo. Hydrogel scaffolds have also been used in vitro for cell expansion and the induction of cardiogenic differentiation. For example, cell-cell interactions of aggregates of CMs, derived from skeletal muscle-derived stem cells (MDSCs), were enhanced in collagen scaffolds. The expression of cardiac genes, including connexin 43 and cardiac troponin-T were also enhanced, suggesting that MDSCs within collagen scaffolds is a useful 3D culture system to directly assess the contractile properties of differentiated CMs in vitro. In addition, evaluation of a composite scaffold made of the natural and synthetic biomaterials, collagen and PGA, in a perfusion bioreactor demonstrated enhanced attachment of cardiac stem cells (CSCs). Moreover, physical stimuli such as mechanical stress promoted 2-fold increases in CMs, in addition to matrix fiber alignment, myofibrillogenesis and sarcomeric banding, while cyclic mechanical stress increased CM hypertrophy (2.2-fold) and proliferation rates (21%) when compared to controls with no mechanical stress.

2.2. Neural Tissue Engineering

The central nervous system (CNS), consisting of the spinal cord and the brain, is a very unique tissue network with an unusual ECM structure and characteristic soft physical properties (elastic modulus of natural brain tissue is around 500 Pa) when compared to muscle (10^4 Pa) and bone (10^9 - 10^{10} Pa), which is susceptible to damage, illnesses, and injuries, including traumatic brain injury, spinal cord injury, stroke, Parkinson's disease, and multiple sclerosis. The mechanical properties, structure, and composition of the ECM are effectors of cell function, thus, soft hydrogel scaffolds are utilized for CNS applications to mimic the biochemical and mechanical properties of the CNS. For instance, hydrogel scaffolds made of acrylamide and PEG with argininegly cine-aspartic acid (RGD) can regulate cell behaviors, such as adhesion, cell renewal, and differentiation of neural stem cells (NSCs). Platelet-derived growth factor (PDGF)-AA immobilized agarose scaffolds have been reported to support differentiation of NSCs and neural progenitor cells (NPCs) to oligodendrocytes. Hydrogel scaffolds made of AcN-RADARADARADARADAIKVAV-$CONH_2$ ($RADA_{16}$-IKVAV) have been shown to serve as a

guiding cue to direct NSC adhesion and neural differentiation with in vitro and in vivo to direct stem cell differentiation toward neural lineages and to promote the signal transmission among neurons because of electrical conductivity. The hydrogel in a rat brain surgery model enhanced survival of NSCs, reduced the formation of glial astrocytes, and improved brain tissue regeneration after 6 weeks post-transplantation. In addition, CNT is used. For example, electrical stimulation was shown to enhance the proliferation and differentiation of NSCs on thin film scaffolds made of laminin and single-wall carbon nanotubes (SWCNT). Bioelectricity also has been shown to affect intercellular signaling of the nervous system, as fibrous scaffolds made of poly-l-lactide/polyaniline (PLLA/PANi), applied with an electric field of 100 mV/mm for a period of 60 minutes, showed extended neurite outgrowth compared to cells grown on non-stimulated scaffolds.

Applications	Scaffold	Components	
Cardiac tissue engineering	Injectable hydrogel [45]	alpha-CD/PEG-PCL-PEG	
Cardiac tissue engineering	Injectable microsphere [53]	bFGF/heparin-alginate	
Cardiac tissue engineering	Hydrogel [54]	collagen	CM
Cardiac tissue engineering	Sponge/nanofiber [55]	PGA/collagen	
Cardiac tissue engineering	Hydrogel [56]	Collagen	
Neural tissue engineering	hydrogel [59]	RGD/acrylamide/PEG	
Neural tissue engineering	Hydrogel [60]	PDGF-AA/agarose	
Neural tissue engineering	Hydrogel [61]	$RADA_{16}$/IKVAV	
Neural tissue engineering	Thin film [62]	Laminin/SWCNT	
Neural tissue engineering	Fibrous mesh [63]	PLLA/PANi	
Bone tissue engineering	Fibrous mesh [65]	PLGA/collagen/hydroxyapatite	
Bone tissue engineering	Hydrogel [66]	Calcium phosphate/collagen	Hu
Bone tissue engineering	Hydrogel [67]	ACP/collagen	
Bone tissue engineering	Sponge [68]	cMWCMT/PLGA	
Bone tissue engineering	Spheroid [69]	N/A	
Liver tissue engineering	Fibrous mesh [71]	PLGA	
Liver tissue engineering	Fibrous mesh [72]	PCL/collagen/polyethersulfone	

Table 1. Scaffold biomaterials and stem cells used in tissue engineering.

2.3. Bone Tissue Engineering

Bone is a connective tissue consisting of a collagenous ECM that is extensively mineralized with hydroxyapatite ($Ca_{10}[PO_4]_6[OH]_2$) and other ions that contribute to the high density and strength of bone, as well as its homeostatic regulation and metabolic function. Collagen, however, has poor structural stability, thus, scaffolds made of hybrid materials, consisting of natural and synthetic components, are utilized in bone tissue engineering. For example, fibrous scaffolds made of PLGA/collagen/hydroxyapatite were shown to provide structural stability and mechanical integrity, as well as improve human MSC (hMSC) binding. Inorganic materials such as calcium phosphate or amorphous calcium phosphate (ACP) may enhance the structural stability of collagen scaffolds. Scaffolds made of a composite of collagen and ACP, for example, have been shown to support the proliferation and osteogenic differentiation of MSCs [67], while rat MSCs (rMSCs) on carboxyl-functionalized MWCNT (cMWCNT)/PLGA composites showed enhanced levels of alkaline phosphatase produced by osteoblasts. In addition, bone is a highly metabolic tissue requiring an abundant vascular supply throughout its structure for homeostasis, growth, and remodeling. As a result, 3D co-culture systems based on biomaterials have been studied for concurrent angiogenesis/vasculogenesis and osteogenesis. A spheroid co-culture system of bone marrow derived mesenchymal stromal cells (bmMSC) and human dermal microvascular endothelial cells (HUVECs) produced well-organized 3D vascular structures in vitro and resulted in increased alkaline phosphatase expression when compared to a control culture system of bmMSCs and fibroblasts.

2.4. Liver Tissue Engineering

The liver is the largest organ in the human body and has major roles in metabolism, detoxification, and protein synthesis. Hepatocytes, the major cell type in the liver, execute most of the metabolic, synthetic and storage functions of the liver. The interactions between hepatocytes and non-parenchymal cells also affect the function of the liver. Within in vitro culture environments, hepatocytes tend to lose their function, suggesting that stem cells could be

an alternative cell source in combination with supplementary factors, such as differentiation-inducting factors, for liver tissue engineering. Evaluation of rat ESCs (rESCs) cultured within a 3D culture system were shown to differentiate into hepatic-like cells with morphological characteristics of typical mature hepatocytes in the presence of supplementary factors, such as recombinant mouse hepatic growth factor, fibroblast growth factor, insulin, transferrin, selenium, oncostatin, and dexamethasone. Additionally, when these stem cellbearing scaffolds were transplanted into severe combined immunodeficient mice, the rESCs remained viable, undergoing further differentiation and maturation of hepatic-like cells in vivo. Studies of PCL/collagen/polyethersulfone composite scaffolds showed that these scaffolds promoted hMSC differentiation to hepatocytelike cells and the expression of hepatocyte-specific markers, such as albumin, α-fetoprotein, cytokeratin-18, cytokeratin-19, and cytochrome P450 3A4 at mRNA levels, where the number of albumin-positive cells cultured on the scaffold (47% ± 4%) was higher than that in the two-dimensional culture system (28% ± 6%) in vitro. However, additional functional assessment of hepatocyte like cells was needed because of the uncertainty of their functionality when compared to adult hepatocytes.

2.5. Other Applications

In addition to cardiac muscle, nerve, bone, and liver applications as described above, the combination of stem cells and tissue engineering could apply to regenerate other tissue types, such as the eyes, cartilage, skin, bladder, and tendon. Clinical trials of strategies using a combination of tissue engineering and stem cells to regenerate bladder, kidney, and urethra tissue are already underway.

The stem cell theory of aging postulates that the aging process is the result of the inability of various types of stem cells to continue to replenish the tissues of an organism with functional differentiated cells capable of maintaining that tissues (or organ's) original function.

All tissue is composed of parenchymal (from Greek, that poured in beside) and stromal (Greek, framework or foundation) cells. Parenchyma are the functional cells of a tissue (e.g., for liver,

hepatic parenchymal cells or hepatocytes; for bone marrow, hematopoietic cells), where stroma comprises primarily connective tissue elements which, together with their products, form the structural framework of tissue. Parenchymal cells can be derivatives of any of the three germ layers, and during development they usually grow into areas populated by stromal cells or their progenitors. Under the strictest definition, stromal cells are derivatives of mesenchyme and include fibroblasts, osteogenic cells, myofibroblasts, and fat cells which appear to arise from a common stem/progenitor cell. Some investigators apply the term stromal cell to all the nonparenchymal cells that contribute to the microenvironment of a tissue and include endothelial cells and macrophages (histiocytes) in this classification as well. However, the ontogency of both endothelial cells and macrophages is distinct from that of mesenchymal tissue-derived cells. A partial listing of tissue cells that may influence the function of organ parenchyma. For the sake of brevity, migrating cells of bone marrow origin will not be discussed in the text (e.g., mast cells, B lymphocytes, natural killer cells), although these cells can influence parenchyma either directly or via cytokine-mediated modulation of stromal cell function.

One key component of the in vitro bone model is the scaffold, which provides a structural and logistic template for the developing tissue, and can markedly affect cell behavior. Several types of porous scaffolds have been shown to support in vitro bone formation by human cells, including those made of ceramics, native and synthetic polymers and composite materials. Scaff old properties important for bone formation include: the size, distribution and shape of the pores , surface roughness ; the presence of cell attachment sites and the biomechanics of both the material and the scaff old structure. In general, the most suitable scaff olds for bone formation are those with large and interconnected pores (which facilitate cell infi ltration and matrix deposition) and rough inner surfaces (which facilitate cell attachment), made of osteoconductive materials (such as bone protein and hydroxyapatite), and with mechanical properties similar to those of native bone (both to enable load-bearing and stimulate osteo genesis). Additional features of interest include aniso tropic structure, capacity for vascularization, and process ability into anatomically correct shapes. Scaffolds can also incorporate and modulate delivery of molecular signals controlling cellular functions. Another key component of bone tissue engineering is the culture system or bioreactor. Bioreactor systems

can be designed to control transport of nutrients and oxygen to cells in clinically sized constructs and provide lineage specifi c biological stimuli in various regions of the graft. Additionally, the development of functional, load bearing characteristics of the graft would be enhanced by the application of biophysical stimulation in order to attain mechanical competence in both the cartilage and bone regions. Advanced bioreactor designs maintain the physiological milieu in the cell microenvironment (pH, temperature, oxygen and nutrient delivery) by perfusion and conditioning of culture medium . Bioreactors can also be designed to recapitulate one or more of the developmentally relevant biophysical signals in a time-controlled manner. For example, increased mass transport and fluid shear by medium perfusion, and cyclic loading have been shown to improve osteogenesis and enable formation of homogenous bone constructs. Ideally, a bioreactor system should be capable of coordinating biological, physiological and mechanical stimuli, and applying them in a spatially and temporally controlled manner to provide lineage-specific stimulation within clinically sized grafts. The clinical and scientific utility of tissue engineering largely depends on our ability to predictably direct cells to differentiate into the right phenotypes in a spatially and temporally defined pattern. The control of environmental conditions provided through the design of bioreactors - in conjunction with scaffolds - can help gain more insight into the interplay of molecular and physical factors that guide the development of bone from various types of osteogenic cells. Understanding of the developmental process may then serve as feedback to the optimization of engineering parameters toward better graft designs, and towards the use of engineered grafts as models of development and disease. Sources of human osteogenic cells, ` there are several basic considerations when choosing a cell source for bone tissue engineering: the choice. Tissue-engineered bone constructs have the potential to alleviate the demand arising from the shortage of suitable autograft and allograft materials for augmenting bone healing. They also can serve as controllable in vitro models of high biological fidelity for studies of bone development, disease or regeneration. Each of the sources of osteogenic human cells - primary cells, MSCs, ESCs and induced pluripotent stem cells - has distinct advantages when used for bone tissue engineering, and the quest for an ‘ideal’ cell source is still in progress.