Chapter Six

Human Stem Cell Research:

The Alchemist's Dream

Introduction

Conceptually, stem cell research can be viewed as a form of modern alchemy that transforms primordial embryonic cells into specialized, differentiated cells, which can be used to replace damaged cells or organs and may revolutionize medicine. There are currently many initial clinical studies seeking to examine how stem cell technology can be applied to correct organ failure, grow new organs *in vitro* for organ transplantation or treat a variety of chronic diseases that plague humans.

The clinical applications of stem cell-based therapy are vast. The potential exists to treat some of the most disabling human diseases including diabetes, Alzheimer's disease, spinal cord injuries, macular degeneration, multiple sclerosis, heart disease, neurological diseases, and cancer. According to the statistics published online by various organizations including the CDC (Center for Disease Control and Protection), there are over 200 million people in the United States suffering from chronic diseases (about 5 million Americans with Alzheimer's disease, 27 million with some form of cardiovascular disease, 26 million with diabetes, 79 million with a pre-diabetic condition, 11 million with macular degeneration, 1 million with Parkinson's disease, 13 million with cancer, and more than 50 million with osteoporosis), which are potentially treatable with stem cell-derived therapies. Moreover, some bioethicists such as Glenn McGee predict that a billion individuals around the world may be treated with human embryonic stem cells before the decade comes to an end.[1] However, the process of applying stem cell technology to treat human diseases is much slower than predicted. As of 2016, the FDA has only approved one application of stem cell technology. Cord blood-derived hematopoietic progenitor cells have been approved for certain diseases such as blood cancer and some (inherited) metabolic and immune disorders.

Stem cell research will also lead to a better understanding of fundamental aspects of biology in the areas of cellular differentiation, trans-differentiation, epigenetics, and de-differentiation**.** In this light, stem cell research simultaneously represents a domain of fundamental discovery in human biology, and also a therapy with the potential to affect human health and quality of life.

However, embryonic stem cell research is also one of the most morally controversial scientific areas of the 21st century because, until recently, these stem cells could not be isolated without destroying the early embryo. While stem cells can also be isolated from adult tissues, the current view is that embryonic stem cells obtained either

[1] http://www.springerlink.com/content/g3h427539krqp648/fulltext.pdf

from non-implanted early embryos or from discarded embryos offers the best potential source for therapeutic application, for reasons that will be explained later. There are almost 500,000 frozen embryos, stored in IVF clinics across the US, which could be donated to stem cell research.

Rarely do democratic governments try to regulate new forms of medical research; however, governments around the world are trying to regulate and restrict basic embryonic stem cell research. Why? The prevailing cultural and religious views in many Western countries claim that once an ovum is fertilized by a sperm, even outside of the womb, the resulting zygote attains human status, making the destruction of such early embryos unethical, immoral, and possibly even a form of murder (see Chapter 5). To better appreciate the dilemmas associated with stem cell research, this chapter focuses on understanding and updating the basic biological principles of stem cell development and research. The bioethical dilemmas associated with stem cell research are examined in Chapter 7.[2]

Defining and Characterizing Stem Cells

In many organisms, life begins from a fertilized egg that divides, grows, and differentiates into all the various specialized cells—such as neurons, muscle cells, pancreatic cells, and blood cells—that an animal needs to function. Cell differentiation begins with the fertilized zygote, and is a process that regulates the functional and structural specialization of cells in all organ systems within a multicellular organism. Specifically, differentiation occurs via differential gene activity, in which each specialized cell type turns on or off selected genes specific for that cell type. Cell specialization, for over 200 histologically different cell types characterized in the human body, is thus determined by the activation and suppression of a specific subset of the ~20,000 genes in the human genome.

As the egg divides and grows, new stem cells are generated to allow for the full embryological development of the organism. Stem cells are **self–renewing,** primitive cells that can develop into functional, **differentiated** cells. Stem cells are naturally occurring in all multi-cellular complex organisms, and are found at every stage of development from conception to death. In adult tissue, stem cells can replenish the wear, damage, and disease that affect tissues during the lifespan of the organism.[2]

All stem cells exhibit two fundamental properties: **self-renewal and plasticity**. Self-renewal is the ability of stem cells to divide indefinitely, producing a population of identical offspring. Plasticity describes the capacity of stem cells to undergo an asymmetric division, on cue, to produce two dissimilar daughter cells. One daughter cell is identical to the parent and continues to contribute to the original stem cell line (Fischbach and Fischbach, 2004), while the other differentiates into one of the many specialized cell types. In general, stem cell proliferation is associated with only one, not both, of the daughter cells differentiating: the other retains its undifferentiated state to

[2] An online course in stem cells is available at http://stemcellbioethics.wikischolars.columbia.edu/.

maintain the reservoir of stem cells.

Before describing the different types of stem cells, it is important to review some basic elements of early human embryology. After fertilization, the haploid nuclei of the egg and sperm in the zygote fuse to form a single nucleus containing 46 human chromosomes. The zygote, derived from the Greek words zugōtos 'yoked, or zugoun 'to yoke', undergoes cellular proliferation to form a compact ball of cells called the morula, which has the appearance of a mulberry (the Latin term *morus* means mulberry). As the morula flows through the oviduct, the cells in the embryo continue to proliferate and the morula enlarges to form a hollow sphere called a blastocyst. Within this hollow sphere, a few specialized cells form an inner cell mass within the cavity. This cellular cluster is a primary source of embryonic stem cells. The time between fertilization and implantation of the human embryo in the uterine wall is approximately 9-14 days.[3]

There are several types of stem cells:

1. Totipotent stem cells are cells that can differentiate into any of the 200 plus specialized cells in the human body. In general, **totipotency lasts for about 3-5 cell divisions** after fertilization until the embryo implants into the uterus and has the potential to develop into a complete fetus and a placenta. As the embryo further develops, germinal totipotent cells are formed migrate into the primitive gonad, also called the genital ridge, and can differentiate into either female or male germ cell precursors.

Textbox 1. In a 2015 study scientists were able to generate totipotent murine stem cells from pluripotent embryonic stem cells by altering how chromatin and histones are formed (Ishiuchi et al., 2015). The totipotent cells resembled embryos at the 2-cell stage, and were capable of creating every cell type in the mouse. Understanding how to generate totipotency, "is essential to understanding of how a maximum degree of cellular plasticity can be achieved and maintained, thereby providing more options for efficient reprogramming and potential therapeutic avenues,". This technology can be seen as opening the door to reproductive cloning where there is strong moral and ethical opposition

2. Pluripotent stem cells have the capacity to differentiate into any other cell type, but cannot be implanted into a uterus to create a fetus because they lack the essential cells of the placenta. When the number of cells in the embryo approaches 32-64 a blastocyst is formed that creates a cell-free center within the expanding cluster of cells. Cells, called trophoplasts, in its outer cell layer differentiates and forms the placenta. Cells in the blastocyst's inner cell mass, called embryoblasts, develop into the fetus and are *pluripotent* because these cells cannot form a placenta. Isolated stem cells from the inner cell mass can be adapted to grow in a Petri dish and can be induced by biological substances or by environmental

[3] http://writ.news.findlaw.com/grossman/20011120.html

conditions to differentiate into any cell type found in the body (Figure 1).

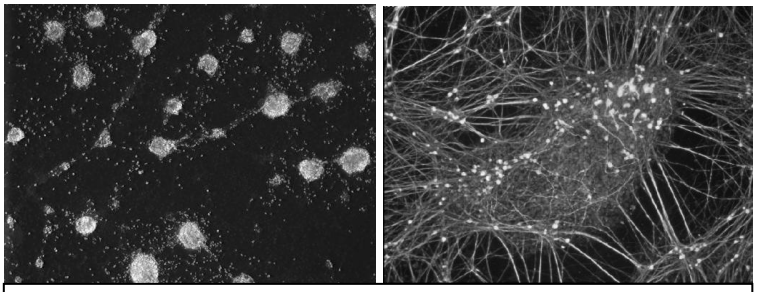


Figure 1. Stem cell differentiating into beta-insulin producing cells (left) and into neurons (right) (from NIH image gallery).

3. Multipotent stem cells are generally found in adult tissue and are technically pluripotent. They were originally thought to be responsible for the regeneration of only a very restricted set of cell lineages. However, it is becoming increasingly evident that some multipotent stem cells show considerable plasticity, and can be triggered to differentiate into a wide variety of specialized cells. Still, they cannot differentiate into as many different kinds of specialized cells as pluripotent stem cells (Wang, et al. 2009). The different underlying biological mechanisms that regulate totipotent, pluripotent, and multipotent stem cells differentiation remains an intense area of ongoing investigation.

Embryonic stem cell research aims to provide a better understanding of the underlying mechanisms of cellular differentiation. For example, once stem cells differentiate into a specific cell lineage, they do not change to other cell lineage pathways. Stem cells that begin differentiating into white blood cells will not change course and become red blood cells. In contrast, many tumor cells can jump cell lineages or de-differentiate. Therefore, there is a great need to understand the complete biology of stem cells in order to identify how physiological and non-physiological products and processes regulate gene expression and differentiation.

One of the biggest historical breakthroughs in human stem cell research occurred in 1998, when researchers led by James Thomson, isolated and grew stem cells derived from human embryos (Thomson et al., 1998). These human pluripotent embryonic stem cells were derived from fertilized embryos that were less than a week old. Five independent stem cell lines were developed that could either be perpetuated in culture for long periods of time or be frozen and recovered at a later date. Dr. Thomson used this technology to develop stem cell lines from 14 blastocysts that were obtained from donated, surplus embryos produced through *in vitro* fertilization. This was the first time human embryonic stem cells had been successfully isolated and cultured in a laboratory. Amazingly, this discovery came just seven years after the first reports of the isolation and culture of embryonic stem cells from mice (Evans and Kaufman, 1981).

At the same time researchers, led by John Gearhart, described a method to isolate and culture immature germ cells from 5 to 8 week-old fetuses that were donated anonymously by women undergoing therapeutic or spontaneous abortions (Shamblott et al., 1998). These scientists placed the stem cells, obtained from the germinal centers of the ovaries or testes, in plastic dishes and added biological factors that enabled the germ

stem cells to continue to divide while remaining in a state of suspended development, preventing differentiation. These germ cell-derived stem cells could also be frozen, recovered, and maintained as stem cells in culture. Interestingly, Gearhart's initial purpose for his research was to develop a tool for studying Down's syndrome.

The success of both Thomson's and Gearhart's research was based on their ability to retain and maintain the two fundamental properties of stem cells: self-renewal and plasticity. Both research groups showed that these cells could be repeatedly frozen and thawed while still maintaining their characteristic undifferentiated stem cell properties.

Once techniques were developed to isolate and culture human embryonic stem cells, many scientists around the world began to generate other human stem cell lines. These stem cell lines have been used as models to understand the regulation of cell differentiation and as potential sources for stem cell replacement therapy. One major clinical objective in cell replacement therapy is to use differentiated cells, such as neurons, to replace cells injured due to trauma (spinal cord injury) or neurodegenerative diseases such as Alzheimer's or Parkinson's disease. There are currently many ongoing clinical trials attempting to use cell replacement therapy for a variety of diseases. However, one major obstacle in these trials is the potential immunological rejection of the transplanted cells by the recipient patient. Ideally, stem cell therapy would be best implemented using the patient's own stem cells. The quest for generating patient-specific stem cells has led to the search for a method to utilize somatic cell nuclear transfer (SCNT- see Chapter 4) to isolate embryonic stem cells from patients. As discussed in Chapter 4, SCNT involves transferring the nuclear genetic material from a patient's own cell into an enucleated oocyte. This "fused" cell is then stimulated to develop into a pre-implanted embryo in order to harvest the embryonic stem cells from the inner mass. Stem cells isolated in this manner would be histocompatible to the patient and therefore could be used for cell replacement therapy. Reports by Noggle et al. (2011) and Tachibana et al. (2013) on the application of SCNT to human cell systems have already stimulated a great deal of research into possible methods of deriving patient-specific stem cells.[4]

In 2004, researchers in South Korea claimed to have successfully cloned a human non-implanted embryo as a source for harvesting embryonic stem cells (Kim and Park, 2013) (Hwang, Ryu et al., 2004; Hwang, Roh et al., 2005). Hwang claimed to have used extremely fresh eggs donated by South Korean volunteers. When workers in his research institute reported that they were coerced to donate their eggs, the scientific community began to learn about the scientific fraud. All of their data was falsified. Conceptually, Hwang was correct in principle, but it took another eight years until Noggle et al., (2011) and Tachibana et al., (2013) were able to apply SCNT technology to generate human embryonic stem cells.

Like SCNT, stem cell research is also susceptible to academic pressure and the risk of scientific fraud. In 2014, a group of scientists from Japan's Riken Center for Developmental Biology reported two papers in Nature. In their first paper, they reported that stem cells could be generated by exposing differentiated adult murine cells to an acid

[4] http://www.medicalnewstoday.com/medicalnews.php?newsid=70950

bath and other external environmental stresses (i.e., low pH conditions), in order to revert these cells into stimulus-triggered acquisition of pluripotency (STAP) cells. In their second paper, which appeared in the same issue of Nature, they claimed that STAP cells could also contribute to the placental tissue. This would demonstrate that STAP cells are not just pluripotent, but also totipotent, unlike embryonic and induced pluripotent stem cells. While these findings caused great excitement, it soon become clear that other laboratories across the globe could not replicate these findings. A six-person committee — three Riken scientists, two university researchers and a lawyer — found that the lead scientist, Dr. Obokata, had manipulated data in an intentionally misleading fashion. The committee branded this research as scientific misconduct.[5] Moreover, the top administrators of RIKEN, Japan's national network of research laboratories, decided to voluntarily return 1 to 3 months of their salaries in order to atone for their responsibility in the STAP stem cell fiasco (See Textbox 2).

Textbox 2: Scientific Fraud

Revelations of scientific misconduct always cause collateral damage. They taint the colleagues and co-authors of the person responsible, and can close down labs. In the case of RIKEN, a leading administrator hanged himself as a result of scientific fraud.

How common is scientific fraud? The PubMed database of biomedical research claims that only 1 in 10,000 recent papers, has been retracted. What is process of retraction? Can there be evidence of fraud but evidence not substantial so paper not retracted? However, other measures of misconduct appear to be more common. Daniele Fanelli, a senior research scientist at Stanford University, pooled data from 18 surveys and found that almost 2 percent of scientists admitted to fabricating their work or manipulating data. When asked whether they'd ever seen misconduct among peers, 14 percent said they had. Scientists have become less likely to admit misconduct," says Dr. Fanelli, "but they're no less likely to report the misbehavior of their colleagues.

Governmental fines issued for fraud are not high. But in August of 2015, the National Science Foundation ordered Northeastern University to pay back $2.7 million for nearly a decade of mishandling a grant from the agency.

Stem Cells Can Be Obtained from Various Tissues

There are six major tissue sources of stem cells: embryos, fetuses, umbilical cord blood, adult organs, amniotic fluid, and teratocarcinomas. Stem cells from the embryo or fetal tissue can either be totipotent or pluripotent, as described earlier. From an ethical perspective, it is also important to identify whether the stem cells are obtained from "spare embryos" created via IVF, cloned embryos (created for research purposes), or aborted

[5] http://www.nature.com/news/stem-cell-scientist-found-guilty-of-misconduct-1.14974

fetuses, since each tissue source of stem cells would elicit different moral perspectives (see Chapter 7). Stem cells from adults are generally *multipotent* and can be obtained from a variety of sources, including the bone marrow and most major organs. There are a few organs, such as the pancreas, from which stem cells have not been obtained. Another source of adult stem cells is human post-mortem tissue, which can be extracted up to 20 hours after death. Unlike embryonic stem cells adult-derived stem cells exhibit a more limited capacity to differentiate into various cell types.

Human amniotic fluid stem cells and umbilical cord blood may be other important sources for both basic science and regenerative medicine. These stem cells exhibit a high proliferation rate, are self-renewing, and may have a lower frequency of tumor production than embryonic stem cells (Roura et al., 2012; Cananzi et al., 2009).

Another source of stem cells is teratocarcinomas, which, historically, were first recognized as yielding pluripotent stem cells. Teratocarcinomas are gonadal tumors. These tumor cells are also one of the main components of human testicular germ cell tumors. One interesting feature of teratocarcinomas is that they contain a wide array of tissues derived from the three primary germ layers that make up an embryo: the endoderm, mesoderm, and ectoderm. Thus, they contain a large assortment of tissue types including cartilage, squamous epithelia, primitive neuroectoderm, ganglionic structures, muscle, bone, and glandular epithelia. The differentiated cells of the tumor are formed from pluripotent stem cells present in the tumor. While there is currently limited application for utilizing these cells as sources for stem cell therapy, these cells have provided great insights into the mechanisms of cell differentiation and tumorigenesis.

In 2007 and 2008, scientists claimed a major breakthrough by inducing adult fibroblasts to de-differentiate into stem cells that have pluripotent characteristics. These scientists were able to reprogram mouse fibroblasts into induced pluripotent stem cells (iPS) by genetically overexpressing four genes (oct4, sox2, klf4, and c-myc) and using subsequent drug selection for the reactivation of a marker for pluripotency (Greenbaum 2010). The process of reprogramming is slow and the frequency of developing into stem cells is low, so it could take up to 20 days to transform fibroblasts into stem cells. In addition, there are reported side effects of using iPS generated stem cells. Yamanaka et al. (Yamanaka and Blau, 2010) found that 20% of the stem cell-derived offspring developed tumors, presumably related to the activation of one of the transfected genes such as Myc (an established oncogene). iPS cells have been obtained from differentiated stomach cells, fat cells, and liver cells and can be obtained even if Myc, which can induce cancer, is omitted. The resulting stem cells do not appear to be substantially different from ES (embryonic stem cells). In 2009 and 2011, there were other improvements in iPS technology (Hong et al., 2009, Kawamura et al., 2009, Li, Collado et al., 2009, Marion et al., 2009, Utikal et al., 2009). Non-integrating adenoviral vectors or plasmids, for example, were used to achieve transient expression of reprogramming factors without disturbing the host genome. But such an approach presents two immediate problems: the requirement for prolonged expression of the pluripotency factors to achieve reprogramming, and the difficulty of repeatedly delivering the full complement of factors using different vectors.

The goal of this research was to develop viral-free systems to generate iPS (Pera 2009). A leap of faith must be taken in order to transition from proof-of-principle in mice to application in humans, and there are still scientific hurdles to overcome. If human stem cells can be generated using iPS technology, patient-specific stem cells could be made without the use of donated eggs or embryos. This technique has an obvious ethical advantage because it does not require the destruction of pre-implanted embryos. Yamanaka, who discovered iPS, received the Nobel Prize for his work in 2011.

In 2012, researchers adapted the iPS technique of Dr. Yamanaka to breed genetically engineered mice with the same cocktail of four reprogramming transcription factor genes. By having the mice drink a particular drug, these genes were turned on and embryonic stem cells appeared in multiple tissues and organs in these mice within a few weeks. The researchers extracted these cells and demonstrated through various tests that they were like those in a new embryo containing just 16 cells (Abad et al., 2013). The next step is to explore whether these *in vivo*-generated iPS stem cells are capable of efficiently generating different tissues in vital organs such as the pancreas, liver, heart, bone marrow, or kidney. Their research aims to devise methods for inducing regeneration locally, as well as in a transitory manner, for a particular damaged tissue.

Another problem with iPS technology is that the stem cells generated do not have the same epigenetic markers as embryonic stem cells. iPS cells differed and retained residual DNA methylation patterns and the transcriptome profiles of their parental somatic cells. In contrast, embryonic stem cells generated via SCNT technology corresponded closely to similar cells generated by classical IVF technology. Thus, human somatic cells can be faithfully reprogrammed to pluripotency by SCNT and may be better suited for cell replacement therapies (Ma et al., 2014). Because of ethical concerns regarding embryonic stem cells and histocompatibility issues, research is focusing more on applying iPS cells to clinical situations (Takahashi, et al., 2016).

A new technology is developing in which one cell type is directly converted into another without going through a “stem cell” intermediate. In a 2014 paper, Dr. Yoo and his colleagues reported that co-expression of various transcription factors, enriched in the developing striatum, can guide the conversion of human postnatal and adult fibroblasts into an enriched population of neurons analogous to striatal medium spiny neurons (Matheus et al., 2014). In addition, they demonstrated that when transplanted in the mouse brain, the reprogrammed human cells persisted in situ for more than 6 months, exhibited membrane properties equivalent to native medium spiny neurons and extended projections to the anatomical targets of these cells.

Disadvantages of Stem Cells Derived from Different Sources

The major disadvantages of embryonic stem cells, apart from ethical considerations, are that they may be rejected if transplanted in an HLA incompatible

person, and that they may form tumors more easily than adult- derived stem cells. Adult tissues contain multipotent stem cells that provide another source for stem cell research. The most common organ for multipotent stem cells is the bone marrow whose stem cells can differentiate into a variety of different cell types. Moreover, the ease with which bone marrow cells can be obtained and our experience using these cells in a variety of

Textbox 3. Funding of human embryonic stem cell research.

A clear pattern has emerged over the years in states such as California (the nation's largest funder of stem cell research apart from the federal government) and Maryland to trend away from funding hESC research and provide overwhelming financial support for ethically non-contentious adult stem cells and other types of non-embryonic stem cell research. Minnesota is the most recent state to provide public money for adult stem cells and other ethically non-contentious, non-embryonic stem cell research stem cell research. In declining to fund hESC research Minnesota is echoing a trend that has been gathering momentum for years. Do you believe this is a valid approach to scientific research?

treatments (e.g., leukemia) have been a great impetus for exploring them as a source of adult stem cells. Yet, bone marrow-derived cells are not as pluripotent as embryonic stem cells. Another possible disadvantage of using stem cells from bone marrow is that about 10-20% of patients lack a sufficient number of recoverable bone marrow-derived stem cells for therapeutic transplantation because of the patients' disease.

The main advantage of using bone marrow or any adult-derived stem cells is their use in autologous therapy, which avoids the risk of tissue rejection. Adult-derived stem cells, however, have some disadvantages in therapeutic applications. One technical hurdle is that they can only be isolated in low numbers. In mouse bone marrow, stem cells represent only 1 in 10,000 cells. In addition, they are more difficult to isolate than embryonic stem cells, are notoriously slow to grow in culture, and have a restricted proliferation potential.

Another issue with adult derived stem cells is their plasticity, or ability to differentiate into other cell types. Adult derived stem cells from certain organs such as bone marrow, muscle, fat, liver, synovial membranes, and brain, express better plasticity or pluripotency than adult cells from other sources. For example, studies (Santarelli et al., 2003) showed that, even in adult rodent brains, stem cells had the capacity to generate neurons (neurogenesis). This finding may explain why patients taking antidepressants require several weeks before a therapeutic effect is seen. During this time, the antidepressants appear to stimulate the generation of new neurons in these patients. This research could lead to developing new compounds that trigger neurogenesis from endogenous adult stem cells in the brain. In fact, a San Diego-based start-up called BrainCells screens drugs that stimulate the proliferation of neural stem cells in the hope of finding new antidepressants or drugs to treat cognitive disorders, such as Alzheimer's.

Since there are several sources of embryonic and adult stem cells, it is critical to

assess which type of stem cells will generate the best therapeutic value. To date, the main disadvantages of adult stem cells are that they are: a) few in number, b) difficult to isolate and maintain in culture, c) slow to proliferate, and d) difficult to stimulate to differentiate into various other tissues types. Until we are able to test stem cells, from various sources, side-by-side in the laboratory and in a variety of experimental paradigms, the answer to whether or not adult embryonic stem cells could serve in a therapeutic mode will remain unresolved.

Stem Cell Differentiation Assessment and Targeting

There are several stages between isolating stem cells and transferring them to patients. Currently, therapeutic applications are focused on five major health problems: diabetes, blood diseases (including AIDS), neurodegenerative disease, spinal cord injuries, and cardiovascular disease. However, the critical stage in the development of these therapies is assessing the capacity of stem cells to differentiate into specialized cells.

Manipulating the extracellular environment can trigger the differentiation of stem cells into specialized cells. Differentiation into specialized cell types, for example, can be initiated by growing the stem cells at high cell growth densities, placing them on different types of non-proliferating feeder cells, adding specific growth factors, or maintaining these cells on either crude or defined extracellular matrices. Scientists are just beginning to discover the control mechanisms for generating specialized cells. A great deal of future investigation remains necessary for a complete identification of all cell culture conditions, or chemical factors, that regulate stem cell differentiation.

In the laboratory, there are several methods to assess the developmental potency of pluripotent stem cells: (1) *in vitro* differentiation in a Petri dish; (2) differentiation into teratomas or teratocarcinomas, and (3) *in vivo* differentiation when introduced into the blastocoele cavity of a pre-implantation embryo. In the first method, scientists use plasma membrane surface markers to determine whether the embryonic stem cells will differentiate into the target specialized cell. In addition to surface markers, current research also focuses on generating gene expression profiles to characterize stem cells and their differentiated progeny. In the second method, pluripotency is demonstrated when human embryonic stem cells are injected into an animal and form teratomas. The third method involves injecting the human stem cells into a developing animal embryo; pluripotency is assessed by analyzing the tissue distribution of the human cells in the animal that is born. It is important to note that testing human embryonic stem cells in this manner involves creating a human-animal chimera that may elicit bioethical concerns (see chapter 8).

In many instances, stem cell differentiation leads to a mixed population of non-differentiated cells and differentiated cell types. The differentiated cells and the non-differentiated stem cells must then be separated from one another. Separation of these two populations is possible because each cell type expresses unique surface proteins.

Clinical Applications of Stem Cell Technology

A specific lure of stem cells in cell and organ replacement therapy is based, in part, on the fact that stem cells offer an unlimited supply of potential cells to use in transplantation, in order to repair either diseased or damaged organs. In addition, stem cells obtained from the patient offer a promising method of cell or organ replacement without the risk of tissue rejection. In contrast, conventional organ transplantation involves finding a donor whose HLA antigens express the greatest compatibility with the patient's own tissue. Since it is usually difficult to find tissue-compatible donors, transplant recipients must often be placed on medications for at least a year, if not longer, to prevent their immune systems from rejecting the transplanted organs. These medications are associated with many side effects that can cause dangerous health risks (Griffith and Naughton, 2002). Although the technology that uses stem cells to generate complete organs is in its infancy, cell replacement therapy may offer a viable clinical alternative for classical organ transplantation in the future.

Other medical uses that may result from stem cell technology include: patient-specific drug development, gene therapy, and the study of underlying mechanisms of disease. As stated above, it appears that cell replacement, as opposed to organ development, is the most immediate therapeutic utilization of stem cells. In the following section, we will briefly review the current research in applying stem cell technology to treat heart disease, diabetes, and Parkinson's disease.

Heart Disease: Research on the clinical application of stem cells to heart disease is being conducted by many centers around the world. Scientists are trying to examine how stem cells can be used as a means to augment cardiac repair and regeneration (Lin and Pu, 2014). On average, an individual who experiences one myocardial infarction (MI) loses about 1 billion cardiomyocytes. Transplanting human embryonic stem cell-derived (ESC) cardiomyocytes into patients with heart disease may enhance cardiac repair and function. One fundamental medical challenge related to the use of stem cells in heart disease is the relative immaturity of current ESC-derived cardiomyocytes. Although these cells contract and generate force, their immaturity likely reduces their efficacy and host integration. In addition, these ESC is allogenic (and not from the patient's own cells) and require the patient to receive drug-mediated immunosuppression to avoid graft rejection. Finally, the safety (lack of teratoma formation or arrhythmogenesis) and longevity of ESC-based grafts will need to be carefully demonstrated.

Cardiac progenitor cells (CPS) are another cell source that might have therapeutic applications for heart disease. These cells differentiate into both vascular cells and myocardiocytes. On the basis of preclinical studies, these cells were tested in humans with ischemic heart failure who underwent coronary artery bypass graft surgery in a randomized, open-label, phase 1 study called SCIPIO. Four months after surgery, autologous CPCs, expanded from myocardial tissue harvested during surgery, were administered by intracoronary infusion. No adverse events related to CPC treatment were noted. However, the clinical outcomes were not so dramatic. CPC-treated patients had

slight, statistically significant improvement in the left ventricular ejection fraction compared to untreated controls at 4 months (36% versus 29%). Thus, there is much more work to be done before stem cell therapy can be applied to treat heart disease.

In most respects, iPSCs behave like ESCs, and thus offer their conceptual advantages. At the same time, iPSCs sidestep the ethical issues that surround ESCs. Because it is possible to generate autologous iPSCs, these cells would also circumvent the need for immunosuppression. However, production of iPSCs will require months of preparation, precluding their deployment for acute or sub-acute illnesses such as MI. Furthermore, the uniform manufacture of iPSC-derived cardiomyocytes from individual patients is a major logistical and regulatory hurdle for the clinical use of iPSC-derived cells (Li and Carlos, 2016).

Diabetes: In Type I diabetes, the beta islet cells of the pancreas, which normally produce insulin, are destroyed by an autoimmune process. The pancreas is an interesting organ because, to the best of our knowledge, it is not clear if this organ contains natural stem cells (Kopp et al., 2016). Scientists are actively differentiating embryonic stem cells into beta islet cells capable of producing insulin, in order to transplant these cells into a diabetic patient. In order for this procedure to work clinically methods must be designed that the diabetic patient's immune system will not destroy the newly transplanted islet cells in the same fashion that it destroyed its own beta cells. Even if beta cell destruction in diabetic patients were to occur, it might not occur immediately, rendering stem cell therapy a viable method to acutely treat diabetics. However, this would require periodic renewal transplantation of stem cells in order to maintain a non-diabetic state.

In a 2007 article, scientists were able to use stem cell therapy in conjunction with anti-rejection therapy to treat a small number of patients with Type I diabetes so that they did not require insulin injections (Voltarelli et al., 2007). This was the first time stem cell therapy was effective in taking diabetic patients off insulin. Since then there have been several studies examining the use of stem cells to treat diabetes (El-Badawy and El-Badri, 2016). In 2016, Doug Melton and his colleagues published a landmark paper that used encapsulated embryonic stem cell-derived islet cells to treat diabetic mice (Vegas, et al., 2016). Encapsulation prevented the recipient animal from rejecting the heterologous islet cells and still their physiological responses to produce insulin. Encapsultation protects allogenic stem cells from the host's immune system by creating a matrix barrier between the transplanted islet cells and the pancreas that allows diffusion of glucose, other nutrients, and insulin but not of larger molecules, cells, or antibodies. Moreover, even if some of the transplanted stem cell derived β cells turn tumorigenic the physical barrier limits their growth, and more importantly these tumorigenic cells cannot escape into the vascular or tissue compartments to cause system wide cancer.

Neurodegenerative Diseases: In Aug of 2014, a neurosurgery team transplanted cells from aborted human fetuses into the brain of a person with Parkinson's disease. This operation broke a decade-long international moratorium on the controversial therapy, which was imposed after many patients failed to benefit from fetal cell transplants. Parkinson's disease is characterized by degeneration of neurons in the substania nigra of the brain that produce the neuro-transmitter dopamine, which is crucial for normal

movement. Conventional treatment, such as the administration of L-dopa, replaces dopamine to treat the symptoms, but does little in slowing down the progression of the disease. These cellular therapies aim to replace the dopamine-producing (dopaminergic) cells with cells from fetal brains or with those derived from human stem cells.

Research is under way to ensure that the stem cells develop into the exact type of dopaminergic cell needed to treat Parkinson's and that they become correctly integrated into recipients' brains. Progress has been so fast that clinical trials are already on the horizon. A Japanese trial, using induced pluripotent stem cells, is planned to start in Kyoto within two years; and two trials using human embryonic stem cells are also planned – one to begin within three years in New York and the other in Europe within four to five years. In 2016, the Colorado Clinic offers stem cell therapy for back pain relief to help patients achieve relief and avoid the need for back surgery. The treatments are offered by Board Certified providers for both spinal disc and joint degeneration. The clinic offers two options. The first is giving the patients platelet rich plasma (PRP) therapy that contains many growth factors. While PRP therapy doesn't have stem cells directly, it does trigger the body's stem cells to engage in a repair process. The second option is amniotic stem cell therapy, in which amniotic fluid is harvested from consenting donors after a scheduled caesarian section. The process is FDA regulated and the fetus is safe. The third option is bone marrow derived stem cell therapy, in which the bone marrow is harvested from the patient's hip area.

Another approach to stem cell therapy is to develop medications that enhance endogenous stem cells, naturally found in many organs, to proliferate. Since most organs in the human body contain their own stem cells, specific cellular hormones or growth factors could be identified that promote differentiation *in situ*. This type of therapy would not require injection of stem cells into patients; this would allow for broad clinical applications and eliminate most bioethical and religious concerns by eliminating the need for embryos. For example, current evidence suggests that the brain contains endogenous stem cells. Thus, a drug that stimulates stem cell proliferation may one day be helpful in treating victims of strokes, Parkinson's, or Alzheimer's disease. Administering cellular hormones that summon the migration of stem cells to sites of injury presents another kind of potential therapy.

Stem cell transplantation in the brain may operate in novel ways. In the past few years, there have been reports (Lindvall and Kokaia, 2010) of stem cells used to treat spinal cord-paralyzed rats. The mechanism by which recovery from paralysis was observed remains unclear. At first, it was believed that the transplanted stem cells differentiated into new neurons that repaired damaged spinal nerves. Now, evidence suggests that the transplanted stem cells stimulate the production of specific growth factors and cytokines that promote regeneration of endogenous nerve (damaged or undamaged) and stem cells (Fernandez, Mannino et al., 2006, Cabanes et al., 2007). In a recent 2014 publication[6], scientists transplanted olfactory ensheathing cells from a paralyzed patient's own olfactory bulbs to his injured spinal cord. These offactory nerve cells are highly regenerative and offer an innovative source for nerve repair. In fact, the

[6] http://dx.doi.org/10.3727/096368914X685131

success of this trial is the first time that cell transplantation has been shown to reverse paralysis in a real-life situation in which the injury involves a combination of damage to the nerve fibre and to surrounding tissues. While the therapy did not completely restore function, it marks a very significant step towards a potential therapy. Dr. Alok Sharma, director, NeuroGen Brain and Spine Institute in India is beginning to apply stem cell therapy to treat patients with autism, cerebral palsy, and mental retardation.

Other Diseases: Another therapeutic benefit has emerged from stem cell research. Several studies (Potier et al., 2010) show that, as a result of bone marrow transplants, donor stem cells can fuse with resident host tissue cells. Therefore, injecting genetically modified stem cells might constitute a novel means of introducing new genes into the host without the use of viral vectors. The injected stem cells, which contain new genes, would fuse with endogenous cells and allow the expression of these new gene products. The use of stem cells as gene transfer vehicles may lack the clinical problems associated with conventional gene transfer using viral vectors, such as inflammatory side effects and the potential to develop certain forms of cancer.

There is a great deal of interest in applying stem cell technology to treat macular degeneration. Macular degeneration is a common eye condition and a leading cause of vision loss among people age 50 and older. It causes damage to the retinal epithelial cells in the macula, a small spot near the center of the retina and the part of the eye needed for sharp, central vision. In a 2014 study published in Lancet, Dr. Robert Lanzia from Advanced Cell Technology reported the first evidence that stem cell therapy can be used to replace the damaged retinal pigment epithelial cells (Schwartz et al., 2014). Over 70% of the transplant recipients had measurable increases in sub-retinal pigmentation, which gradually increased over time. These results are indicative of high-rate stable engraftment of the newly transplanted retinal pigment epithelial cells. Stem cell technology may therefore prove to be an effective treatment of this disease.

In July 2013, Japan's regulatory authorities gave the go-ahead for a team led by ophthalmologist Masayo Takahashi at the RIKEN Center for Developmental Biology in Kobe to collect cells to be used in a clinical iPS cell pilot study. Skin cells from a woman in her seventies with macular degeneration were reprogrammed to become retinal tissue. These cells were then transplanted into the eye, and RIKEN has reported that the patient experienced no serious side effects. This patient was the first person to receive iPS generated stem cells.

Creating human organs. The use of stem cells to generate rudimentary organs has taken off in the past five years. Using carefully timed chemical cues, researchers have produced three-dimensional structures that resemble tissue from the eye, gut, liver, kidney, pancreas, prostate, lung, stomach, breast, and brain. These bits of tissue are called organoids because they mimic some of the structure and function of real organs.

Organoids are furthering our knowledge of human development, serving as disease models and drug-screening platforms, and might eventually be used to rescue damaged organs. A key breakthrough in creating organoids has been embedding stem cells in matrigel, a soft jelly that resembles the extracellular matrix of many organs. Organoids do not function as well as human organs. Some lack key cell types; others imitate only the earliest stages of organ development or vary from batch to batch (see Willyard, 2015 for a review). Because organoids can be grown from human stem cells and from patient-derived induced pluripotent stem cells, they have the potential to model human development and disease. Furthermore, they have potential for drug testing and even future organ replacement strategies.

Ageing. The effects of aging on stem cells is an important area of research. Recent research (Goodell and Rando, 2015) has focused on the ways that genetic mutations, epigenetic changes, and the extrinsic environmental milieu influence stem cell functionality over time. One recent study reports the ways these factors interact, and how these interactions decrease stem cell health over time. The hope is to uncover potential strategies to enhance stem cell function and increase tissue resiliency into old age. Peripheral blood from young individuals, for example, is generated from around 1000 active stem cells. By the age of 70, the clonal diversity collapses, resulting in dominance of one HSC clone, such that about 20% of individuals have one clone that dominates 20 to 80% of blood cell production. Interestingly, the injection of plasma from young mice into the circulation of aged mice has recently been shown to induce a more youthful state of cells in the brain of the old animal. These findings indicate that at least some aspects of cellular aging may be reversible, perhaps through reprogramming of the epigenome.

Textbox 4. FDA Approvals of Clinical Trials

Regenerative medicine investigators at the Cedars-Sinai Medical Center have received U.S. Food and Drug Administration (FDA) approval to test a novel combination stem cell-gene therapy they've developed to stall amyotrophic lateral sclerosis (ALS) progression. Federal regulators also have told Athersys Inc. that its design of its planned Phase 3 clinical trial should proceed into clinical testing as a therapy for stroke victims using adult stem cell therapy.

Stem Cell Therapy in Sports

Several world famous sports figures, including tennis star, Rafael Nadal, NFL's Peyton Manning, NBA's Pau Gasol, and MLB's Bartolo Colon, have undergone stem cell treatment to repair injuries using either bone marrow or fat as the source. The ailing hockey legend Gordie Howe received stem cells grown by Stemedica, and his story attracted international attention. Athletes, whether playing or retired, have a special need for the regenerative abilities that stem cells might provide. They break bones, strain

ligaments, bang knees, and wear out cartilage. If their restorative capability is proven, stem cells could be considered the latest form of sports medicine.

Since Howe's treatment in late 2014, two other athletic legends have received Stemedica's cells — former quarterbacks Bart Starr and John Brodie. These cells can grow into new bone, cartilage, muscle, or connective tissue and help speed injury recovery in an athlete's knee, back, or shoulder. The NFL considers stem cell therapy a medical treatment rather than a performance-enhancing substance. As of 2014, the FDA limits stem cell therapy to the injection of the unaltered harvested cells directly to the site of the injury. Stem cell therapy is even being used in race horses; Sprinter Smoko is reported, in November 2014, to have had stem cell therapy at Murdoch Veterinary Hospital in order to repair a strained suspensory ligament in his off-foreleg. Most of these athletes had to go abroad for treatment. The alleged success in treatment has put a great deal of pressure on the FDA to initiate more clinical trials in the USA.

Stem cell therapies are extremely expensive and profitable. In fact, it is estimated that more than 600 unauthorized stem cell clinics were operating in the United States in 2016 and charge at least $30,000 per treatment. The global stem cell market is projected to grow from about $6.7 billion in 2016 to nearly $12.3 billion in 2021, registering a five-year compound annual growth rate of 13.1% for the period. In the UK, insurance cover for stem cell therapy has been offered for the first time to "democratise" a process that would ordinarily cost hundreds of thousands of pounds. A company called CellPlan is selling coverage for up to $1m (£680,000) for families who have banked their children's umbilical cord blood. Stem cells from the cord blood can be used to treat 82 diseases including leukaemia in close family members.

When Should Clinical Trials of Stem Cell Technology Begin?

While many animal studies serve as models for human diseases and have demonstrated the potential clinical applications of stem cells, translating these studies to humans is often a difficult process. There are many genetic and physiological differences between humans and mice that could account for the failure of therapeutic applications in humans.

There are several clinical trials in progress, or being planned, which aim to examine the clinical efficacy of stem cell therapies. On the commercial side, a leading regenerative medicine clinic on the West Coast, TeleHealth, is now offering multiple stem cell therapy treatments for arthritis and soft tissue injury such as tendonitis of the shoulder. The injection treatments are covered by insurance, and are offered with Board Certified doctors. This clinic claims that stem cell injection treatments possess the potential for actually repairing the cartilage damage in arthritic joints or tendon damage in an injured shoulder.

Public pressure is certainly one reason for the initiation of these clinical trials. The ethical question is whether or not the scientific basis to enter clinical trials is justified. In 2013, an expert panel of scientists had issued a report advising the Italian Government

against continuing to support a controversial stem cell therapy, deeming it 'unscientific'. The clinical protocols in question consisted of using patients' own mesenchymal stem cells, derived from bone marrow, to treat neurodegenerative conditions such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis, as well as muscle-wasting disorders. The panel found the submitted protocols incomplete. Records of preclinical studies were not included. Furthermore, there was a lack of data attesting to the quality of cellular preparation, as well as a lack of data demonstrating the expected expression of proteins in stem cells as they form new neurons. The panel felt that there was not sufficient scientific merit to approve this type of stem cell therapy.

There is a fascinating report, published in October of 2014, stating that a man who was paralyzed from the chest down in a knife attack in 2010 could walk, using a frame support, after receiving stem cells obtained from his olfactory bulb (Tabakow et al., 2014). The treatment used olfactory ensheathing cells which are specialized cells that form part of the sense of smell. These cells enable nerve fibers in the olfactory system to be continually renewed.[7]

All of these stories highlight the potential gains in medicine that people believe will arise from stem cell therapy. Yet, the public must recognize that translational applications of research into clinical trials develop slowly. The Food and Drug Administration (FDA) is concerned that the hopes patients have for stem cell based cures may leave them vulnerable to unscrupulous providers of stem cell treatments that are illegal and potentially harmful. The FDA cautions consumers to make sure that any stem cell treatment they are considering is approved by the FDA or is being studied under a clinical investigation that has been submitted and allowed to proceed by the FDA. As of 2016, the FDA has approved only one stem cell product, Hemacord, a cord blood-derived product manufactured by the New York Blood Center that is used for specified indications in patients with disorders affecting the body’s blood-forming system.

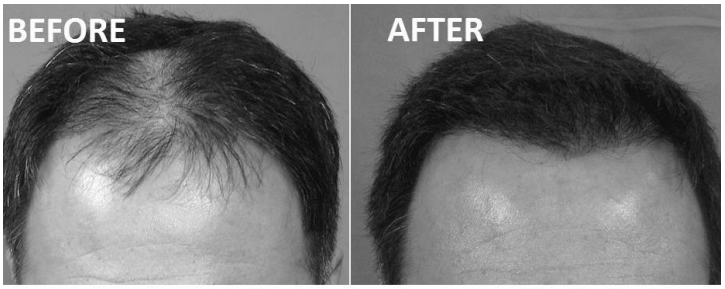
Non-Medical Applications of Stem cells

Stem cells are being studied as a potential treatment modality for a variety of non-medical conditions. Stem cells are being used for a not-quite-surgical procedure that can recontour human faces using a mixture of the patient's own fat and stem cells. This procedure is reported to enable the implanted fat cells to better "take hold" in their new location and become part of the face. In addition, these added stem cells appear to increase the blood supply to the skin to enhance its appearance.

Stem cell technology also enabled scientists at Columbia University to develop a technique to grow human dermal papilla cells, in 3-D culture, to grow de novo hair follicles in human skin, paving the way for a new approach to treating baldness (Higgins et al., 2013). In addition, in a 2014 Nature paper (Yang et al., 2014), scientists describe the method by which they were able to convert adult cells into epithelial stem cells (EpSCs) that formed hair shafts. How did the team produce these cells? The researchers

[7] A video of this report is available at https://www.youtube.com/watch?v=rhFHQMrrz4E.

converted the human skin cells into induced pluripotent stem cells by adding three genes. These iPS cells are able to change into any cell type, so the researchers converted them into epithelial stem cells, which are normally found in a part of hair follicles.



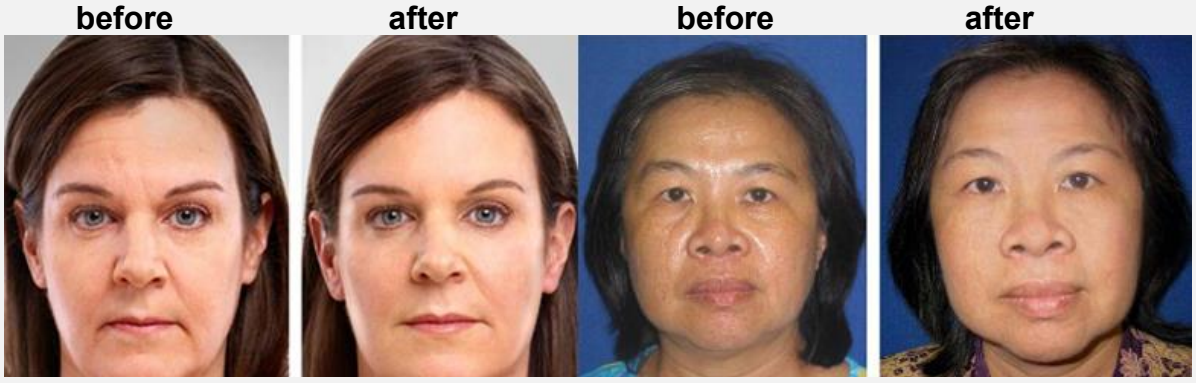
Obtained from http://www.stemrx.in/hair.html

Commercial companies recognize the potential profits of hair restoration. Histogen, Inc., a company whose focus includes hair restoration, presented clinical evidence, at the International Society of Hair Restoration Surgeons (ISHRS) Annual Scientific Meeting in Amsterdam from July 22-26, 2009, that stem cell technology can stimulate hair growth. According to Histogen, HSC is a solution containing naturally secreted embryonic proteins – growth factors that induce new hair follicle formation, hair growth, and hair thickness when injected into the scalp (Meyer-Blazejewska et al., 2011).

Dr. Daniel McGrath is an Associate of the American Academy of Cosmetic Surgery. He runs a clinic that specializes in hair restoration. He removes a small amount of an individual's blood, from which the platelet-rich plasma is obtained and mixed with a wound-healing powder called "a-cell", and injected back into the scalp. Finally, the doctor uses some massage and small needles to create tiny wounds, which trigger a healing hair-restoring response. Dr. McGrath claims that 80 percent hair re-growth or regeneration across the board is observed in his patients. One treatment costs about $3,500.

Textbox 5: Anti-aging Stem Cell Therapy

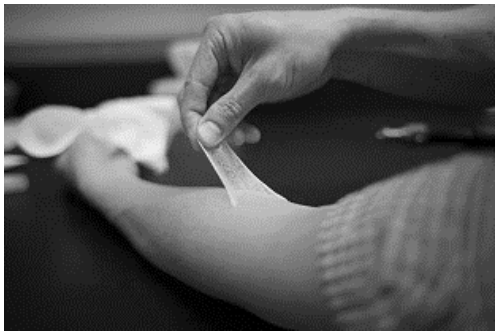
Swiss Medica Clinic provides the latest Stem Cells treatments and procedures to rejuvenate your face, body, organs and increase the feeling of well-being.



Many companies now offer stem cell therapy as its new treatment for Anti-aging (see Textbox 3). Their therapy is based on the theory that aging results from the progressive depletion of stem cells, so the introduction of new stem cells and adjunctive treatments has the potential of slowing down or reversing this process. Another serum product, marketed by Lifeline Skin Care, is based on the unproven concept that human non-embryonic stem cell extracts containing ingredients derived from unfertilized human eggs donated to the ISCO, can renew your skin to a youthful complexion. These anti-aging stem cell serums are marketed to stimulate the skin's abilities to repair itself.

An unusual application of stem cell technology comes from a California company called Ageless Derma.[8] Their skin care product, Swiss Apple Stem Cell Mask, is derived from apple stem cells and incorporates the cells of a long-living rare apple with other natural revitalizing ingredients, resulting in a gentle mask that effectively returns youthful life to the complexion. The cost of this mask is under $40, as compared to a $10,000 product sold by Angle and Weightman, whose face cream contains stem cell extracts that refinishes and re-hydrates human skin.

In June, 2011, the FDA approved a therapy that uses a person's own skin cells to help improve the appearance of smile lines that can extend from the bottom of the nose to the sides of the mouth. The treatment, called laViv, was developed by Fibrocell Science and involves taking a sample of skin cells called fibroblasts, which make collagen, from behind the person's ear. The sample is sent to the company's laboratory, where the fibroblasts are multiplied in cell culture, a process that takes 11 to 22 weeks. The cells are then sent back to the doctor, who injects them into the smile lines (or frown lines), which are technically known as nasolabial folds. The treatment was evaluated in two clinical trials, with a total of 421 patients, in which participants received either three treatments with laViv or three treatments with an injection that did not contain the cells. Six months after the third treatment, both the patients and their doctors, neither of whom knew whether the treatment or control was given, assessed the results.



One consequence of stem cell research is the development of other technologies that are less expensive and are not as ethically challenging. In May of 2016, a report appeared that describes a new and innovative technology called "second skin" (Yu, Kang et al. 2016). While the research was done to help patients who suffer from a variety of skin conditions, the application to the general public might be enormous. Second skin is made of silicon and oxygen compounds called siloxanes that link to form polymers in a thin, skin-like layer which, while removable, can stay intact for at least 24 hours. The ingredients of this product are made from common chemicals that have been deemed safe by the U.S. Food and Drug Administration. This product will help patients with eczema and psoriasis. However, for the aging public that spends billions of dollars on anti-aging creams this revolutionary product that can take some of the signs of aging

[8] http://www.agelessderma.com/contact-us.aspx.

away — at least temporarily. The layer is formed by applying two creams in succession: first, a cream containing the siloxanes; second, a cream containing a platinum catalyst that causes polymer cross-linking and consequently hardening of the material. The research was funded by a small, privately owned biotechnology company in Cambridge, Mass., Living Proof, and the product is being developed by another small, privately owned Olivo Laboratories, which owns the patents.

The use of stem cells in bone restoration is also emerging as a potential therapy for several diseases. Research has shown that mesenchymal stem cells, which reside in bone marrow, are rich sources of adult stem cells that can be used in tooth regeneration and repair (Huang et al., 2009, Mantesso and Sharpe, 2009). Dental pulp stem cells form vascularized pulp-like tissue surrounded by a layer of odontoblast-like cells expressing dentin proteins similar to those found in natural dentin. When seeded onto human dentin surfaces and implanted into immunocompromised mice, dental pulp stem cells create dentin-like structures deposited on the dentin surface.

In 2013, Google founder Sergey Brin funded a project to generate test tube or cloned beef hamburgers created from stem cells extracted from the muscle of three cows.[9] This technology to generate laboratory-cloned beef meat for human consumption is based on stem cell research. Producing laboratory–cloned beef hamburgers involves harmlessly obtaining a small sample of muscle tissue from a living animal and isolating individual muscle stem cells called myosatellites. Myosatellites can reproduce fairly quickly in the laboratory and, when cultured under the appropriate in vitro culture conditions, fuse to form muscle fibers. Layered together, these strands of muscle cells and fibers form the essential components necessary to produce cultured edible meat.

In 2013, Professor Mark Post of Maastricht University created the world's first lab-grown cloned beef hamburger. Culinary experts tasted this hamburger and concluded that it had the taste and texture of real meat, although it was a little dry. The dryness was probably due to the lack of fat cells in the meat, since it is difficult to culture adipose cells together with muscle cells. This first beef hamburger cost $350,000. Currently, the cost of the cloned beef has been reduced by 80% to $70,000. The ultimate goal is to produce a five-ounce burger, referred to as a googleburger, for only $10. Recent scientific innovations, such as the creation of artificial veins in synthetic organs, can increase the fat content and improve the taste of the burger while continuing to lower the expenses.

Cloned animal-derived hamburgers present a more sustainable option for meat production then classical hamburgers. Firstly, cows are very inefficient requiring 100g of vegetable protein to produce only 15 grams of edible animal protein. Second, cloned beef hamburgers will reduce animal wastes, a significant source of land and water pollution, and reduce the emission of methane – a gas responsible for global warming. Third, cloned beef can be genetically modified to produce healthier meat that is low in saturated fats and high in omega 3 fatty acids. Finally, cloned beef doesn't require massive animal killings and therefore minimizes the threat of animal cruelty**.**

[9] http://www.theguardian.com/science/2013/aug/05/synthetic-meat-burger-stem-cells

Medical Risks of Stem Cell Therapy

Critical safety issues must be considered in stem cell-based therapies (Heslop et al., 2015). Currently, most federally-funded programs related to stem cell technology generate embryonic stem cells that are derived from existing or newly established cell lines and are not tissue compatible to the patients. Therefore, patients receiving these transplanted embryonic stem cells will require immuno-suppressive drugs to prevent tissue rejection.

There have been reports suggesting that certain stem cell therapies involving hematopoietic stem cell transplantation have more inherent health risks than ordinary bone marrow transplantation. In addition, the time required for stem cell therapy to reconstitute the immune system may take several months after autologous transplantation and up to a year or longer after allogeneic transplantation (Wingard et al., 2010).

Tissue rejection can be avoided if patients' own stem cells are used as a source of therapy. As mentioned above, there are several ways in which patients can provide their own stem cells. In addition to pluripotent stem cells obtained from bone marrow, therapeutic cloning offers another way to generate histocompatible stem cells that would not require patients to receive immuno-suppressive drugs. Somatic cell nuclear transfer utilizes technology to transfer a nucleus from a specific cell of a patient and then fuse it with an enucleated oocyte. The resulting zygote would then be allowed to differentiate into a blastocyst *in vitro* and would serve as the source for isolating stem cells from the inner mass.

In addition to tissue compatibility, transplanted pluripotent stem cells can form tumors in animals. Researchers have identified what they call cancer stem cells in blood cancers such as leukemia, breast, and brain cancers (Zhang and Rosen, 2006). In other words, the mutations that drive certain cancers to develop in the body may originate in the body's small supply of naturally occurring stem cells. There is also evidence that cancer stem cells, which only form a small portion of the total tumor, are, in fact, the primary cells responsible for maintaining tumor growth (Spillane and Henderson, 2007). Many tumor cells have been shown to exhibit stem cell-like properties, such as reverting back to a less differentiated state and exhibiting the ability to rapidly proliferate. While it remains unclear why a small percentage of implanted stem cells form tumors, it may be related to differentiation processes that have gone unregulated.

Scientists are trying different approaches to overcome the cancer problems associated with the use of stem cells. One method is to utilize adult-derived stem cells for therapy, as these cells are considered less tumorigenic than embryonic stem cells. Another approach is to transform embryonic stem cells into specialized differentiated cells before transplantation into the patient. The hope is that once the stem cell has completed differentiation, its potential to proliferate uncontrollably will be significantly reduced**.**

If the stem cell-cancer problem is not overcome in the near future, patients may

have to accept that a side effect of potentially life-saving stem cell therapies is that a proportion of transplanted stem cells may turn into tumors within 10-20 years. Long development times have been observed in several types of cancers, including colon and prostate; these cancers take more than a decade to fully develop from the time that the earliest cancer nodule is detected. Furthermore, if stem cells are used to treat a 65-year-old patient who has Parkinson's or Alzheimer's disease, then the risk that the patient might develop cancer within 10-20 years may be one that this patient is willing to take. In contrast, given that stem cell therapies may lead to the development of cancers, their use may not be warranted in a child or young adult candidate.

As mentioned above, encapsulation is an innovative technology that may provide a solution to all of the obstacles noted above. In this manner, tissue rejection and tumorigenesis are avoided, but the release of appropriate cytokines and growth factors, which could regulate tissue repair or endogenous stem cell regeneration, is maintained.

A recent study showed that human mesenchymal stromal cells remain viable within alginate for at least 2 months (Barminko et al., 2011). They also demonstrated the benefits of transplanting immobilized stem cells as an immunomodulatory vehicle within rats that had experienced spinal cord injury. The immobilized human mesenchymal stromal cells were able to promote pro-inflammatory macrophage attenuation at the site of injury (Barminko et al, 2011). Another study revealed transplanted encapsulated mesenchymal stem cells are protected from MHC-mediated attack by activated T cells (Ansari et al, 2015). One concern with encapsulation is the looming threat that the pores of the encapsulating biomaterial will become clogged by clotting factors or large biological, thereby restricting the entry and exit of biologicals.

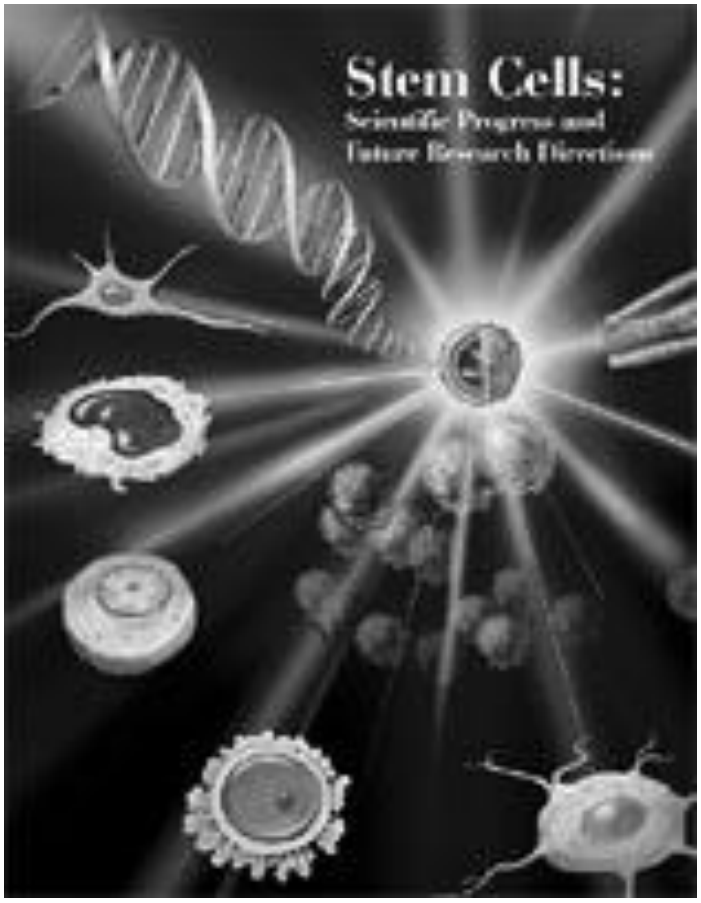
Another medical risk associated with the use of undifferentiated stem cells is called epigenetic instability (Benayoun et al., 2015). The long-term maintenance and continual passing of stem cells that is needed to preserve embryonic stem cell lines can result in aberrant methylation (or silencing) of gene promoter regions. A final safety issue is that there may be infectious agents present in the cell-feeder layers used to maintain stem cells. Currently, stem cells are most easily maintained in culture by growing them in chambers where other transformed cells, such as fibroblasts (obtained from other species), serve as feeder layers. The feeder cells supply essential nutrients required for the stem cells to maintain their state of self-renewal. In addition, these feeder layers prevent the stem cells from differentiating by secreting a variety of extracellular matrix proteins or cytokines. The human stem cells are physically separated from the cellular feeder layer by semi-permeable membranes. Embryonic feeder cells provide convenient growth and efficient study of embryonic stem cells in the laboratory but raise the risk of interspecies virus transfer. There is ample evidence that some polio vaccines used during the mass vaccination campaigns of the 1950s and 1960s may have been contaminated with the simian virus SV-40, which has been reported to be associated with a variety of human tumors. SV-40 contamination may have occurred because the vaccine was developed using monkey kidney cell lines (Petricciani et al., 2014). These types of reports suggest that feeder-cell-independent culture conditions, or serum free conditions, have to be developed to prevent infectious agents from contaminating the stem cell preparations. Thus, one goal of embryonic stem cell research is to find a way to derive

and culture cell lines without the use of feeder layers or animal serum (Crocoo et al., 2013). In fact, several groups have reported the benefits of maintaining human stem cells on hydrogel (hyaluronic acid) in the absence of any feeder layers (Liu, et al., 2012).

Conclusions

As of 2015, undifferentiated human stem cells have not cured any disease. To cure diseases, stem cells must be differentiated into more specialized cells that can be transferred to patients. Much more work is needed to understand how or whether stem cell transplants will benefit patients. One critical unknown is whether the stem cells infused into the animal or patient proliferate to replace the damaged tissue or whether these stem cells merely fuse with existing endogenous cells to affect a therapeutic response. Cell fusion has been observed between adult stem cells obtained from bone marrow and nerves from the central nervous system, in animals with spinal cord injuries that were given stem cells. Are the fused cells dead-end products that disappear with time, or are they intermediate steps in the normal process of tissue repair? The capacity of cells to fuse with one another is not unique to stem cells. Fused cells are normally found in several organ systems including the liver, intestine, placenta, skeletal muscle, cardiac smooth muscle, and bone marrow (megakaryocytes).

In summary, stem cell therapy holds exciting promise because it may greatly impact the treatment of a variety of diseases. Stem cell research offers more than just the potential to create new cell transplant protocols or cure disease; in the short term, research into stem cell differentiation will facilitate a better understanding of normal and abnormal cell differentiation, gene regulation, and embryological development from a single cell into a complete organism. The potential for a better understanding of basic biology and for the development of new biotechnologies from stem cell research appears quite promising and justifies the investment of money, time, and effort in stem cell research.



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