CHAPTER 11

# Tissue Engineering: The Future of Stem Cells

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## Summary

Tissue engineering is an interdisciplinary field that applies the principles and methods of bioengineering, material science, and life sciences toward the assembly of biologic substitutes that will restore, maintain, and improve tissue functions following damage either by disease or traumatic processes. The general principles of tissue engineering involve combining living cells with a natural/synthetic support or scaffold to build a three-dimensional living construct that is functionally, structurally and mechanically equal to or better than the tissue that is to be replaced. The development of such a construct requires a careful selection of four key materials: 1) scaffold, 2) growth factors, 3) extracellular matrix, and 4) cells. Much progress has been made in the field of tissue engineering, but further work toward organ and tissue replacement is necessary. The optimal cell source, scaffold design, and in vitro bioreactors, the use and development of microfabrication technology to create vascularized tissues and organs are still being investigated. The search for and use of an appropriate multipotent or pluripotent stem cell in tissue engineering is an emerging concept. Certainly, many areas of stem cell research and their potential clinical applications are associated with controversies; therefore, it is important to address the ethical, legal, and social issues early.

This paper will provide an overview of tissue engineering and stem cells, and describe the current progress with stem cell research in tissue engineering, the potential implications on medical treatment and the economic impact with the passage of Proposition 71.

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## *Introduction*

Artificial transplantation or transplanted organs is a successful therapy for otherwise incurable end-stage diseases or tissue loss. However, such interventions are challenged by organ shortage, the necessity of lifelong immunosuppression and its potential for serious complications. Tissue engineering has emerged as a rapidly expanding approach to address these problems and is a major component of regenerative medicine. Tissue engineering is an interdisciplinary field that applies the principles and methods of bioengineering, material science, and life sciences toward the assembly of biologic substitutes that will restore, maintain, and improve tissue functions following damage either by disease or traumatic processes (1-3).

The general principles of tissue engineering involve combining living cells with a natural/synthetic support or scaffold that is also biodegradable to build a three-dimensional living construct that is functionally, structurally and mechanically equal to or better than the tissue that is to be replaced (4). The development of such a construct requires a careful selection of four key components: 1) scaffold, 2) growth factors, 3) extracellular matrix (ECM), and 4) cells (1-5). Scaffold materials are three-dimensional tissue structures that guide the organization, growth and differentiation of cells. Scaffolds must be biocompatible and designed to meet both nutritional and biological needs for the specific cell population (6). Growth factors are soluble peptides capable of binding cellular receptors and producing either a permissive or preventive cellular response toward differentiation and/or proliferation of tissue (7). ECM must be capable of providing the optimal conditions for cell adhesion, growth, and differentiation within the construct by creating a system capable of controlling environmental factors such as pH, temperature, oxygen tension, and mechanical forces (5). These conditions are determined by the particular cell lines and the properties of the scaffold (5). Finally, the development of a viable construct involves a suitable supply of cells that are ideally nonimmunogenic, highly proliferative, easy to harvest, and have the ability to

differentiate into a variety of cell types with specialized functions (1-5, 8). There are two primary methods to harvest cells and culture. In cases where direct harvest is not feasible, as seen in many patients with extensive end-stage organ failure or cells with limited proliferative capacity in culture, stem cells are envisioned as being an alternative source of cells (2-10).

Despite the growing interest in embryonic stem cell research, progress has been hampered by ethical and legislative debates. The political, ethical, and religious opposition toward embryonic stem cell research, which primarily uses discarded non-transferred human embryos for their derivation, have biased research toward adult stem cells and severely restricted federal funding in the United States. In the mist of such controversies, California has become the leading state to support stem cell research by passing Proposition 71 or the California Stem Cell Research and Cures Initiative. It promises to provide California a possible solution to bridge critical research funding gaps by committing $3 billion for stem cell research and to potentially accelerate the understanding of their therapeutic potential. It does not however support human reproductive cloning. The purpose of this paper is to provide an overview of tissue engineering and stem cells, and describe the current progress with stem cell research in tissue engineering and the potential implications on medical treatment and the economic impact with the passage of Proposition 71.

## *Fundamentals of tissue engineering*

In 1933, the concept of tissue engineering was first introduced when mouse tumor cells demonstrated survival when encased in a biocompatible polymer membrane and implanted into the abdominal cavity of chick embryos (11). Several decades later, Chick *et al.*, demonstrated that pancreatic beta cells from neonatal rats, cultured on synthetic capillaries and perfused with medium, released insulin in response to changes in

glucose concentration (12). In the early 1980s, Burke *et al.*, successfully created artificial skin with fibroblasts seeded onto collagen scaffolds for the treatment of extensive burn injury (13). Clinically, this is still being utilized today.

Efforts are now being undertaken for engineering a variety of tissue and organ types with an emphasis on the application of stem cells. Ultimately, the goal of tissue engineering is to regenerate tissues and restore organ function through cell implantation and matrix incorporation into the patient (2, 4).

Current approaches to tissue engineering can be stratified into substitutive, histioconductive, and histioinductive (1). Substitutive approaches (*ex vivo*) are essentially whole organ replacement, whereas histioconductive approaches (*ex vivo*) involve the replacement of missing or damaged parts of an organ tissue with ex-vivo constructs. In contrast, histioinductive approaches facilitate self-repair and may involve gene therapy using DNA delivery via plasmid vectors or growth factors.

A number of criteria must be satisfied in order to achieve effective, long-lasting repair of damaged tissues. 1) An adequate number of cells must be produced to fill the defect. 2) Cells must be able to differentiate into desired phenotypes. 3) Cells must adopt appropriate three-dimensional structural support/scaffold and produce ECM. 4) Produced cells must be structurally and mechanically compliant with the native cell. 5) Cells must successfully be able to integrate with native cells and overcome the risk of immunological rejection. 6) There should be minimal associated biological risks (9).

The source of cells utilized in tissue engineering can be autologous (from the patient), allogenic (from a human donor but not immunologically identical), or xenogenic (from a different species donor) (5). Autologous cells represent an excellent source for use in tissue engineering because of the low association with immune complications. Autologous cells are however not cost effective and batch controlled for universal clinical use (1). In contrast, allogenic cells offer advantages over autologous cells in

terms of uniformity, standardization of procedure, quality control and cost effectiveness(1).

Cell sources can be further delineated into mature (non-stem) cells, adult stem cells or somatic stem cells, embryonic stem cells (ESCs), and totipotent stem cells or zygotes (4). The utility of mature cells is restricted because of its low proliferative and differentiating potential. Adult stem cells are resident stem cells found in specific niches or tissue compartments and are important in maintaining the integrity of tissues like skin, bone and blood (9). They are undifferentiated cells that can be programmed to differentiate into specific tissue types. At least 20 major categories of somatic stem cells have been identified in mammals. They can be found in bone marrow, blood, cornea and retina of the eye, dental pulp, liver, skin, GI tract, and pancreas. ESCs are derived from the inner cell mass of the pre-implantation blastocyst (14). They are undifferentiated, immature cells that are capable of unlimited self-renewal with the ability to differentiate (15).

## *Stem cells (3 types)*

Rapid advances are being made in stem cell research with a focus on their therapeutic potential for regenerative medicine and other biomedical applications. In combination with tissue engineering, stem cells hold a number of promises in further advancing contemporary medicine.

Traditionally, adult stem cells were believed to form a small number of cells restricted to a particular germ layer origin; however, some evidence now indicates that adult stem cells isolated from various tissues have greater plasticity than previously thought. Several researchers have attributed this apparent plasticity of adult stem cells to developmental signals-mediated differentiation. For example, Azizi *et al.*, demonstrated that marrow stromal stem cells transplanted into the rat brain can acquire a neural

phenotype (16). Neural and muscle-derived stem cells isolated from cloned mice were able to differentiate into all cell types of hematopoietic lineage (17, 18). A year later Clark *et al.*, reported that murine neural stem cells could differentiate into all three germ layers with appropriate culture modifications (15, 19, 20). In 2000 Lagasse *et al.*, demonstrated that the injection of murine bone marrow stem cells into a mutant mouse line with progressive liver failure were able to form hepatocytes and restore liver function (21, 22). Recently, however, others reported cell fusion, rather than signal-mediated differentiation, as a possible mechanism for transdifferentiation. For example, Alvarez-Dolado *et al.*, demonstrated the evidence for cell fusion of bone marrow-derived cells with neurons and cardiomyocytes and the lack of transdifferentiation without cell fusion in these tissues, suggesting that cell fusion may be necessary for the observed plasticity of adult stem cells (23). This finding is not without controversies. Later, others demonstrated that stem-cell plasticity is a true characteristic of neural stem cells, suggesting that transdifferentiation can be accomplished without cell fusion (24). In summary, the concept of adult stem cell plasticity is new, and the phenomenon is not thoroughly understood.

The ESC is defined by its origin, that of a blastocyst before uterine implantation. Three unique characteristics define primate ESCs: 1) They are derived from the four to five day-old embryos that have been produced in an *in vitro* fertilization clinic. 2) They possess a prolonged undifferentiated quality that is capable of self-renewal. 3) They demonstrate stable developmental potential to form the derivatives of all three embryonic germ layers ('pluripotent') even after prolonged culture. Pluripotent stem cells have been derived in mice and primates from pre-implantation embryos and bone marrow stroma. They can be induced to differentiate into all cell types and are able to colonize tissues of interest after transplantation. Human ESCs are mostly obtained from discarded embryos generated in fertility clinics.

In the presence of leukemia inhibitory factor (LIF) or embryonic fibroblast feeder layers, ESCs can be maintained and expanded in an undifferentiated, pluripotent state *in vitro*

almost indefinitely (14, 25). Upon withdrawal of LIF, ESCs spontaneously differentiate to form distinct embroid bodies, which contain differentiating cells of ectodermal, endodermal and mesodermal lineage (25). Much of the development in manipulating ESCs have been based on mouse ESC models, and there are numerous examples of improved tissue function with this usage.

Human ESCs, like no other ESCs, differentiate into somatic cell types that make up the human body. The potential benefits to health care are enormous, ranging from generating neurons for treating patients to Parkinson's disease to learning about the molecular processes that drive tumor development.

## *Current progress*

The current utilization of stem cells in tissue includes skin, blood vessels, cartilage, heart tissue, liver, pancreas and neural tissue (26).

Skin defects are primarily treated by the use of epidermal and dermal constructs (1). Dermal fibroblasts are obtained from neonatal foreskin, expanded *in vitro*, seeded onto a scaffold of polylactic or polyglycolic acid before being cultured in a bioreactor system to generate a dermal layer (27). A bilaminate construct is produced by coating the dermal layer with multiple layers of keratinocytes. The long-term viability of these skin grafts depends on the population of the engrafted skin stem cells (26).

Various techniques have been utilized to promote neovascularization. More recently, cell-based therapies known as endothelial progenitor cells or angioblasts have been utilized (20).

The lack of vascularity renders cartilage less susceptible to ischemic insult than tissues with greater dependency on direct vascularity. Autologous cell products have been used effectively for cartilage repair. Alternatively, both adult stem cells and ESCs are being investigated for their potential to differentiate into the appropriate cells for repair of damaged bone, cartilage, and tendon (28, 29).

The complexity and specialized conducting infrastructure of the heart have posed a challenge to duplicate in engineering systems. Furthermore, such effort is further limited by a low proliferative potential of cardiomyocytes. Available engineered heart products are biocompatible, non-biodegradable mechanical. These devices are ineffective for long-term replacement. Nonetheless, the possibility of development of an engineered heart is exemplified by the successful manufacturing of tissue-engineered valves and myocardial infarct scar remodeling. Combining the present engineering efforts with the potential clinical applications of stem cells may accelerate the rate of the development of these engineered products. Kehat *et al.*, first described the generation of a reproducible spontaneous cardiomyocyte differentiating system from human ESCs (30). Injection of bone marrow cells in the contracting cardiac wall bordering myocardial infarcts has been demonstrated to regenerate myocardium (31). The development of human ESC lines and their ability to differentiate to cardiomyocytes holds great promise. Nevertheless, bridging the gap between the theoretical potential of this unique system and any actual clinical applications will require much work.

Presently, liver transplantation is the only successful treatment modality for end-stage liver failure. The limited availability of donor livers has turned our attention to alternative sources including tissue engineering. A successful construct employs the use of highly porous biodegradable discs to deliver hepatocytes (32). This approach is limited by the low proliferative potential of hepatocytes and the suboptimal cell survival from the poor access to vascularity for nutrient provisions and removal of metabolic waste (1). Several types of stem cells, such as human adult hematopoietic and mesenchymal stem cells, have been demonstrated to differentiate into hepatocytes under

the appropriate environment, and hence may serve as a unlimited cell source for therapy (22, 33).

Diabetes may be potentially treated by generating insulin-producing cells from stem cells and grafting them into the pancreas in diabetic patients (34, 35). A much better understanding is necessary for the mechanisms that govern the expansion and differentiation into beta cells (36). Clinical islet cell transplantation for the treatment of type 1 diabetes has been introduced with enthusiasm (37). The procedure is considered minimally invasive and potentially offers the possibility of being performed under donor-specific tolerant conditions. Recently, multipotent precursor cells from the adult mouse pancreas have been identified (38), and may be a promising candidate for cell-based therapeutic strategies. Unlike isolated beta cells which release insulin in a monophasic, all-or-none manner without any fine-tuning for intermediate concentrations of blood glucose, pancreatic precursor cells may be cultured to produce all the cells of the islet cluster in order to generate a population of cells that will be able to coordinate the release of the appropriate amount of insulin to the physiologically relevant concentrations of glucose in the blood (39).

The potential of ESCs in the treatment of brain diseases is also under active investigation. Ma *et al.*, recently demonstrated the first CNS stem cell-derived functional synapse and neuronal network formation on a three-dimensional collagen gel (40). Mesenchymal stem cells are ideal for replacing the dead neural cells due to their strong proliferative capacity, easy acquisition, and considerable tolerance of genetic modifications (41). Following the transduction of brain-derived neurotrophic factor (BDNF) via recombinant retroviral vectors into the human mesenchymal stem cells, nearly all cells were demonstrated to express BDNF, and the quantity of BDNF in the culture medium was increased by approximately 20,000-fold (42).

Another area under investigation is genetic manipulation of adult stem cells as a potential therapeutic strategy to surmount the problem of organ shortage. Oncology

studies have identified numerous genes involved in controlling cell growth. By introducing growth-promoting genes, many stem cells can be immortalized and gain the ability to differentiate. For example, immortalized human hepatocyte stem cells grafted into rats with acute liver failure can keep the animals alive (43). There is however some concern regarding oncogenic risks with using growth-promoting genes. This problem may be unlikely by introducing three levels of safeguards: 1) deletion of the transferred oncogene by site-specific recombination followed by different selection, 2) allogenic transplantation requiring temporary immunosuppression, and 3) introduction of a "cell-suicide" gene that can be activated by administering a drug (22, 43).

While the selection of appropriate cell source remains a key to the successful creation of stable, complex three-dimensional constructs, equally important is the design of robust scaffolds and ECM that mimics a native physiologic environment and a capillary network in a three-dimensional structure. In collaboration with Draper Laboratories (Cambridge, MA), Shieh *et al.*, have developed a computational model of vascular circulation (3).

## *Limitations*

One of the major challenges of the field is to design an appropriate capillary network to allow gas exchange, provide nutrition, and remove metabolic waste from the implanted constructs (1-3, 26). It will be necessary to create a tubular system with a minimum diameter of 10 μm to allow vascular ingrowth, especially important for multilayered solid organ constructs with a volume greater than 2 to 3 cubic mm (1, 3). An alternative is to implant a vascularized construct in an ectopic, well-vascularized location, such as omentum (1). Special attention will be given to the control of the local milieu of each cell type as different cell types often require different culturing environments, making it

difficult to design a multilayered organ system construct. A challenge will be to create a scaffold capable of supporting various cell types (1, 9).

The appropriate expression sequence, dosing, and duration for growth factors also needs to be defined if we are to maximize ESCs utility in tissue growth and maintenance in the pluripotent state (10, 44).

## *Ethical/technical considerations*

There are three main research programmes in stem cell research. 1) Research on adult stem cells. 2) Research on ESCs from discarded embryos that are produced via *in vitro* fertilization. 3) Research on ESCs obtained via therapeutic cloning. There is little controversy surrounding human adult stem cells. On the other hand, human ESCs face great controversies, the extent of which is dependent on the type of research programmes.

Various autologous adult stem cells, including mesenchymal, hematopoietic, neural, muscle, and hepatic stem cells, are actively being investigated. They are more likely immunocompatible and are not associated with ethical concerns unlike their ESC counterparts. Challenges remain in regard to optimization of isolation techniques that minimize contamination, permanently maintain the desired cell types following differentiation, and increase the production of a large number of cells that are adequate for the construction of a tissue or organ (3). Furthermore, much of the data cited have not been reproducible (15). The utility of adult stem cells would effectively avoid the ethical problem of using ESCs. But much needs to be learned about adult stem cells in regard to their replication and differentiation. ESCs may offer technical advantages over other sources of somatic precursors. For example, ESCs can be generated in abundant quantities in the laboratory and can be grown in their undifferentiated state for many

generations. In contrast, researchers have had difficulty finding laboratory conditions under which some adult stem cells, especially hematopoetic stem cells from blood or bone marrow, can proliferate without becoming specialized. This technical barrier to proliferation has limited the ability of researchers to explore the capacity of certain types of adult stem cells to generate sufficient numbers of specialized cells for transplantation purposes.

Unlike adult stem cells, ESCs can be grown indefinitely in tissue culture (15). There is an increased risk for immune rejection because of genetic mismatch, which in turn would demand lifelong high-dose immunosuppressant treatment. Furthermore, the creation of human ESCs derived from discarded, non-transferred human embryos using an immunosurgical technique has resumed arguments that have not been resolved (14).

A SART-RAND study identified at least 400,000 frozen embryos in storage since the late 1970s (45). Only 2.8% of these embryos have been designed for research. With a 15% efficiency rate for generating a human ESC line from blastocysts and incorporating some losses to the freeze-thaw process, only approximately 275 human ESC lines can be created from the excess (46). The pluripotential virtue of stem cells itself renders limitations: the efficiency of differentiation appears to be compromised for certain cell types, such as hepatocytes and cardiomyocytes, and the quality of differentiation is still questionable.

The use of ESCs as an important cell source for tissue engineering has great potential for the treatment of diseases, but there are alternatives, such as the use of adult stem cells and ESCs from therapeutic cloning under active investigation (46). Worldwide legislation reflects the dilemma, ranging from the more permissive (the United Kingdom—its Human Embryology and Fertilization Authority can license stem cell research, and the legislature has defined the threshold for embryos to assume moral status to be 14 days) to the nominally more restrictive (the United States—only minimal federal financial funds granted to research that meets the strict NIH guidelines, but stem

cell research is legal without any restriction in the private sector) to the truly restrictive (Germany, Austria, Ireland, France—laws on reproductive medicine ban the extraction from stem cells from a human embryo) (47, 48).

The host immune response to allogenic embryonic stem cells poses a major challenge in addition to ethical and religious concerns regarding ESCs. This has led researchers to investigate creation of a universal donor cell by making histocompatible proteins on the cell surface thus reducing the cells' antigenicity. This is the heart of nuclear transfer technology or "therapeutic cloning". However, the technology has its own shortcomings. It has a low efficiency rate which in turn requires a large number of oocytes (49). Its inability to control differentiation requires a better understanding of ESC growth, differentiation, and application in engineering tissues. Furthermore, therapeutic cloning protocols *in vitro* culture systems must be optimized before contemplating the use of this technology for cell therapy (49).

All three research programmes discussed here are directed at increasing our knowledge about basic cell biology, creating new therapies through stem cell culture and control of cell differentiation, and producing commercially viable stem cell products either by the direct patenting of stem cell lines or by combining stem cell technologies with genetic engineering or other patentable interventions (50). The primary discussion on stem cells is concerned with the ethical issues raised by each of these programmes and with whether these issues should influence regulatory decisions regarding public financial support for research.

## *Proposition 71 passage in California*

Proposition 71, the California Stem Cell Research and Cures Initiative, recently passed by statewide ballot in November 2004. As a result, the California Institute for

Regenerative Medicine (CIRM or Institute) was created in order to provide breakthrough cures for devastating diseases (51). These diseases include, but are not limited to, diabetes, heart diseases, cancer, osteoporosis, multiple sclerosis, Alzheimer's disease, Parkinson's disease, Lou Gerig's disease and spinal cord injuries by utilizing tax-free state bonds to fund stem cell research (52, 53). The institute would ensure that strict ethics codes will be adopted and enforced, including utilizing guidelines developed by the National Institutes of Health. Mirroring the existing state law, the institute would strictly prohibit human reproductive cloning (53).

In addition to finding cures for various diseases, the goal of the institute is also to reduce the rapidly rising healthcare costs in California and boost its economy. Proposition 71 is designed not to burden taxpayers, authorizing tax-free state bonds that will provide an average of $295 million per year over ten years to support stem cell research in California (53). It assumes that a large share of Health care costs is caused by diseases and injuries that can potentially be cured with stem cell therapies.

Opponents to Proposition 71 have argued that the bill places an unnecessary and unwise financial burden on an already-insufficient state budget (54). According to some estimates, state bond debt, which amounted to $33 billion in May of 2004, might rise to more than $50 billion by July of 2005, largely due to the costs of Proposition 71 (54). In addition, the initiative has yet to assure the Californian taxpayers whether the state will actually receive any royalties from discoveries paid for by state dollars. Questions about significant conflicts of interest on the Independent Citizen's Oversight Committee, (the "board of directors" of CIRM) and even about the scientific integrity of the stem-cell lines available for research have been raised (54).

In spite of this opposition, many defend the economic merits of Proposition 71. As no payments would be demanded within the first five years of the bill's implementation, the state would remain free from financial stress and allowed time to balance its budget. No tax increases would accompany the legislation (53, 54).

The potential effects that the passage of California's Proposition 71 would have on other states and the nation as a whole are not to be underestimated. New Jersey, the nation's first state to create a publicly funded institute for stem cell research and the second state to pass legislation legalizing the research, has found it necessary to review its policy and is considering an increase in their research grants. Other states, including Massachusetts, Maryland, Wisconsin and Connecticut, are also jumping in with proposals to fund research cloning.

These changes are happening against the recent passage of the "declaration" of banning all forms of human cloning by the United Nations (55). A recent Swiss law that mirrors laws in most European countries, allows medical research on stem cells taken from human embryos but bars cloning (56). Britain and Belgium allow embryo creation for research. Britain has an independent embryo authority to scrutinize and monitor all projects and has issued only two cloning licenses since 2001 (56).

What has made the United States such a fertile ground for expanding embryo research is not its liberal laws but the lack of them. Congress has tried but failed to pass legislation largely because of irreconcilable differences over when life begins. Both Presidents Bill Clinton and George W. Bush banned the National Institutes of Health on ethical grounds from funding the creation of human research embryos, although their orders apply only to federally funded work. In 2001, Bush additionally narrowed NIH research to embryonic stem cells already harvested from spare IVF embryos. These restrictions in turn triggered an influx of private and state funding, and the dramatic Proposition 71.

## *Future of tissue engineering with stem cell research*

Although much progress has been made in the field of tissue engineering, further work toward organ and tissue replacement is necessary. The optimal cell source, scaffold design, and *in vitro* bioreactors, the use and development of microfabrication technology to create vascularized tissues and organs are still being investigated. The search for and use of an appropriate multipotent or pluripotent stem cell in tissue engineering is an emerging concept. Certainly, many areas of stem cell research and their potential clinical applications are associated with controversies; therefore, it is important to address the ethical, legal, and social issues early. Many technical questions are yet to be answered and require close interdisciplinary collaborations of surgeons, engineers, chemists, and biologists, with the ultimate goal of functional tissue restoration. As more scientific knowledge will be gained from stem cell research, hopefully, some of the current ethical and technical concerns will be answered or removed in the future.

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