Chapter 63

Embryonic Stem Cells in Tissue Engineering

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Tissue engineering is an interdisciplinary science that involves the use of biological sciences and engineering to develop tissues that restore, maintain, or enhance tissue function. However, to realize the dream of creating off-the-shelf organs for transplantation, a renewable source of cells is required. Embryonic stem (ES) cells have the potential to provide such a source of cells for tissue engineering applications, because of their ability to differentiate to all somatic cells, and their unlimited proliferative capability. However, to use ES cells in tissue engineering a number of challenges must be addressed regarding methods to direct ES cell differentiation, overcome host's immune rejection, prevent tumor formation, scale-up the production process, and control scaffold properties better. This chapter will introduce tissue engineering approaches, and the role of ES cells in various tissue engineering applications.

INTRODUCTION

Traditionally, approaches to restore tissue function have involved organ donation. However, despite attempts to encourage organ donations (Platt, 1998; Niklason and Langer, 2001), there is a shortage of transplantable human tissues such as bone marrow, hearts, kidneys, livers, and pancreases. Currently, more than 74 000 patients in the United States are awaiting organ transplantation, while only 21 000 people receive transplants annually (Petit-Zeman, 2001).

Tissue engineering-based therapies may provide a possible solution to alleviate the current shortage of organ donors. In tissue engineering, biological and engineering principles are combined to produce cell-based substitutes, with or without the use of materials. One of the major obstacles in engineering tissue constructs for clinical use is the limit of available human cells. Stem cells isolated from adults or developing embryos are a current source for cells for tissue engineering. The derivation of human embryonic stem (hES) cells in 1998 (Shamblott *et al.*, 1998; Thomson *et al.*, 1998), has generated great interest in their potential application in tissue engineering. This is because of the ability of ES cells to grow in culture, and to give rise to differentiated cells of all adult tissues. However, despite their therapeutic potential, both adult and ES cells present a number of challenges associated with their clinical application. For example, although adult stem cells can be directly isolated from the patient, and are therefore immunologically compatible with the patient, they are typically hard to isolate and grow in culture. In contrast, ES cells can be easily grown in culture and differentiated to a variety of cell types, but ES-derived cells may be rejected by the patient, and undifferentiated ES cells may form tumors.

The goal of this chapter is to analyze the potential of ES cells in tissue engineering. The chapter discusses the importance of ES cells as a source of cells for tissue engineering by using examples from the current research in the field. Furthermore, the chapter discusses some of the fundamental principles and seminal work in tissue engineering.

TISSUE ENGINEERING PRINCIPLES AND PERSPECTIVES

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences to develop biological substitutes, typically composed of biological and synthetic components, which restore, maintain, or improve tissue function (Nerem, 1991; Langer and Vacanti, 1993). Tissue-engineered products would provide a life-long therapy, and would greatly reduce the hospitalization and healthcare costs associated with drug therapy, while simultaneously enhancing the patients' quality of life.

In general there are three main approaches to tissue engineering (Langer and Vacanti, 1993):

1. to use isolated cells or cell substitutes as cellular replacement parts;

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2. to use acellular materials capable of inducing tissue regeneration;
3. to use a combination of cells and materials (typically in the form of scaffolds).

Although host stem cells could be involved in all of these approaches, ES cells can be directly involved in the first and the third approach.

Isolated Cells or Cell Substitutes as Cellular Replacement Parts

Isolated cells have been used as a substitute for cell replacement parts for many years. In fact, the first application of stem cells as a cellular replacement therapy is associated with bone marrow transplantation or blood transfusion studies, in which donor hematopoietic stem cells repopulated the host's blood cells (Till and McCulloch, 1980). Other stem cells have demonstrated their potential in various diseases. For example, bone marrow-derived cells have been shown to: (1) give rise to endothelial progenitor cells that were used to induce neovascularization of ischemic tissues (Kocher *et al.*, 2001; Edelberg *et al.*, 2002; Luttun *et al.*, 2002; Walter and Dimmeler, 2002); (2) to regenerate myocardium (Orlic *et al.*, 2001); (3) to give rise to bone, cartilage and muscle cells (Pittenger *et al.*, 1999); (4) migrate into the brain to give rise to neurons (Brazelton *et al.*, 2000; Mezey *et al.*, 2000). In addition, myoblasts isolated from skeletal muscle that on injection into the heart restored heart muscle function (Dorfman *et al.*, 1998; Taylor *et al.*, 1998), and neural stem cells that resulted in the treatment of Parkinson's disease (Fricker, 1999; Okano *et al.*, 2002; Storch and Schwarz, 2002), are some examples of other potential adult stem cell-based therapies. Tissue engineering products based on cells have been developed in the form of skin substitutes through the use of allogeneic cells (from companies such as Organogenesis and Advanced Tissue Sciences). In addition, the injection of mesenchymal stem cells is underway for cartilage and bone regeneration (Bruder *et al.*, 1998)

Embryonic stem cells provide an alternative source of cells for cellular substitutes. *In vitro* ES cells have been shown to give rise to cells of hematopoietic (Bigas *et al.*, 1995; Palacios *et al.*, 1995; Kaufman *et al.*, 2001), endothelial (Hirashima *et al.*, 1999), cardiac (Rohwedel *et al.*, 1994; Fleischmann *et al.*, 1998; Kehat *et al.*, 2001), neural (Brustle *et al.*, 1999; Reubinoff *et al.*, 2001; Schuldiner *et al.*, 2001; Zhang *et al.*, 2001), osteogenic (Buttery *et al.*, 2001), hepatic (Hamazaki *et al.*, 2001), and pancreatic (Assady *et al.*, 2001; Lumelsky *et al.*, 2001) tissues. Although ES cells provide a versatile source of cells for generation of many cell types, so far only a few experiments have demonstrated the use of ES cells to replace functional loss of particular tissues. One such example is the creation of dopamine-producing cells in animal models of Parkinson's disease (Bjorklund *et al.*, 2002; Kim *et al.*, 2002). These ES cell derived highly-enriched populations of midbrain neural stem cells generating neurons that showed electrophysiological and behavioral properties similar to neurons. Although functional properties of neurons derived from human ES cells still need to be investigated, it has been shown that human ES cells derived neural precursors can be incorporated into various regions of the mouse brain, and differentiate into neurons and astrocytes (Zhang *et al.*, 2001). Also, human ES cells that were differentiated to neural precursors were shown to migrate within the host brain, and differentiate in a region-specific manner (Reubinoff *et al.*, 2001). ES cells were also tested for future use in the heart. It was shown that mouse ES-derived cardiomyocytes were morphologically similar to neighboring host cardiomyocytes (Klug *et al.*, 1996; Kehat *et al.*, 2001, 2002; Min *et al.*, 2002; Yang *et al.*, 2002; Mummery *et al.*, 2003). In addition, mouse ES cells that were transfected with an insulin promoter (driving expression of the neo gene, a marker for antibiotic resistance), have been shown to give rise to insulin-producing cells that can restore glucose levels in animals (Soria *et al.*, 2000). Although these functional data were obtained by using genetically-modified ES cells, insulin production from ES cells (Assady *et al.*, 2001; Lumelsky *et al.*, 2001) suggests that these cells may potentially be used for the treatment of diabetes.

ES cells have also been shown to give rise to functional vascular tissue. Early endothelial progenitor cells, isolated from differentiating mouse ES cells, were shown to give rise to three blood vessel cell components, hematopoietic, endothelial, and smooth muscle cells (Yamashita *et al.*, 2000). Once injected into chick embryos these endothelial progenitors differentiated into endothelial and mural cells, and contributed to vascular development. We have shown that human ES cells can differentiate into endothelial cells, and isolated these cells using platelet endothelial cell adhesion molecule-1 antibodies. *In vivo*, when transplanted into immunodeficient mice, the cells appeared to form microvessels (Levenberg *et al.*, 2002).

Using a Combination of Cells and Materials

Tissue engineering approaches that use cells and scaffolds can be categorized into two categories; open and closed systems (Uludag *et al.*, 2000). These systems are distinguished based on the exposure of the cells to the immune system on implantation.

Open Systems

In open tissue engineering systems, cells are immobilized within a highly-porous, three-dimensional scaffold. The

scaffold could either be comprised of synthetic or natural materials, or composites of both (Langer and Vacanti, 1993; Lanza *et al.*, 1997; Langer and Vacanti, 1999; Vacanti and Langer, 1999). Ideally, this scaffold provides the cells with a suitable growth environment, optimum oxygen and nutrient transport properties, good mechanical integrity, and a suitable degradation rate. The use of scaffolds provides three-dimensional environments, and brings the cells in close proximity so that it provides the cells with sufficient time to enable self-assembly and formation of various components that are associated with the tissue microenvironment. Ideally the material is degraded as cells deposits their extracellular matrix molecules. The materials used for tissue engineering are either synthetic biodegradable materials, such as hydroxyapatite (Laurencin *et al.*, 1996; Friedman and Costantino, 1998; Pelissier *et al.*, 2003), calcium carbonate (Pelissier *et al.*, 2003; LeGeros, 2002; Li *et al.*, 2002), poly(lactic acid) (PLA) (Laurencin *et al.*, 1996; Mooney *et al.*, 1996), poly(glycolic acid) (PGA) (Mooney *et al.*, 1996), poly lactic-glycolic acid (PLGA), poly(propylene fumarate), and polyarylates, or natural materials such as collagen (Chevallay and Herbage, 2000) and alginate (Rowley *et al.*, 1999). Natural materials are typically more favorable to cell adherence, while the properties of synthetic materials, such as degradation rate, mechanical properties, structure, and porosity, can be better controlled (Langer and Vacanti, 1993).

Open tissue engineering systems have been successfully used to create a number of biological substitutes, such as bone (Cancedda *et al.*, 2003; Ohgushi *et al.*, 2003), cartilage (Rahman and Tsuchiya, 2001; Baek *et al.*, 2002; Sherwood *et al.*, 2002), blood vessels (Niklason and Langer, 1997; Niklason *et al.*, 1999), cardiac (Shinoka *et al.*, 1998), smooth muscle (Kim *et al.*, 1999), pancreatic (Cui *et al.*, 2001), liver (Hasirci *et al.*, 2001), tooth (Young *et al.*, 2002), retina (Lu *et al.*, 2001), and skin (Herson *et al.*, 2001; Badiavas *et al.*, 2002) tissues. Several tissue-engineered products are under clinical trials for Food and Drug Administration (FDA) approval. Engineered skin or wound dressing and cartilage are two of the most advanced areas with regard to clinical potential (Niklason and Langer, 2001). For example, a skin substitute that consists of living human dermis cells in a natural scaffold consisting of type I collagen already received FDA approval for use in a diabetic foot ulcer (Griffith and Naughton 2002). In addition, various cartilage and bone products are currently also in clinical stages, and bladder and urologic tissue are being tested in various stages of research (Oberpenning *et al.*, 1999).

Despite the ability of stem cells to differentiate into cells with the phenotypic and morphological structure of desired cell types, there have been very few scaffold-based tissue engineering studies that use ES cells. In the case of adult stem cells, scaffolds have been utilized in conjunction with mesenchymal stem cells (Boo *et al.*, 2002; Howard *et al.*, 2002; Weber *et al.*, 2002; Bensaid *et al.*, 2003; Yamada *et al.*, 2003), neural stem cells (Park *et al.*, 2002), and oval cells (Suh *et al.*, 2003). One such example is the transplantation of neural stem cells onto a polymer scaffold that was subsequently implanted into the infarction cavities of mouse brains injured by hypoxia-ischemia. These stem cells generated an intricate meshwork of many neurites, and integrated with the host (Park *et al.*, 2002). We have seeded neural stem cells onto specialized scaffolds (Lavik *et al.*, 2001, 2002), and have demonstrated spinal cord regeneration and improved hind-leg function of adult rats from a hemisection injury model (Teng *et al.*, 2002). Also, MSCs have been differentiated on polyethylene glycol (PEG) or PLGA scaffolds, and have been shown to give rise to cartilage or bone, depending on the medium conditions (Martin *et al.*, 2001).

ES cells may be differentiated in culture, desired cell types selected and subsequently seeded onto scaffolds. We have used this technique to study the behavior of ES cell-derived endothelial cells in tissue engineering constructs (Levenberg *et al.*, 2002). Human ES cell-derived endothelial progenitors that were seeded onto highly porous PLLA/PLGA biodegradable polymer scaffolds formed blood vessels that appeared to merge with the host vasculature when implanted into immunodeficient mice (Figure 63-1).

There may be other approaches to using ES cells or their progeny with scaffold-based tissue engineering systems. For example, it may be possible to differentiate ES cells directly on scaffolds in culture. Finally, it may be possible to differentiate genetically-engineered ES cells seeded onto scaffolds *in vivo* (see Figure 63-2).

Coercing cells to form tissues while differentiating is an important issue that has not been widely explored. This may be achieved by seeding ES cells directly onto the scaffolds, followed by inducing their differentiation *in situ*. Porous biodegradable polymer scaffolds can be used to support the ES cells, and they represent a promising system for allowing formation of complex 3D tissues during differentiation. The scaffold provides physical cues for cell orientation and spreading, and pores provide space for remodeling of tissue structures. These scaffolds should ideally provide the cells with cues to guide their differentiation into the desired cell types. The possible advantages of this system could involve the assembly of the cells as they differentiate. This differentiation pattern may mimic the developmental differentiation of the cells much more closely, and therefore may induce differentiation into the desired tissue. Ultimately, *in vitro* differentiated constructs can potentially be used directly for transplantation.

An approach which has not been considered as an alternative to *in vitro* differentiation of ES cells is to use the adult body's microenvironment to induce the differentiation of ES cells. *In vivo* differentiation of ES cells is not yet a feasible option, due to the tumorigenic nature of ES cells, as well as the heterogeneous cell population that results from

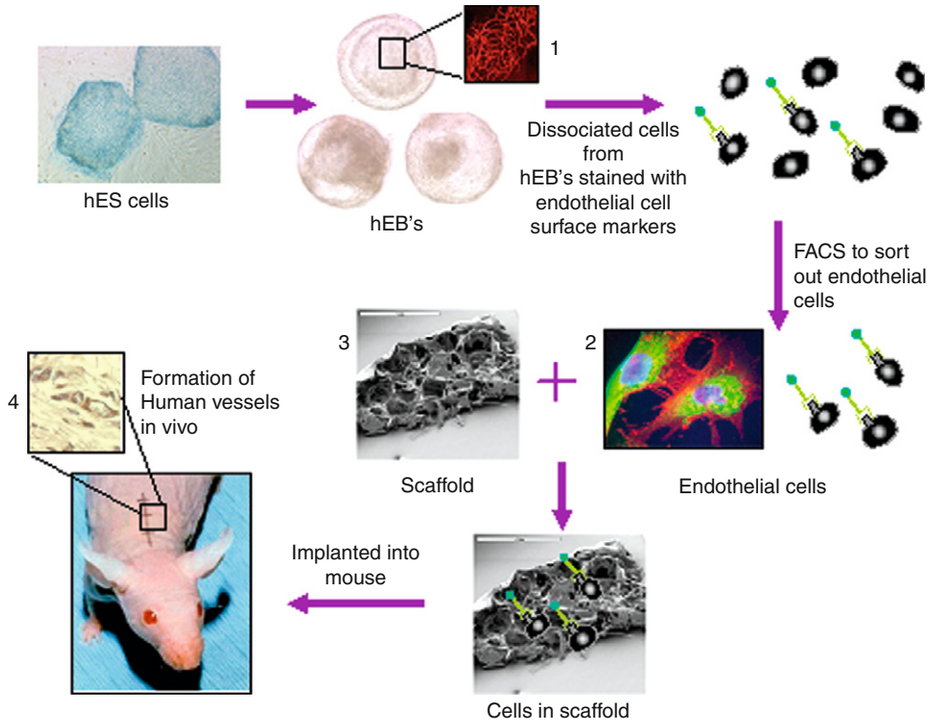

FIGURE 63-1 Approaches for using ES cell for scaffold based tissue engineering applications. ES cells can be used in tissue engineering constructs in a variety of methods. ES cells can be expanded in culture and then seeded directly onto scaffold where they are allowed to differentiate. Alternatively, stem cells can be directed to differentiate into various tissues and enriched for desired cells prior to seeding the cells onto scaffolds.

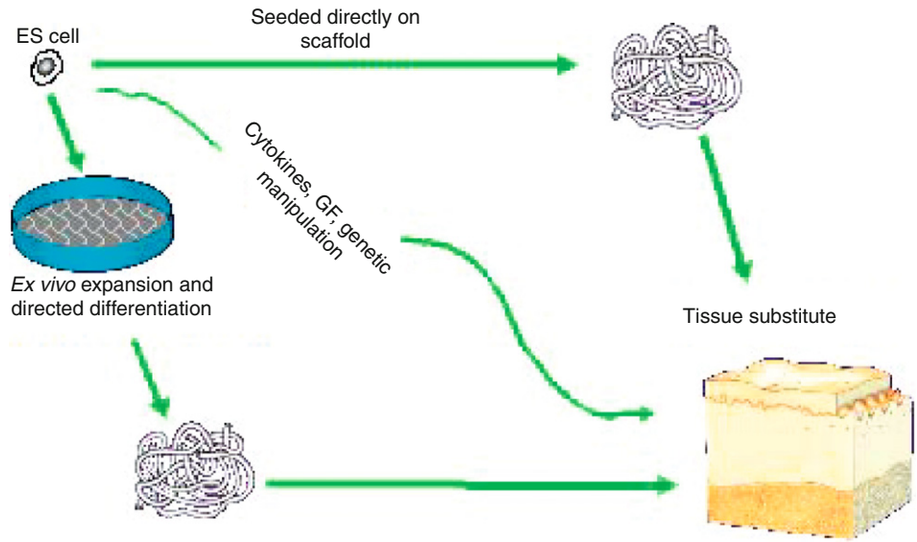

FIGURE 63-2 Embryonic endothelial cells on scaffolds *in vivo*. hES cells were induced to form EBs, in which differentiation into endothelial cells and formation of vessel-like network was observed (1). Embryonic endothelial cells were isolated from hEBs by staining dissociated EB cells with endothelial surface marker and sorting out positive cells using flow cytometry (FACS). Isolated endothelial cells (2) were seeded on polymer scaffolds (3) and implanted into immunodeficient mice. The embryonic endothelial cells appeared to form vessels *in vivo* (4). Confocal image of vessel network formation within 13-day-old hEB, stained with PECAM-1 antibodies. Isolated embryonic endothelial cells grown in culture stained with PECAM-1 (red) and VWF (green) antibodies. Scanning electron microscopy (SEM) of PLLA/PLGA scaffolds. Immunoperoxidase (brown) staining of seven day implants with anti-human PECAM1 antibodies showing vessels lined with human endothelial cells.

their non-directed differentiation. However, it may be possible to use a cell's apoptotic response mechanism to induce selective pressure for the desired cells *in vivo*. Thus, genetically-modified ES cells that undergo apoptosis on differentiation into undesirable cell types could be used to direct the differentiation of these cells, while similar approaches could be adopted to control their proliferative behavior.

Closed Systems

One of the main difficulties associated with open tissue engineering systems is the potential immunological issues associated with the implanted cells. Closed systems aim to overcome this difficulty by immobilizing cells within polymeric matrices that provide a barrier for the immunological components of the host. For example, cells can be immobilized within semi-permeable membranes that are permeable to nutrients and oxygen, while providing a barrier to immune cells, antibodies, and other components of the immune system (Lim and Sun, 1980; Uludag *et al.*, 2000). Furthermore, the implants can either be implanted into the patient, or used as extra-corpical devices. Closed tissue engineering systems have been used particularly for the treatment of diabetes (Chicheportiche and Reach, 1988; Zekorn *et al.*, 1992; Sefton *et al.*, 2000), liver failure (Uludag and Sefton, 1993; Chandy *et al.*, 1999; Wang *et al.*, 2000), and Parkinson's disease (Aebischer *et al.*, 1991; Esposito *et al.*, 1996; Wang *et al.*, 1997; Vallbacka *et al.*, 2001). This system may prove to be particularly useful in conjunction with ES cells, since the immobilization of ES cells within a closed system may overcome the immunological barrier that faces ES cell-based therapies. For example, ES cell derived β-cells that can response to insulin, or domaine-producing neurons can be used in clinics without fear of rejection. In addition, closed systems protect the host against potentially tumorigenic cells, as it limits the cells within the polymeric barrier. Currently, engineering and biological limitations, such as material biocompatibility, molecular weight cut-off, and the immune system's reaction to shed antigens by the transplanted cells, are some of the challenges that prevent these systems from widespread clinical applications.

LIMITATIONS AND HURDLES OF USING EMBRYONIC STEM CELLS IN TISSUE ENGINEERING

Despite significant progress in the field of tissue engineering, there are a number of challenges that are limiting the use of ES cells in tissue engineering. These challenges range from understanding stem cell biology questions, to how to control stem cell fate, to engineering challenges on scale-up, to business questions of feasibility and pricing.

Directing the Differentiation of Embryonic Stem Cells

Perhaps the biggest challenge in using ES cells in clinical applications is the lack of knowledge of how to direct their differentiation ability. All studies that have shown the generation of specific cell types have not shown a uniform differentiation into a particular cell type. This may be attributed to the intrinsic property of ES cells to differentiate stochastically in the absence of proper temporal and spatial signals from the surrounding microenvironment.

Techniques can be used to increase the ratio of cells that give rise to the desired lineages include genetic and microenvironmental manipulations. Genetic techniques can be categorized into positive or negative regulators. The positive regulators include the constitutive or controlled expression of transcription factors that have been shown to drive the differentiation into particular tissues. For example, the overexpression of Nurr transcription factor has been shown to increase the frequency of ES cells that differentiate into functional neural cells (Kim *et al.*, 2002). Alternatively, the negative regulators could be incorporated to induce the apoptosis of cells that differentiate to varying pathways. For example, neomycin selection and suicide genes that are activated by certain transcription factors can be used (Soria *et al.*, 2000). Clearly, these techniques will benefit from further understanding of the inner workings of transient cells, and knowledge of the differentiation pathways and lineages. Further analysis into stem cell and progenitor hierarchy through high-throughput analysis of microarray or proteomics data should accelerate this process (Phillips *et al.*, 2000; Ivanova *et al.*, 2002; Ramalho-Santos *et al.*, 2002).

Another important criterion is the functionality of ES cell-derived cells as a source of tissues. The importance of rigorous testing has become clear in studies in which nestin-positive putative pancreatic cells stained positive for insulin using antibodies, due to cellular uptake from the surrounding medium (Rajagopal *et al.*, 2003). Thus, the incorporation of protein and functional tests should accompany the morphological and phenotypic analysis that is often used in ES cell literature to characterize differentiated cells.

Isolating the Desired Cell Types for Therapy

One of the main problems with ES cell-based therapies is finding suitable techniques to isolate the desired cells from a heterogeneous population of cells. One approach is to allow for the random differentiation of the ES cells, followed by isolation using a cell surface marker. We have used this method for isolation of ES cell-derived endothelial cells using PECAM-1 receptor (Levenberg *et al.*, 2002). Also, ES cell-derived hematopoietic progenitors

have been isolated in a similar manner using CD34 marker (Kaufman *et al.*, 2001). Another potential method is through reporter gene knockin modifications. These modifications have already been used on ES cells to allow for labeling of cells at various stages of differentiation (Eiges *et al.*, 2001; Zwaka and Thomson, 2003). The utility of other techniques, such as magnetic separation, or use of neomycin selection, must be further examined for selecting various ES cell derived progeny.

Scale-up of Embryonic Stem Cells in Tissue Engineering

Although laboratory scale ES cell cultures have been shown to produce differentiated progeny for both rodent (Chen *et al.*, 2000) and human (Itskovitz-Eldor *et al.*, 2000) ES cells, it is generally accepted that these culturing methods are not feasible for large-scale production of ES cells for therapeutic applications. Production of a sufficient quantity of differentiated cells from ES cell is an important challenge in realizing the clinical potential of ES cells. The large-scale production of ES cells will likely be specific to the type of tissue being generated, and must remain reproducible, sterile, and economically feasible. Furthermore, this scale-up process must maintain the appropriate control over bioprocess conditions, such as mechanical stimuli, medium conditions, and physicochemical parameters (such as temperature, oxygen, pH, and carbon dioxide levels), as well as growth factor and cytokine concentrations.

ES cell differentiation protocols have generally used two-dimensional cultures and/or embryoid bodies. Although each technique provides specific advantages, differentiation of ES cells in embryoid bodies produces a wider spectrum of cell types (Magyar *et al.*, 2001). This has been attributed to the embryoid bodies' ability to better mimic the temporal pattern of cell differentiation as seen in the embryo. However, in some applications, the combined use of EB's and adherent cultures has resulted in better cell yields. For example, to induce ES cells to differentiate to cardiomyocytes, an EB formation in suspension cultures followed by a differentiation in adhesion cultures has been shown to optimize the percentage of cells that give rise to cardiomyocytes (Guan *et al.*, 1999; Klinz *et al.*, 1999). Similarly, the production of hepatocytes has been shown to be induced by first culturing the cell in EB's, followed by culturing on two-dimensional cultures (Hamazaki *et al.*, 2001).

The formation of EB's in laboratories has generally been performed using techniques that have not been ideal for large-scale production. For example, many studies have used the "hanging drop protocol" (Maltsev *et al.*, 1999), in which ES cells are placed within a "hanging drop," and allowed to form an aggregate which can then be differentiated. Other techniques have formed EB's by placing the cells on non-adherent tissue culture dishes, which once more place a limit on the quantity of cells produced. A technique which may allow for the scale-up of embryoid body cultures is the use of suspension cultures using spinner flasks (Dang *et al.*, 2002). Such cultures have been shown to enhance the supply of oxygen and nutrients to the cells within the embryoid body, by exposing the surface of the cell aggregate to continuous supply of fresh medium (Wartenberg *et al.*, 1998; Dang *et al.*, 2002).

To prevent the difficulties associated with EB heterogeneity, EBs have been immobilized in alginate microbeads. The microencapsulation of cells within these microbeads resulted in differentiation of cells into cardiomyocytes and smooth muscle cells (Magyar *et al.*, 2001). In addition, ES cells may adhere to beads with desired extracellular matrices and be differentiated. This approach also enhances the transport of medium and oxygen to the cells, in comparison to two-dimensional cultures, and provides additional mechanical stimuli which may be an improved alternative to two-dimensional culture systems.

To enhance the supply of medium to tissue-engineered scaffolds or embryoid bodies, methods other than passive diffusion maybe required, such as the use of perfusion systems, in which the medium is flowed through the scaffold. Perfusion bioreactors have already been developed for a variety of tissue-engineering applications, such as cartilage and cardiac (Carrier *et al.*, 2002; Davisson *et al.*, 2002). For example, perfusion through scaffolds has been generated in rotating wall vessels (Freed *et al.*, 1997; Margolis *et al.*, 1999; Vunjak-Novakovic *et al.*, 1999; Martin *et al.*, 2000; Botchwey *et al.*, 2001), or through pumping the medium directly through the scaffolds (Davisson *et al.*, 2002) to grow chondrocytes for cartilage generation.

It is known that mechanical forces affect the differentiation and functional properties of many cell types (Niklason *et al.*, 1999; Vunjak-Novakovic *et al.*, 1999; Li and Xu, 2000; Martin *et al.*, 2000), therefore, ES cell based cultures that aim to direct the differentiation of ES cells require proper mechanical stimuli for the tissue. Understanding the effects of mechanical stimuli on ES cell differentiation is still primitive, but tissue-engineering systems have been developed that incorporate the effects of mechanical forces. For example, functional autologous arteries have been cultured using pulsatile perfusion bioreactors (Niklason *et al.*, 1999). Thus, the use of mechanical stimuli may further enhance the ability of these cells to respond to exogenous signals. Other environmental factors that maybe required are the use of electrical signals and spatially regulated signals, to induce the differentiation and allow for maturation of the desired tissues. Hopefully, with time, such techniques will become particularly important in allowing for scaled-up ES cell based tissue-engineering applications. The development of bioreactors that control the spatial and temporal signaling that induces ES cell differentiation requires collaborative effort between engineers and biologists, and is currently in the early stages of its development.

Tissue Engineering Limitations

Synthetic scaffolds that support tissue growth by serving as the extracellular matrix for the cells do not represent the natural ECM associated with each cell type and tissue. ES cells and their progeny reside in a dynamic environment during development; thus, synthetic or natural substrates that aim to mimic the developing embryo must present similar signaling and structural elements. A number of approaches are currently under development which may prove useful for ES cells. The use of "smart" scaffolds that release particular factors (Murphy *et al.*, 2000) and/or control the temporal expression of various molecules released from the polymer could be used to induce the differentiation of ES cells within the scaffolds (Richardson *et al.*, 2001). It has been shown that, by dual delivery of vascular endothelial growth factor (VEGF)-165 and platelet-derived growth factor (PDGF)-BB, each with distinct kinetics, from a single, structural polymer scaffold, a mature vascular network can be formed (Richardson *et al.*, 2001). An alternative approach to modify the surface that is exposed to the cells is to immobilize desired ligands on to the scaffold. For example, RGD peptides, the adherent domain of fibronectin, can be incorporated into polymers to provide anchorage for adherent cells (Cook *et al.*, 1997).

Another difficulty with the current materials is their lack of control over the spatial organization within the scaffold. To create tissues that resemble the natural structure of biological tissues, the spatial patterning of cells must be recapitulated. For ES cells differentiated in scaffolds, this modeling and structure may be directly obtained as cells differentiate. The spatial arrangement of cells grown in EBs is typically organized with cells of particular tissues appearing in clusters. For example, blood precursors occur in the form of blood islands, similar to their normal appearance in embryonic development. In the system in which ES cell-derived cells are plated onto scaffolds, spatial rearrangement can occur via direct patterning or cell "reorganization." In the direct cell patterning system, cells can be seeded into the scaffold at particular regions within the cells. For example, the direct attachment of two different cell types on different sides of the scaffold has been used to generate cells of the bladder (Lanza *et al.*, 2000). Cell patterning techniques, as have been developed for soft lithography for controlled co-culture of hepatocytes and fibroblasts, could be scaled-up to tissue-engineering scaffolds to allow for more controlled and complex direct patterning (Bhatia *et al.*, 1997, 1998a, b).

CONCLUSIONS AND FUTURE PERSPECTIVES

ES cells have generated a great deal of interest as a source of cells for tissue engineering. However, there are a number of challenges that exist in making ES cell-based therapy a reality. These include directing the differentiation of ES cells (using controlled microenvironments or genetic engineering), to ensure their safety and efficacy *in vivo*, to ensure that the cells are immunologically compatible with the patient and will not form tumors, to improve protocols for isolating desired cell types from heterogeneous populations, to enhance current tissue engineering methods. Further research is required to control and direct the differentiation of ES cells, in parallel with developing methods to generate tissues of various organs, and this may lead to realization of the ultimate goal of tissue engineering. We are getting close to a day when ES cells can be manipulated in culture to produce fully-differentiated cells that can be used to create and repair specific organs. Clearly, significant challenges remain, and the ability to overcome these difficulties does not lie within any scientific discipline, but rather involves an interdisciplinary approach. Innovative approaches to solve these challenges could lead to an improved quality of life for a variety of patients that could benefit from tissue engineering approaches.

KEY WORDS

Biomaterials Any natural or synthetic materials that interface with living tissue and/or biological fluids.

Cell therapy The transplantation of cells from various sources to replace or repair damaged tissue and/or cells.

Differentiation The developmental process in which cells change their genetic programs to become more restricted in their potential and to mature into functional cells of various tissues and organs.

Embryonic stem cells Cells derived from the embryo that can self-renew and give rise to all the cells of the adult organism.

Scaffold A porous structure typically made out of degradable polymers within which cells are seeded. Scaffolds provide geometrical structure for cells to reorganize and form 3D multicellular tissues.

Tissue engineering The interdisciplinary field that applies the principle of engineering and the life science toward the development of biological substitutes that restore, maintain, or improve tissue function.

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