J. Biomedical Science and Engineering, 2014, 7, 67-74 **JBiSE**
http://dx.doi.org/10.4236/jbise.2014.72010 Published Online February 2014 (http://www.scirp.org/journal/jbise/)

Application of tissue engineering in stem cell therapy

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Received 17 October 2013; revised 25 November 2013; accepted 7 December 2013

ABSTRACT

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.

KEYWORDS

Stem Cell; Cell Behavior; Scaffold; Tissue Engineering; Tissue Regeneration

1. INTRODUCTION

1.1. Tissue Engineering

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

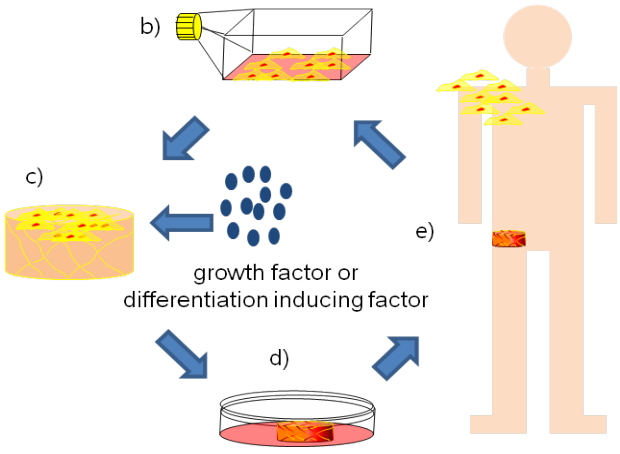

Figure 1. a) Tissue harvested from a patient's body to obtain cells b) *in vitro* cell culture c) a scaffold seeded with cells d) *in vitro* cell culture for cell colonization of the scaffold e) implantation of cell-colonized scaffold within the patient.

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.

1.2. Stem Cells

Although it is difficult to grow some cell types such as cardiomyoctes (CMs) and hepatocytes in large quantities, stem cells are undifferentiated biological cells that can produce more stem cells (self-renewal) and can differentiate into specialized cells (cell potency). Two broad types of stem cells exist: embryonic stem cells (ESCs) isolated from the inner cell mass of blastocysts, and adult stem cells found in various tissues, including the dermis [3], bone marrow [4], blood [5], muscle [6], adipose tissue [7], etc. Use of ESCs has been limited for tissue engineering because of the legal and ethical concerns regarding use of human ESCs [8]. These issues are less prevalent for adult stem cells, and as a result, adult stem cells, such as skeletal stem cells [9], neuronal stem cells [10] and mesenchymal stem cells (MSCs) [11] from human or non-human sources in tissue engineering have been widely investigated. For example, MSCs are used in tissue engineering because of their availability in various sources (bone marrow [12], muscle [6], trabecular bone [13], dermis [3], adipose tissue [7], periosteum [14], blood [5], and synovial membrane [15]); and their ability to differentiate to multiple connective tissue cell types (osteocytes [16], chondrocytes [17], adipocytes [18], and myocytes [19]) and other cell types (hepatocytes [20] and neuron [21]) in response to extracellular stimuli, including differentiation-inducing factors from protein and chemical origins. Biological growth factors from protein sources can induce differentiation of stem cells; chemical agents are also used for the specific differentiation of stem cells *in vitro*. Transforming growth factors beta 1 and 3 (TGF-β1 and TGF-β3), for example, have been reported to enhance the differentiation of MSCs to chondrocytes [22] and the chemical agent β-mercaptoethanol (BME) has been used for neural transdifferentiation of MSCs [23].

1.3. Scaffolds

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) [24]. 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 [25]. Various processing techniques have been used for fabricating scaffolds which have biocompatibility and appropriate surface properties to support cellular attachment, proliferation and differentiation [25]. Examples of scaffold fabrication methods include emulsion/freeze-drying [26], solvent casting/particular leaching [27], computer-aided design/computer-aided manufacturing [28], electrospinning [28], nanofiber self-assembly [29], and photolithography [30].

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 [31-33] and natural biomaterials extracted from animal sources, such as fibrin [34], collagen [35], gelatin [36], chitosan [37], alginate [38], hyaluronic acid [39], etc. Synthetic biomaterials fabricated from laboratories or factories, such as polycaprolactone (PCL) [40], polylactic acid (PLA) [41], poly(glycolic acid) (PGA) [42], poly(d,l-lactic-co-glycolic acid) (PLGA) [43], polyvinyl alcohol (PVA) [44], polyethyleneglycol (PEG) [45], polyurethanes, carbon nanotubes (CNT) [46], TiO_2 nanotubes [47], 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 [1].

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 [48]. 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 [49]. In addition, tissue engineering treatments for cartilage repair have been established clinically but are not widespread because of limitations in efficiency, consistency and applicability [50]. 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 [51]. 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 [52]. 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 [45]. 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* [53]. 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* [54]. 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) [55]. 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 [56].

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 [57,58]. The mechanical properties, structure, and composition of the ECM are effectors of cell function, thus, soft hydrogel scaffolds are utilized for

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

Applications	Scaffold	Components	Stem cells
Cardiac tissue engineering	Injectable hydrogel [45]	alpha-CD/PEG-PCL-PEG	N/A
Cardiac tissue engineering	Injectable microsphere [53]	bFGF/heparin-alginate	N/A
Cardiac tissue engineering	Hydrogel [54]	collagen	CMs derived from skeletal MDSCs
Cardiac tissue engineering	Sponge/nanofiber [55]	PGA/collagen	CSCs
Cardiac tissue engineering	Hydrogel [56]	Collagen	hiPSCs
Neural tissue engineering	hydrogel [59]	RGD/acrylamide/PEG	NSCs
Neural tissue engineering	Hydrogel [60]	PDGF-AA/agarose	NSCs/NPCs
Neural tissue engineering	Hydrogel [61]	$RADA_{16}$/IKVAV	NSCs
Neural tissue engineering	Thin film [62]	Laminin/SWCNT	NSCs
Neural tissue engineering	Fibrous mesh [63]	PLLA/PANi	NSCs
Bone tissue engineering	Fibrous mesh [65]	PLGA/collagen/hydroxyapatite	hMSCs
Bone tissue engineering	Hydrogel [66]	Calcium phosphate/collagen	Human adipose-derived stem cells
Bone tissue engineering	Hydrogel [67]	ACP/collagen	hMSCs
Bone tissue engineering	Sponge [68]	cMWCMT/PLGA	rMSCs
Bone tissue engineering	Spheroid [69]	N/A	bmMSC/HUVEC
Liver tissue engineering	Fibrous mesh [71]	PLGA	mESC
Liver tissue engineering	Fibrous mesh [72]	PCL/collagen/polyethersulfone	hMSC

CNS applications to mimic the biochemical and mechanical properties of the CNS [58]. For instance, hydrogel scaffolds made of acrylamide and PEG with arginine-glycine-aspartic acid (RGD) can regulate cell behaviors, such as adhesion, cell renewal, and differentiation of neural stem cells (NSCs) [59]. Platelet-derived growth factor (PDGF)-AA immobilized agarose scaffolds have been reported to support differentiation of NSCs and neural progenitor cells (NPCs) to oligodendrocytes [60]. Hydrogel scaffolds made of AcN-RADARADARADARA-DAIKVAV-$CONH_2$ ($RADA_{16}$-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 [61]. 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) [62]. 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 [63].

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 [64]. 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 [65]. Inorganic materials such as calcium phosphate [66] or amorphous calcium phosphate (ACP) [67] 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 [68]. 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 [69].

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 [70]. 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 cell-bearing scaffolds were transplanted into severe combined immunodeficient mice, the rESCs remained viable, undergoing further differentiation and maturation of hepatic-like cells *in vivo* [71]. Studies of PCL/collagen/polyethersulfone composite scaffolds showed that these scaffolds promoted hMSC differentiation to hepatocyte-like 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* [72]. 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 [73], cartilage [74], skin [75], bladder [75], and tendon [76]. Clinical trials of strategies using a combination of tissue engineering and

stem cells to regenerate bladder, kidney, and urethra tissue are already underway [77].

3. CONCLUSIONS AND FUTURE PERSPECTIVES

Advancements in the fields of stem cell biology and biomaterials science and engineering have been combined to produce strategies by which stem cell attachment, proliferation and differentiation *in vitro* are supported and enhanced. Although the combination of stem cells and tissue engineering is currently in the research phase and still far from clinical application, the combination of tissue engineering and stem cell biology has greatly enhanced the possibility of tissue regeneration. However, many different biomaterials such as nanobiomaterials that have not adapted for use with stem cell culture could be studied in near future. Meanwhile, the application of tissue engineering to stem cell therapy *in vivo* is highly expected to progress in the near future. Other future consideration for tissue engineering based on stem cell therapy is how to develop many methods of incorporating and producing a vascular network in a scaffold, allowing it to integrate with actual tissue or organs and restore function lost after injury. It could be achieved by co-culturing with endothelial cells or by embedding angiogenic factors such as vascular endothelial growth factors to promote angiogenesis into scaffolds. Overall, this review paper has provided a good starting point for future development of tissue engineering combined with biomaterial scaffolds for stem cell therapy.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Research Foundation funded by the Korean government (MEST; 2010-0020260, NRF-2012R1A2A2A01045085, 2012M3A9C6049720).

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