Stem Cells and Society

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STEM CELLS AND SOCIETY

An Interactive Qualifying Project Report

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the

Degree of Bachelor of Science

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January 13, 2012

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ABSTRACT

Although a great diversity of stem cells can be isolated from the human body for research or medical purposes, their applications, ethics, and legalities are still not fully understood by many. This is especially the case for human embryonic stem cells, as there are deep and complex ethical implications regarding their isolation from human embryos. In this study, these issues are investigated as an excellent example of the interface between science and society. It is of the utmost importance that these potentially life-saving techniques and their legalities across the globe be understood by society to make educated decisions about their use.
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PROJECT OBJECTIVES

The ultimate goal of this IQP report is to provide a cohesive and up-to-date summary of the advancements and controversies of stem cell research and the important impacts this novel biological technology has had on society. Chapter-1 serves to introduce the basics of these unique cells by describing their classifications and potencies, as well as discussing different types of stem cells isolated from numerous parts of the body. The purpose of Chapter-2 is to examine the various applications of these stem cells in medical treatments by examining trials involving both human and animal models. Chapter-3 changes the focus from biology and instead outlines the ethical concerns associated with embryonic stem cell research and the positions of the five major world religions on the topic. The intent of Chapter-4 is to similarly describe the current legal policies on this research declared by governments in both the United States and abroad. This IQP is concluded with a statement from both authors which summarizes the authors’ perceptions of the stem cell debate including the ethical and legal concerns.
Chapter-1: Stem Cell Types

Emily Domain

What Are Stem Cells?

Stem cells are a unique type of cell that forms the basis of the development, growth and survival of a living organism. Though the term is often used to describe controversial embryonic stem cells, there are many different types of stem cells, classified by their original location and/or method of formation. All types share the same basic characteristics that set them apart from other types of cells in the body. The first of these traits is that the stem cell is unspecialized; it does not have the ability to do any specific biological function, such as conduct neuronal signals or secrete hormones. Instead, the role of the stem cell is to divide and maintain its supply until it is needed within the organism. This extended self-renewal is called proliferation, and is the second fundamental feature of stem cells. The proliferation of stem cells is unique and asymmetric; it allows the body to develop other replacement stem cells along with more specialized cells to replace damaged or aging tissue. When a new specialized cell is required, a series of extracellular chemical signals coaxes a stem cell to transform into the required type of cell (Stem Cell Basics, 2009). This process, known as differentiation, is the quality most intriguing to researchers and physicians, as stem cells could potentially be stimulated to differentiate into any type of specialized cell that a sick or injured body could need (Newton, 2007, pg. 22).
**Stem Cell Classification**

Though all stem cells share these basic characteristics, not every type of stem cell can differentiate to the same extent. Therefore, it is convenient to classify stem cells based on their plasticity, or their potential to transform into specialized cells that perform a specific biological operation (Newton, 2007, pg. 19). A stem cell that can differentiate into any of the over 200 kinds of specialized cells in the body, including extra-embryonic tissues, is said to be *omnipotent* or *totipotent* (Newton, 2007, pg. 4). An omnipotent stem cell can transform into all cell types in the body (including those of a developing embryo) plus the placenta and the supportive placental tissues. In mammals, the only truly omnipotent stem cells are those of the newly fertilized egg through the 2, 4, or 8-cell stage, which occurs within about 2 days post-fertilization.

The cells of the pre-implantation embryo continue to undergo mitosis and around day-5, they form a blastocyst, a hollow ball containing about 100 cells. At this stage, the cells have begun to differentiate into two layers: the outer layer of cells is composed of trophoblasts (cells of the placenta tissue) and the inner cell mass (ICM) composed of a more limited type of stem cell described as *pluripotent*. Pluripotent stem cells can become any type of cell in the body originating from the “three primary germ layers which are normally established during gastrulation of the embryo” (Chamany, 2004, pg. 13). These germ layers, the ectoderm, the mesoderm, and the endoderm, are formed after the implantation of the blastocyst in the uterine wall, when the pluripotent stem cells in the inner cell mass organize themselves.

![Figure 1: Photograph of a Human Blastocyst with Evident Trophoblasts and Inner Cell Mass (ICM). (Conaghan, 2001)](image-url)
into three distinct layers of cells (Stem Cell Basics, 2009). The ectoderm eventually forms the skin, nerves and mucus glands; the mesoderm yields the blood, bones and muscle; and the endoderm gives rise to the pancreas, liver, and other internal organs. Thus, a pluripotent stem cell can become any specialized cell in the body that is not part of the placental tissue or the reproductive organs, which are formed by another collection of cells called primordial germ cells. Embryonic stem (ES) cell research, promising yet controversial, is based upon these pluripotent stem cells (Chamany, 2004, pg. 2-3).

Outside the realm of embryonic development, there also exists a variety of stem cells in the adult body which are classified as multipotent. These “adult stem cells” are more limited in their ability to differentiate, as they can give rise to cells in only one of the three primary germ layers (Chamany, 2004, pg. 13). For example, stem cells in the blood (hematopoietic stem cells) can differentiate into any red or white blood cell or platelet, but are usually limited to only these blood-related cells (Domen et al., 2006, pg. 14). Cells of this potential, called progenitor cells, have also been discovered in many places in the human body, including the brain, liver and pancreas (Chamany, 2004, pg. 5). The investigation and use of adult stem cells, or somatic stem cells, does not carry as much controversy as embryonic stem cell research, but their medical uses are also more limited due to their restricted differentiation potential (Newton, 2007, pg. 19).

There is also a class of adult stem cell, called a precursor cell, which is described as unipotent. These cells can only differentiate into one type of specialized cell and are often intermediates in the differentiation of a multipotent stem cell into a specialized cell (Newton, 2007, pg. 122). Unipotent skin and hair precursor cells, for instance, result from the mitosis of a multi-potent skin stem cell during differentiation (Cotsarelis, 1991, pg. 82).
A Brief History of Stem Cell Science

Prior to the dramatic increase in stem cell discoveries in the mid-20th century, scientists had speculated the existence of such an entity since the 1700’s. Interest in the regeneration of limbs and other structures in plants and animals launched the scientific investigation of stem cells. Early biologists sought to understand why simple plants and other organisms “knew” how to regenerate tissues, while the highly developed human body could not (Newton, 2007, pg. 6). This ability was not hypothesized to be attributed to a unique cell until the early 1900’s, when Alexander Maximow, a Russian hematologist, applied the idea of regeneration to blood cells in the human body. Maximow posited that a “common stem cell of different blood elements” was responsible for the development and replacement of all types of blood cells. This idea was later supported in 1960 by the research of Ernest Armstrong McCulloch and James Edgar Till at the Ontario Cancer Institute who found that mice injected with bone marrow developed nodules on their spleens (the location of blood formation in mice) whose presence corresponded with the amount of marrow injected (Newton, 2007, pg. 8). The team discovered that these nodules were made of a single repeating “colony forming unit”- the hematopoietic stem cell predicted by Maximow.

Another early discovery that led to the development of the idea of stem cells was the existence of the teratoma. Originally known simply as “monstrosities”, teratomas are large tumors that consist of clumps of cells from all three primary germ layers. These monstrous looking tumors have the potential to contain characteristics of any specialized cell in the body (Newton, 2007, pg. 9). For example, in the accidental discovery that led embryologist Leroy Stevens to research these bizarre tumors, Stevens dissected a laboratory mouse with a teratoma that “contained both skeletal and cardiac cells, the latter beating in unison, as they would in a
mature heart” (Newton, 2007, pg. 11). Stevens’ goal was then to observe the earliest step in the formation of teratomas in mice, and in 1964 identified the cause as an abnormal sperm cell growing in the genital ridge of an embryo. He called this cell a “pluripotent embryonic stem cell” and went on to demonstrate that some cells in the teratoma could proliferate indefinitely and remain undifferentiated (Newton, 2007, pg. 12). When these embryonic carcinoma (EC) cells were injected into the organs of a mouse, Stevens observed that they would quickly differentiate and again form a mature teratoma (Newton, 2007, pg. 13-14). Further research by Beatrice Mintz and Karl Illmensee in the 1970’s showed that EC cells extracted from cancerous teratomas and transplanted into normal developing mouse blastocysts became fully integrated into the tissue of the developing mouse, indicating that a stem cell’s environment can influence its differentiation (Chamany, 2004, pg. 4).

These experiments supported the existence of a cell with the unique characteristics of a stem cell, but it would be another decade before scientists physically isolated and cultured a stem cell. In 1981, the team of Martin Evans and Matthew Kaufman from the University of Cambridge successfully extracted embryonic stem cells from the inner cell mass (ICM) of a mouse blastocyst (Evans and Kaufman, 1981; Newton, 2007, pg. 14). They also demonstrated the ability of these extracted murine ES cells to proliferate indefinitely and differentiate in vitro, a breakthrough that began the modern era of stem cell research and application (Chamany, 2004, pg. 4).

Types of Stem Cells

Embryonic Stem Cells

Embryonic stem (ES) cells are “invariably derived from the ICM of 5-day-old embryos (blastocysts) or fetal gonadal tissue” (Alison, 2005, pg. 2). At this stage of embryogenesis, the
embryo has not yet implanted in the uterus, and the cells of the inner cell mass (ICM) have not yet begun to differentiate into the primary germ layers (Yu, 2006, pg. 1). The blastocyst is only composed of about 100 cells at this point, 30-40 of those being the pluripotent stem cells of the ICM. These cells begin to differentiate soon after the implantation of the embryo, but can be held in a state of indefinite proliferation if isolated and cultured correctly in vitro (Yu, 2006, pg. 3). These embryonic stem cells are considered the most plastic of all stem cells (except the totipotent cells of the early blastomere), and thus have the most to offer stem cell biologists (Vats et al., 2005, pg. 83). Their great promise is matched by their great controversy, as the unavoidable destruction of the embryo during the removal of the ICM raises ethical questions about the formation and destruction of life (discussed in Chapter-3). As a result, most governments have strict regulations on the operation and funding of embryonic stem cell research projects (discussed in Chapter-4).

The embryonic stem cell was the first type of stem cell to be isolated, as demonstrated by the mouse model of Evans and Kaufman in 1981. The isolation of the first human ES cell, after years of ethical objections and scientific dilemmas, was performed independently by two teams: one under the direction of John Gearhart at Johns Hopkins University (Shamblott et al., 1999), and another under James A. Thomson at the University of Wisconsin (Thomson et al., 1998; Newton, 2007, pg. 16). Gearhart and coworkers extracted pluripotent primordial germ cells from the immature gonads of aborted fetuses that later were found to have less desirable proliferation properties than true ES cells (Chamany, 2004, pg. 14). Alternatively, the team under the

![Figure-2: Embryonic Stem Cell.](Embryonic, 2012)
direction of James Thomson used “leftover” fertilized eggs from *in vitro* fertilization (IVF) techniques to isolate ES cells (Newton, 2007, pg. 17). IVF involves the fertilization of an oocyte (egg cell) by a sperm cell outside the body, the growth of the embryo to the blastocyst stage, and for reproductive purposes the implantation of the blastocyst into the uterus for gestation. To increase the probability of creating a healthy blastocyst, this therapeutic technique requires the fertilization of numerous oocytes at a time. The remaining embryos are then frozen to be preserved for further implantations (Chamany, 2004, pg. 16). It was from these “extra” embryos that the first human embryonic stem cell was isolated.

IVF clinics are still the primary source of human ES cells for therapy and research, through the donation of their surplus blastocysts with donor consent. In 2006, there were an estimated 400,000 spare embryos in storage from IVF procedures in the United States. Of these, 88.5% were claimed for use in further implantation, while 2.8% were donated to scientific research, a total of approximately 11,000 embryos (Yu, 2006, pg. 3). However, the most promising embryos created through IVF are used in implantation, leaving the more unsound and frail embryos for donation. Of these donations, it is estimated that only 275 blastocysts are healthy enough to be used for scientific inquiry, as the chance of blastocyst formation in humans is only 1 in 18, and the freeze/thaw cycle tends to destroy cell membranes and other proteins. For this reason, in some countries outside the United States where laws allow embryos to be created solely for research purposes, IVF clinics and research institutes have begun seeking the help of volunteer oocyte donors. These are women who undergo the IVF procedure and donate their embryos to researchers rather than having them implanted for gestation (Chamany, 2004, pg. 17).
Parthenote Stem Cells

Embryonic stem cells isolated from leftover IVF blastocysts are not ideal for therapeutic use due to their ethical issues. Also, since the blastocyst is a hybrid of male and female genetic donors, the stem cells will also be a combination of genetic material and would be subject to resistance in the recipient body. To circumvent this issue, scientists have been investigating the use of artificial parthenogenesis to create homozygous ES cells (Kim et al., 2007, pg. 1). Parthenogenesis is a form of asexual reproduction in which an unfertilized egg duplicates its own genetic material and begins to divide, developing into an embryo. In nature, this process occurs in some insect species to create large amounts of worker insects, but it does not normally occur in mammals. Parthenogenesis can be artificially induced in mammals by an electric pulse to an unfertilized egg, mimicking the charge brought upon by the flow of calcium ions during normal fertilization (Chamany, 2004, pg. 18). The ES cells isolated from this blastocyst will “express only one of two sets of parental histocompatibility antigens” and therefore run less risk of rejection in the body, especially if implanted into the same donor female (Kim et al., 2007, pg. 1). The embryos produced by artificial parthenogenesis cannot fully develop with only one set of genetic material, so some scientists believe this avoids the moral dilemma over the destruction of embryonic life (Chamany, 2004, pg. 18).

Somatic (Adult) Stem Cells

A large variety of adult stem cells are found throughout the body. These multipotent cells are “capable of producing a limited range of differentiated cell lineages appropriate to their location” (Alison, 2005, pg. 6). The role of these stem cells is to maintain the health and vitality
of the tissue in which they are located. Stem cells can remain in an undivided state (quiescent) in the “stem cell niche” for long periods of time until they are needed in the body (Stem Cell Basics, 2009). The stem cell niche is the protective microenvironment of the adult stem cells, generally a part of the tissue that undergoes the least amount of stress. The stem cell niche is thought to control much of stem cell behavior, via the use of “cells and extracellular matrix components” (Alison, 2005, pg. 9). To date, somatic stem cells have been identified in the human “brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis” (Stem Cell Basics, 2009).

The first direct evidence of the stem cell came from adult hematopoietic (blood) stem cells. In the groundbreaking work of Till and McCulloch in the 1960’s, the team observed tumors in mice injected with irradiated bone marrow, which were formed by a single colony-forming unit: a hematopoietic stem cell (Newton, 2007, pg. 8). They deemed this cell to be pluripotent, but by today’s standards it is considered multipotent; it is the source of all blood cells in the body, but generally cannot differentiate beyond this type of cell (Domen et al., 2006, pg. 15). The majority of the hematopoietic stem cells (HSCs) in humans are located in the bone marrow, but this location is used more infrequently in medicine due to the pain and cost of the isolation procedure. In the late 1980’s, HSCs were also isolated from the blood of the umbilical cord, a tissue that is normally discarded after the birthing process. This tissue is rich in HSCs, which appear to have greater proliferation potential than those isolated from adult bone marrow (Domen et al., 2006, pg. 22).
Human bone marrow also contains another type of stem cell called mesenchymal stem cells (MSC). These cells have the ability to differentiate into all connective tissue cell types, including bone, cartilage, and tendons (Baksh, 2004, pg. 305). The earliest demonstration of the existence of these cells was in 1961 by A. J. Friedenstein, who showed that bone marrow cells can undergo osteogenesis, producing bone cells rather than the expected blood cells (reviewed in Friedenstein et al., 1976; Baksh, 2004, pg. 302). MSCs have since been isolated from “trabecular bone, adipose tissue, synovium, skeletal muscle, lung, deciduous teeth, and umbilical cord cells” (Baksh, 2004, pg. 304). An interesting aspect of these cells is that they appear to exist in a variety of different potentials, including a state of pluripotency which is usually only found in embryonic stem cells (Baksh, 2004, pg. 306). Although further differentiated mesenchymal cells are more commonly found in the body, it has been demonstrated that some MSCs can form cells in all three primary germ layers (Baksh, 2004, pg. 308). However, it is estimated that there exists only one MSC for every 34,000 differentiated somatic cells in the body, and the probability of that cell being completely undifferentiated is smaller yet (Beyer and Meirelles, 2008, pg. 255).

Another type of somatic stem cell is responsible for an organ long known to regenerate in humans: the skin. The first direct evidence of this epithelial skin cell came in 1981 by the application of a thymidine label to basal (skin) cells (Bickenbach, 1981). J. R. Bickenbach demonstrated that a small group of basal label-retaining cells (not undergoing DNA replication or cell division) carried this label for 240 days, an extremely long cell cycle for skin. Upon examination of these cells, they were observed to be “small, contain few organelles, occupy a fixed position in the tissue architecture, and are clonogenic [colony-forming] in vitro”, all characteristics of a somatic stem cell (Cotsarelis, 1991, pg. 83). Epithelial skin cells are found in
the epidermis and the hair follicle bulge, and give rise to all the precursor cells for skin and hair (Cotsarelis, 1991, pg. 81-82). These stem cells have also been found in the cornea, and there is evidence that corneal tissue can be coaxed to differentiate into skin cells (Dhouailly, 1991, pg. 87). Approximately 10% of basal cells are epithelial stem cells, and it is believed that they are the only type of cell that lasts the entire life of the epidermis (Bickenbach, 1991, pg. 84).

The discovery of adult stem cells in other organs has forced scientists to reevaluate the regeneration potential of organs once thought to be “terminally differentiated”, such as the heart and the brain (Beltrami et al., 2003, pg. 763). The first evidence for neuronal stem cells was observed in 1989 (Temple, 1989), and by the mid 1990’s, it was discovered that the brain can regenerate neurons under conditions of stress via a neural stem cell, contradicting the original assumption that the brain had no regenerative potential. These stem cells can give rise to neurons and the supportive brain cells, oligodendrocytes and astrocytes (Rebuilding… 2009). Neural stem cells are particularly exciting to scientists in the area of drug delivery and gene therapy, as they could guide therapeutic agents directly to target areas in the brain and other tissues (Müller et al., 2006). In 2003, Antonio Beltrami and his team at New York Medical College demonstrated that the adult heart also has regenerative potential. They successfully isolated and cultured in vitro the first cardiac stem cell from an adult rat. These cells can differentiate into any type of cardiac cell: myocytes, smooth muscle, and endothelial cells (Di Felice et al., 2009, pg. 449-450).

Somatic stem cells have also been isolated from many other locations in the body, and some show promise of pluripotentiality. In the adult liver, hepatocytes are normally non-
proliferative (do not undergo mitosis) but in response to cell loss they enter the cell cycle and begin to regenerate liver tissue. Thus, they can be “regarded as a functional stem cell for the liver” (Vats et al., 2005, pg. 87). Even liposuction waste contains an estimated “50-100 million stem cells per 250 g” which can be used to generate fat, bone, and cartilage cells (Alison, 2005, pg. 15). Stem cells have also been extracted from amniotic fluid, which surrounds a developing fetus and absorbs the cells that it sheds. These cells are called amniotic fluid-derived stem cells (AFS) and have been coaxed to differentiate into cells from all three primary germ layers. These cells do not create the full complement of proteins expected from a pluripotent stem cell, and consequentially have not been observed to form a teratoma upon injection (Battey, 2006, pg. 83).

**Induced Pluripotent Stem Cells**

Given the limited plasticity of most adult stem cells and the controversy surrounding embryonic stem cells, it has been necessary to search for alternative methods of acquiring pluripotent stem cells. In 2005, the focus shifted from locating pluripotent stem cells to “reprogramming” already differentiated cells back into their stem-like state. Current research in induced pluripotent stem (iPS) cells centers on the isolation of the genes responsible for pluripotency and the methods used to introduce them to a differentiated cell. The pioneer in this area is Shinya Yamanaka of Japan, who first isolated iPS cells from mice in 2006 (Takahashi et al., 2006) and later from humans in 2007 (Takahashi et al., 2007). In his 2007 work with human cells, Yamanaka de-differentiated a human fibroblast (skin cell) by the insertion of just four genes: OCT3/4 SOX2, KLF4, and c-MYC. He did this by introducing an altered retrovirus that carried the genes for pluripotency, which were then incorporated in the chromosomes of the fibroblast. However, it was found that c-MYC was linked to the formation of tumors, and
retroviruses inherently tend to disrupt cancer-inhibiting genes, so biologists also experimented with adenoviruses (which do not enter chromosomes) and “piggyBacs”, genetic carriers that can leave the chromosome after reprogramming finishes. In 2009, Sheng Ding of the Scripps Research Institute began research into the insertion of proteins, rather than the genes that code for them, into the somatic cell. He did this by attaching the proteins to the end of a positively charged polyarginine molecule, which carries the proteins across the cell membrane. Unlike genes which remain in the cell and can become reactivated, these proteins break down rapidly and leave behind no genetic material to become cancerous, making them a safer option than gene insertion (Aldhous, 2009).

**Cloned Stem Cells**

In an attempt to derive ES cells genetically identical to a specific patient and minimize chances of immune-rejection, Hwang Woo Suk of South Korea proposed using somatic cell nuclear transfer (SCNT) in 2005. This technique involves the insertion of a nucleus isolated from a differentiated adult somatic cell (usually a skin cell) into an enucleated egg (Newton, 2007, pg. 17). Factors present in the cytoplasm of the oocyte (egg cell) promote the restoration of the pluripotent state of the nucleus, and the embryo divides to the blastocyst stage from which ES cells are obtained (Battey, 2006, pg. 87). This cloned embryo, however, is still subject to the debate over the creation and destruction of life (Newton, 2007, pg. 18). In 2005, the South Korean team claimed success with the SCNT approach in humans, but the work was later discredited for data fabrication (Hwang et al., 2005).

A proposed solution to this came in 2006 when Dr. Rudolph Jaenisch at MIT developed altered nuclear transfer (ANT), a modification of SCNT that inhibited the gene Cdx2, which is
responsible for the implantation of the embryo in the uterus. Technically, this meant that the embryo could not give rise to life and thus to some scientists it would avoid the moral and ethical objections of normal embryos. However, ANT remains controversial and research has not yet progressed outside of mouse studies (Battey, 2006, pg. 84-85).

**Proliferation and Differentiation of Stem Cells**

A single isolated stem cell is of limited use in science and medicine. Instead, the focus is on the creation of stem cell lines: a collection of cells derived from the isolated stem cell, which can grow and proliferate *in vitro* and can adopt a variety of cell fates (Chamany, 2004, pg. 13). This cell line propagates indefinitely in the correct culture medium, maintaining its “stemness” and plasticity until needed. An important aspect of these cell lines is their potential to be patient-specific, either by the derivation of iPS cells from the patient’s skin cell, or by the use of SCNT (not achieved yet in humans). This would greatly reduce the risk of rejection if the recipient was the same as the skin cell donor, as the cells carry the DNA of only the host and the risk of an immune reaction is lowered (Chamany, 2004, pg. 6). It is these resulting immune-matched stem cell lines that will be used for applications in therapeutic and regenerative medicine. Lines of other stem cell types, unable to be used therapeutically, could be utilized in drug development and testing, and inquiries into the nature of embryogenesis (Newton, 2007, pg. 21-22).

Another current focus of stem cell research is the creation of an environment that can promote either the proliferation or differentiation of a cultured stem cell and its resulting lineage (Newton, 2007, pg. 20). Just as stem cells rely on chemical signals inside their niche in the body, stem cells cultured *in vitro* rely on extracellular proteins, called extrinsic factors, to “provide the necessary induction or inhibition signals to promote the adoption of one cell fate
versus another” (Chamany, 2004, pg. 4). Before these signals can be introduced, the stem cells need a surface to which they can attach in vitro, called the feeder layer.

In early ES cell cultures, the cells of this layer (called feeder cells) were irradiated mouse embryonic fibroblasts (connective tissue). These provided a proper scaffold and nutrients, but some scientists worried about the mixture of animal proteins with the human ES cells, so later experiments used irradiated human cells as a feeder layer with some success (Newton, 2007, pg. 20). With the feeder layer established, extracellular signals, such as cytokines, growth factors, amino acids, proteins, and active ions can be used to influence the mitotic pathway of the cell (Vats et al., 2005, pg. 89). The division of the stem cell can be made to be symmetrical, where a stem cell produces two cells of the same kind: either two differentiated daughter cells (clonal extinction) or two daughter stem cells (clonal expansion). Stem cells can also divide asymmetrically, where a stem cell produces one differentiated daughter cell and one daughter stem cell (Newton, 2007, pg. 19; Gordon, 2005, pg. 69).

The introduction of the correct extrinsic factors can manipulate the stem cells to favor a specific mitotic path, like clonal expansion for the development of a stem cell line or clonal extinction for differentiation into a tissue, by activating or inhibiting key parts of the cell’s DNA (Newton, 2007, pg. 13). An early example of this is the use of leukemia inhibitory factor (LIF) in the late 1980’s by researchers at the Ludwig Institute for Cancer Research in Australia (Chamany, 2004, pg. 4). This protein promotes the proliferation of mouse embryonic stem cells, even in an environment that lacks feeder cells (Yu, 2006, pg. 4). However, it has the opposite effect on human ES cells, causing them to rapidly differentiate (Chamany, 2004, pg. 11). The current goal in cell therapy is to identify a definitive set of culture conditions to promote proliferation along any differentiation path desired without inducing any genetic mutations (Yu, 2006, pg. 5).
Chapter-1 Works Cited


Chapter-2: Stem Cell Applications

Diego Prentice-Webb

Not only do stem cells serve humans as an important learning device to help better advance our knowledge in areas such as embryogenesis, cellular differentiation, and cell repair, but they also serve as life-changing medical instruments used by doctors around the world. With over a half a century of research already completed on them, doctors have been successful in using them to treat genetic disorders such as diabetes and a diverse array of cancers, and have changed lives transplanting them into patients who have suffered mechanical or physiological damage to their organs and tissues. It is vital that this research continues so that stem cells may be exploited to aid patients around the world. The purpose of this chapter is to describe the applications of stem cells for treating several example diseases, as an introduction to their benefits to society, which is an important aspect of their ethics.

By far, the most widely studied stem cell type, the hematopoietic stem cells (HSC), was first characterized over 50 years ago in the bone marrow (Till and McCulloch, 1961). Their ability to self-renew and differentiate into all types of blood and immune cells led scientists to begin investigations on their potential as treatments for irradiated mice with fatally low levels of red and white blood cells. Since their discovery, HSCs and many other stem cell types have been used in various medical applications including treatments for patients with diabetes and leukemia.
Diabetes Treatment with Stem Cells

Currently afflicting roughly 2.8% of the global population (Wild et al., 2004) and 25.8 million Americans (American Diabetes Association, 2011), diabetes mellitus has been around for decades leaving many with no choice but to rely on daily insulin shots and other medications. Patients with type 1 diabetes are unable to produce and secrete insulin (a hormone which causes liver, muscle and fat tissue to absorb excess glucose from the blood and convert it into glycogen) due to an abnormal autoimmune response against the β-cells that normally manufacture it. The lack of insulin causes the patients to have a constantly elevated blood glucose level (BGL) leading to hyperglycemias and ketoacidosis if untreated. Similarly, patients suffering from type 2 diabetes also experience high BGL, but in this case as a result of cells not responding correctly to insulin. Treatment options for diabetes vary from a lifetime dependence on insulin injections or an insulin pump, to a dangerous pancreatic and/or islet cell transplantation in combination with immunosuppressants.

In the past decade, studies have emerged using both autologous (same individual) and allogeneic (histocompatible) stem cells to become functional insulin-secreting cells in patients. Initial in vitro studies differentiated pluripotent human embryonic stem (hES) cells into embryoid bodies (EB) by suspending them in bacterial-grade petri dishes after disaggregation. The cells were then left to spontaneously differentiate into a vast array of cell phenotypes, including one similar to (but not identical to) that of β-cells positive for insulin secretion (Assady et al., 2001). Later, variations in the method for inducing functional β-cell analogues from other embryonic stem cell lines were developed using transcription factors to aid the differentiation and chemical signals which regulate normal pancreas cell formation (Kroon et al., 2008). Additionally, phosphoinositide kinase inhibitors were shown to improve the quantity of insulin
produced. These differentiated cells led to the successful rescue of non-obese diabetic (NOD) scid mice helping to establish normal BGL, suggesting that transplantation of these cells can be a potential treatment in vivo (Hori et al., 2002).

In addition to differentiated ES cells, other types of stem cells also show promise for treating diabetes, including stem cells derived from bone marrow. Hematopoietic stem cells (HSCs) have been shown to have the capability to differentiate into insulin-secreting endocrine cells when cultured with a high-concentration glucose-containing medium. Once transplanted into the kidneys of NOD/scid mice, these cells which also express other pancreas-specific transcription factors and proteins other than insulin, successfully mimicked endocrine cells by working just like normal islets, maintaining a constant, healthy BGL, and therefore preventing hyperglycemia (Oh et al., 2004).

Despite the success in using stem cells to treat animal models of diabetes, there have been some concerns regarding the use of human stem cells in murine studies. For example, in some experiments the transplanted differentiated hES cells have caused the growth of teratomas in the graft areas (Kroon et al., 2008). It has also been suggested that because the differentiated cells are cultured in vitro in a two dimensional space as opposed to natural β-cells which have grown in vivo in a microenvironment containing other cell types in a three dimensional space, a poor formation of islet of Langerhans can result producing low insulin levels (Guo and Hebrok, 2009). Thus, before stem cell treatments and transplantation can be a reality for diabetes patients these barriers must be overcome. However, it is important to note that advancements continue in this field; there are currently 62 clinical studies across the globe using stem cells to treat both type 1 and 2 diabetes (National Institute of Health, 2011).
Repairing Damaged Heart Tissue with Stem Cells

The longest standing culprit of death in America since 1918 is cardiovascular disease (CVD) which includes stroke, myocardial infarction (MI), high blood pressure, coronary heart disease (CHD), and heart failure. At present, CVD affects an estimated 82,600,000 American adults (Roger et al., 2011) and is the cause of more than a third (33.6%) of all mortalities in the country in 2007 (Xu, 2010). Complications arise when heart tissue is deprived of oxygen, causing cardiac muscle cells (cardiomyocytes) to die, resulting in ventricle wall thinning and stretching, an overload of blood flow and pressure and heart failure (Goldthwaite, 2009).

Repair or regeneration of these lost cardiac cells is therefore vital for a patient to successfully recover from CVD. Modern pharmacological and surgical innovations have greatly improved in the intervention of patients suffering from acute CVD, but therapies that can regenerate lost myocardial (heart muscle) tissue are extremely limited and must be explored. Since host cardiomyocytes cannot proliferate fast enough to be used to repair MI, the cells that naturally differentiate into myocardial tissue, known as myoblasts, became a key point of interest for scientists trying to heal tissue after heart failure. Myoblasts were initially found to regenerate myocardial tissue after transplantation onto areas of infarction in murine models (Murty et al., 1996) and were later found to do the same in humans; in 2001 the first transplantation of autologous myoblasts was performed in tandem with a bypass surgery on a patient with severe heart failure caused by extensive myocardial infarction and anterolateral ischemia, providing him with improved heart function (Menasché et al., 2001). This novel study encouraged others to pursue similar transplant options at it showed that stem cells can serve as a viable treatment option for patients with CVD.
Currently, *in vitro*, animal *in vivo*, and human clinical studies are all being performed to understand the efficacy, safety, and mechanism by which diverse forms of stem cells can help heal heart tissue after CVD, including but not limited to, mesenchymal stem cells and ES stem cells. At present, the most popular form of stem cell therapy used during *in vivo* studies is bone marrow mononuclear cells. When locally administered to areas of infarction in mice, these cells have been shown to spark the development of myocytes and vascular structures in the surrounding areas (Orlic et al., 2001). Schächinger *et al* (2006) are one the few groups that have managed to compile a large scale clinical study (more than 200 patients) that uses both negative controls (placebos) and positive controls (infusion of stem cells). The team discovered that the left ventricular ejection fraction (a measure of the heart’s ability to pump blood out of a ventricle per heart beat) was significantly raised in patients receiving intracoronary infusions of BMCs compared to patients receiving the placebo treatment, suggesting improved left ventricular tissue recovery from MI after treatment with BMCs.

Due to the immense array of phenotypes that embryonic stem cells can differentiate into, similar studies have emerged investigating the viability of human ES cells as a treatment for human CVD. Human ES cells have been shown to differentiate *in vitro* into spontaneously beating cardiomyocytes (Kehat et al., 2001) as well as improving cardiac function in post-infarcted rats (Min et al., 2002). However, due to the previously mentioned tendency to form cancer at the graft site, and their ethical and legal obstacles, there are no ongoing studies using ES cells to treat human patients with CVD complications.

It is evident from the above discussion that stem cell therapies are promising to help aide patients who have suffered from CVD, but there is much more to be learned about the specific mechanism behind which each unique stem cell type can form and help repair cardiac tissue. In
addition, before stem cell therapy for CVD can become a standard clinical practice, more information must be brought to light regarding the potential difference of using one stem cell type over another, all done using randomized, placebo-controlled trials (the study done by Schächinger et al (2006) serves as a great model in respect to this) to remove any uncertainties (Boyle, Schulman and Hare, 2006). Regardless of advancements in treatments, the severity and frequency of having CVD should not be taken lightly by anyone. To avoid it, everyone should regularly exercise, have a healthy diet, and avoid smoking.

**Stroke Recovery**

A stroke is a potentially life threatening and life changing attack on the brain which occurs when there is an abrupt and severe decrease in oxygen reaching our vital brain cells. This deficiency can cause the rapid death of any type of brain cell, and occurs when an artery becomes clogged by a blood clot, causing a decrease in blood flow (ischemia, the leading cause of stroke) or by an artery rupturing (hemorrhage). An estimated 795,000 Americans suffer from a stroke every year, while more than 137,000 die from it, costing the nation an estimated $73.7 billion for relevant medical treatments (National Stroke Association, 2010). Since an estimated two million brain cells die every minute during a stroke, the most important thing that can be done to treat stroke and avoid permanent damage is the immediate transportation of the patient to a hospital. Standard clinical practice for ischemic stroke involves the swift activation of the physiological process of thrombolysis (the lysis of blood clots) by the administration of a clot dissolver drug, such as tissue plasminogen activator (tPA) or aspirin, so that blood flow can be restored. Although this treatment has shown to reduce the risk of disability after an attack, it must be administered within 3 hours of the stroke, and it can also result in an excess of blood flow in the brain which can prove fatal (Wardlaw et al., 2009). To complicate matters even
more, the treatment of hemorrhagic stroke is extremely difficult, so only a handful of patients are able receive the dangerous neurosurgery.

Outside of these therapies, little development in medication for patients has occurred. When studying potential treatments in murine models, it is relatively easy to administer treatment quickly, while in human practice, the administration of drugs occurs much later (Willing et al., 2007). However, present research is focusing on the safety of administering stem cells for treating stroke, helping to regrow brain cells. It is known that some types of stem cells can differentiate into cells that express neuronal proteins found in normal CNS cells (Brazelton et al., 2000). Further, studies have shown that transplanted bone marrow cells in rats with induced stroke will migrate towards the site of damage which differentiate into cells exhibiting marker proteins of astrocytes and oligodendrocytes (Chen et al., 2008). Likewise, the injection of green fluorescent protein-tagged hematopoietic stem cells in rats that have undergone induced stroke were detected to first migrate to the CNS where they differentiated into microglia-like cells resulting in a reduction in infarct volume. The HSCs also translocated to the spleen where they were found to counterattack ischemia-mediated effects by increasing proinflammatory cytokine and chemokine receptor levels (Schwarting et al., 2008).

**Stem Cells and Tissue Engineering**

The basis for tissue engineering has been around for many years, combining molecular biology, material science, and engineering. This field refers to the replacement of damaged or lost organs usually by means of donor matrices. Two options can be used when replacing an organ: 1) acellular matrices can be implanted which depend on the recipient’s own cells to direct tissue growth, and 2) matrices can be implanted that have been seeded with cells. Cells used to
populate a matrix can be heterologous (different species), allogenic (same species, different individual), or autologous (of the individual), and are traditionally cells that make up the organ in question. Recently surgeons have been looking towards using stem cells as a potent source to seed matrices for patients.

Tracheal removal and repair for patients with malignant or benign tumors is a highly limited operation, as only 30% of the total trachea length in children, and up to 6 cm long in adults, is considered feasible for removal. Non-biological grafts have been unsuccessful so far, proving too complex and lengthy to be a suitable replacement. Tracheal homografts have been known to induce long-term stenosis, and have unpredictable growth patterns, so a better strategy for transplantation is required. In 2008, a team of surgeons in Spain, led by Dr. Paolo Macchiarini, were treating a 30 year old Colombian woman, Claudia Castillo, whose trachea and left main bronchus suffered from major damage caused by tuberculosis resulting in severe dysphonia (Macchiarini et al., 2008). A patient suffering from dysphonia has a reduced ability to use their vocal organs to produce normal speech or phonation. After several unproductive procedures, including the placement and various replacements of a Dumon stent in the patient’s left bronchus to try and alleviate her stenosis, a complete replacement of the left main bronchus with a bioengineered human trachea was proposed. Their plan was to use autologous stem cells harvested from the patient either from the bloodstream or directly from the bone marrow, and then grow them in a laboratory and graft them onto the trachea. Unlike conventional organ transplants, there is no need for the use of immunosuppressant drugs after using autologous cells. This approach is of great importance to the future of transplant operations as it greatly reduces the risk of rejection of the new organ due to an immune response. However, many patients do not possess an adequate population of healthy autologous adult stem cells needed to line these
organs, which limits the procedure from being available to a wide population of patients. As a result, it has been suggested that it may be more beneficial to use human ES cells for the seeding, as they remain almost indefinitely in their undifferentiated state, have an enhanced proliferative capacity and exhibit greater pluripotency (Metallo et al., 2008).

Regardless, Dr. Macchiarini and his team planned to use a donor windpipe which had been cleaned of all its cells and lined the inner walls of the trachea using the patient’s own mesenchymal stem cells harvested from her bone marrow. A 7 cm trachea was obtained from a recently deceased donor and stripped of all connective tissue and HLA antigens. Mesenchymal stem cells were obtained from the patient’s bone marrow aspirate, and cultured in DMEM containing 10% fetal bovine serum, L-glucose, penicillin, streptomycin, and basic fibroblast growth factor for 72 hours. Chondrocyte differentiation was then induced by methods described by Kafienah et al. (2007), the medium was replaced with DMEM containing recombinant human transforming growth factor-β 3, recombinant parathyroid hormone-related peptide, dexamethasone and insulin, and the cells were incubated for another 72 hours. The internal face of the matrix was then seeded with donor epithelial cells while the external surface was colonized by differentiated chondrocytes. The characterized matrix was then rotated along its longitudinal axis in culture medium for 96 hours before implantation.

A left posterolateral thoracotomy (fifth intercostals space) was performed, leaving the distal trachea and left main bronchus free for Dr. Macchiarini’s team to insert the patient’s new trachea. The result was a landmark achievement, as for the first time ever a patient received a completely bioengineered organ and recovered fully (Macchiarini et al., 2008). In 2010, the procedure was successfully repeated with a 10 year old boy who was born with Long Segment Congenital Tracheal Stenosis, a rare condition which leaves the child with an abnormally narrow
airway (University College London, 2010). The great strides taken by Dr. Machhiarini and his team have paved the way for the future of organ transplantation, and have sprung hope in patients requiring donor organs who cannot receive them. Similar organ transplants using matrices populated by stem cells should therefore eventually become a viable option for patients in the future.

Cancer Stem Cells, A Target for the Future?

To culminate our review of stem cell applications, we move towards using a different approach in examining how stem cells can be exploited to help cure disease. In this case, stem cells are not being transplanted or used to produce new cells to help cure an ailment, but our knowledge of them is being applied to investigate the cause and nature of perhaps the greatest weakness of the human cell: cancer. Although it is currently known that both internal factors (inherited mutations, weakened immune system, DNA replication errors) and external factors (carcinogens, pathogens, radiation) are responsible for transforming cells, the explanation for how all these extremely different factors can trigger a cascade reaction that ultimately causes multiple mutations leading to an unstoppable force of cell divisions is still not clear. However, after decades of research it is becoming more clear that cancer is a disease resulting from our own habits rather than just genetic deformities, as research has shown that up to 90-95% of all cancers are a result of environmental factors (see Figure-1), especially tobacco use and poor diet.
Regardless of the causing factors, all cells in the human body are vulnerable to becoming cancerous, as their DNA is susceptible to genetic mutation whether on the nucleotide level or at the chromosomal level. Mutations that cause cancer either change or create novel oncogenes, which are genes that code for proteins that normally stimulate cell growth and division, so that they are overexpressed producing inappropriate cell reproduction. When the genes that code for proteins that try and prevent this excessive cell division by triggering apoptosis (cell death) (known as tumor suppressors) become non-functional from mutations, the cell will become immortal and tumorigenic also.

One peculiar and deadly feature of cancer is that it can metastasize or migrate through the blood or lymph to another part of the body, and begin to grow there, severely reducing a patients expected survival rate. It is difficult to grasp how a small amount of malignant cells can survive this highly complex process which requires that the cancer cells free themselves from the primary tumor, migrate, exit the circulation, adhere to the foreign tissue, develop a blood supply, and finally maintain their growth there. Additionally, scientists have also questioned how cancers
of the same type have varying levels of resistance to radiation and chemotherapy, even in early stages of tumor progression.

These queries could be answered with the cancer stem cell (CSC) model. In 1994, Lapidot et al. discovered that a rare subpopulation of stem cell-like cells (only 1 in 250,000 cells) found in acute myeloid leukemia (AML) could be harvested and transplanted onto scid-mice, growing extensively with a morphology characteristic to that seen in cancer patients. Since then, cancer stem cell (CSC) populations have been found in breast cancer (Al-Hajj et al., 2003), brain cancer (Galli et al., 2004), prostate cancer (Collins et al., 2005) and pancreatic cancer (Li et al., 2007) each exhibiting self-renewal, differentiating capabilities, and cell-surface markers seen in stem cells, suggesting that they must play some role in tumorigenicity or tumor maintenance. When isolated and transplanted into mice, these CSCs have been shown to successfully generate tumors with remarkably similar phenotypes seen in patients signifying that they could be tumor initiating cells and be responsible for metastasis as they can self-renew without differentiating, something normal tumor cells do not possess. Furthermore, these specialized cells have shown enhanced resistance to chemotherapeutic agents (Liu et al., 2006) suggesting that cancer’s extraordinary ability to resist cell death via therapy may be linked to the number of CSCs present in the tumor (Clarke et al., 2006).

Physicians are given very limited options in how to treat patients with cancer as they are left to choose between surgical removal, which may not even fully cure the patient, or using radiation treatment or chemotherapy, which can devastate normal tissue and still cause little damage to the cancer due to resistance. Thanks to the CSC hypothesis, physicians have now been given the opportunity to target new pathways and/or cell surface markers that are unique to CSCs which will hopefully prove to be a much better treatment method than existing practices.
Whether it is proven that CSCs are indeed the cause of cancer, their potential as targets for drug-induced destruction is immense. Drug development assays should focus on using CSC lines in parallel with accepted cancer cell lines for treatment since it has been shown that the tumors initiated by CSCs after transplantation into mice more accurately mimic actual tumors seen in patients than some cancer cell lines (Galli et al., 2004).

Chapter - 2 Bibliography


Chapter-3: Stem Cell Ethics

Emily Domain

The recent advancements in stem cell research have caused a need to reevaluate personal and doctrinal convictions worldwide. A number of oppositional arguments have arisen in the past decade, most of them citing evidence for ‘respect of the embryo’ and proposing the fundamental question of when life begins (Newton, 2007, pg. 32, 34). This chapter seeks to outline the positions of the five major world religions on this question and on embryonic stem cell research as a whole. Other related dilemmas that arise from stem cell research, such as cloning, induced pluripotent stem cells and artificial parthenogenesis, will also be addressed.

Embryonic Stem Cell Ethics

In the realm of stem cell research, there is little debate over the research and therapeutic use of adult stem cells. In fact, all major world religions agree on the use of somatic stem cells, as long as the research is dedicated to the relief of human suffering. The controversy instead focuses on the use of embryonic stem cells (ESCs), whose isolation involves the destruction of a 5 day old blastocyst embryo (Alison, 2005, pg. 2). The destruction of this embryo for research purposes results in a profound debate over the rights of the embryo. One's opinion of the proper rights granted to this blastocyst depends on that person or system's assumption of the state of the pre-implantation embryo at that time. The determination of this state of being as a living entity, a ball of tissue cells, or something in between, is driven deeply by one's personal conviction as to where and when life begins. This is the belief that also determines where one stands on the continuum of support for embryonic stem cell research.
On one end of the spectrum are those individuals who are morally opposed to embryonic stem cell research by any means. This can be for a variety of reasons, but the most common is the “conceptionalist” view: that a human embryo is a person and should thus be given all the rights of a fully developed human being. This viewpoint is based on the conviction that life begins at conception, and that the destruction of this being for science is equivalent to murder. Those opposed to the scientific destruction of an embryo also cite a modification of the conceptionalist view called the “potentiality argument”. Supporters of this argument believe that since the embryo has the potential to be a human life, it should be considered as such (De Wert and Mummery, 2003). They argue that, regardless of whether the blastocysts destroyed in ESC research are human life, interfering in the development of the embryo is immoral since it prevents the formation of a person (Devolder, 2005, pg. 176).

Oppositely, other individuals argue that the embryo deserves no protection at all, and is usable for scientific inquiry. In their opinion, the 5 day old blastocyst has not yet attained the status of ‘life’ and is a non-person. Its destruction is thus of little or no moral dilemma. To support this idea, defenders of this opinion point to the “limited individuality” of the pre-implantation embryo. Prior to about the 14\textsuperscript{th} day of development, the embryo can split and form twins, or two embryos can fuse together. Supporters of this idea argue that the destruction of the embryo at day 5 is well within the time that the embryo can alter its nature, and therefore its purpose has not yet been established. Another fact used to defend ESC research is that 50-60\% of pre-implantation embryos are nonviable and cannot form a human being, so their destruction should not hypothetically be a moral issue (De Wert and Mummery, 2003). This is not to say that all supporters of embryonic stem cell research necessarily view embryonic tissues as disposable,
but simply that opinions on the point of the formation of life differ. It is these different opinions that lead to the difference in religious stances on embryonic stem cell research.

Of course, the support for embryonic stem cell research is not solely divided into the extremes of approval and opposition. There exists a wide spectrum of intermediate viewpoints into which most people and religious organizations fall. These factions generally agree that the embryo deserves some protection, but not necessarily the same protection given to a full term child. In the religious world, this brings about the question of spiritual development. Religions who view spiritual development as a step-wise process, like physical embryogenesis, generally condone ESC research up to a certain developmental point. There are also “conditional supporters” of inquiry into ESCs: those who condone the use of embryonic stem cells derived under certain conditions, but not derived using other conditions. For example, there are those who support the use of spare embryos from *in vitro* fertilization (IVF) clinics, but not the use of embryos created specifically for scientific investigation (Devolder, 2005, pg. 167).

Ultimately, the question of stem cell ethics can be represented as one’s personal conviction on three basic ethical matters. The first is connected to the principle of proportionality: does one feel that the potential of these stem cells to benefit the living outweighs the destruction of this unborn entity? This argument is generally connected with one’s opinion on the beginning of life as at conception or another developmental stage (Devolder, 2005, pg. 172). The second important question is based the slippery-slope argument, which suggests that if therapeutic cloning is allowed for the harvesting of stem cells, there is no way to prevent *reproductive* cloning (the creation of living genetically identical individuals). An ethical dilemma, reproductive cloning is the application of somatic cell nuclear transfer (SCNT) techniques to fertility treatments, resulting in a fully cloned human being. The third fundamental
concern in stem cell ethics is based on the principle of subsidiarity. This states that other alternatives exist, specifically adult stem cells and induced pluripotent stem cells, and asks whether embryonic research should be the scientific focus, or if any other options successfully validate the other two basic moral questions (De Wert and Mummery, 2003).

Positions of the Five Major World Religions on Embryonic Stem Cells

Christianity

Among the most adamant opposition of embryonic stem cell research is the Roman Catholic Church, which believes that life begins at the moment of conception, thus the destruction of the blastocyst in ESC research is equivalent to murder. However, this has not always been their view on the beginning of life. Since Christianity is based on Jewish teachings, the early Catholic Church originally held the same beliefs as the Jews about the beginning of life: that “humanness” is achieved when the fetus is fully developed. The first collection of church laws, written in 1140 by the canon lawyer Gratian, stated that abortion was not immoral until the fetus was “formed”, or began to resemble a human being. The Church’s current position on the beginning of life at conception was implemented in 1854 by the dogma of the Immaculate Conception under Pope Pius IX (Newton, 2007, pg. 27-28). This dogma stated that Mary, mother of Jesus Christ, was without “original sin” (the human tendency to act immorally) from the moment of her conception in the womb, and was thus fit to give birth to the son of God. The church, then, moved to establish conception as the beginning of life (Robinson, 2007).

Opponents of stem cells research in the Roman Catholic Church draw many examples from the teachings of Pope John Paul II, especially his encyclical Evangelium Vitae. This work cites Bible passages to support the idea that life begins at conception. Of these passages, the most
influential is Jeremiah 1:5, “Before I formed thee in the belly I knew thee; and before thou camest forth out of the womb I sanctified thee.” John Paul cites this passage to say “All human beings, from their mothers’ womb, belong to God who searches them and knows them, who forms them and knits them together with His own hands, who gazes on them when they are tiny shapeless embryos and already sees them the adults of tomorrow…” (Newton, 2007, pg. 28). John Paul also describes the story of Mary’s visitation with her cousin Elizabeth (Figure-1), where in recognition of Mary, Elizabeth’s fetus “leap[ed] in her womb”. John Paul uses this as evidence for the sanctity of prenatal life, concluding that the destruction of a human embryo for experimentation is “a crime against their dignity as human beings, who have a right to the same respect owed to a child once born” (Newton, 2007, pg. 28).

Other denominations of Christian faith have conflicting viewpoints on stem cell research. Orthodox Christianity shares the same opinion as the Roman Catholic Church, that the destruction of an embryo is a sin against human life, and is morally and ethically unjustifiable. Positions of Protestant denominations vary among the different churches. The first Christian faction to take a stand in favor of stem cell research was the United Church of Christ in 2001, followed by the Episcopal Church in 2003. Both denominations have been actively petitioning to the US government for legislation to give federal funding to embryonic stem cell projects. Alternatively, there are denominations like the Southern Baptist and Lutheran Church of...
Missouri, which oppose stem cell research on the basis that an embryo is “totally and fully human in every way” (Newton, 2007, pg. 30).

Judaism

As Judaism was the precursor to Christianity, it is often surprising to learn that the general Jewish stance on embryonic stem cell research is one of support rather than moral objection. In fact, all major denominations of Judaism (Orthodox, Conservative, and Reform) agree upon this support (Newton, 2007, pg. 31). The reason for this approval of ESC research is that the teachings of Judaism argue for a different opinion than Catholic teachings over the instant when life begins. Jews carry beliefs from prior to the installment of the Catholic dogma of the Immaculate Conception, and maintain that humanness is not attained until the fetus begins to resemble a human being.

According to the Talmud, the Jewish guide to moral and civil law, human ensoulment occurs around day 40 of gestation, and prior to that, the fetus exists “as if it were simply water”. As a result, the destruction of the 5-day old blastocyst to isolate stem cells is not a moral predicament. After the 40th day of gestation, the fetus is considered “like the thigh of its mother”: something that normally would not be removed but can be extracted if it is causing harm. This is used to support the Jewish stance on abortion: that the act is considered more like self-injury than murder, and is permissible when the fetus poses a threat to the mother. Stem cells extracted from fetal tissue aborted under Jewish law can be used for research and to better the already existing human population. The Jewish faith also supports the *in vitro* creation of embryos for research. Genetic material and embryos that exist outside the womb are not protected under Jewish law, as they are not considered part of the human body until implantation. Since these IVF embryos
cannot form a human being outside the womb, they are given even less protection than early in vivo embryos, and are able to be used for therapy or research purposes.

Jewish faith dictates that the body is an earthly loan from God, and as outlined in the Torah, the first five books of the Hebrew bible, there are certain requirements to care for it. One of these requirements is *pikuah nefesh*, the obligation to preserve human life and health. It is considered a “duty to God to develop and use any therapies that can aid us in taking care of our bodies, which ultimately belong to God”, including stem cell research. Because of this instruction, Judaism views ESC research as a necessity rather than something to be combated (Dorff, 2002).

*Islam*

Unlike Christianity or Judaism, the Islamic faith has no specific doctrine concerning stem cell research. As Islam is also derived from Judaism, most Muslims share the opinions of the Jews, but there is also inner disagreement, as no single teaching unites the Muslim position. The moral ethics of embryonic stem cells research must then be inferred by each adherent from the Muslim body of principles, the Shari’ah (Frazzetto, 2004). Like the Jewish Talmud, the Shari’ah makes a distinction between *potential* life and *actual* life. Muslims recognize the value of the embryo and consider it to have the potential to become a human being, but do not view it as such before birth. For example, Muslim jurists have ruled that if someone attacks a pregnant woman and kills her fetus, their punishment will be less severe if the attack takes place within the first 40 days of pregnancy than if it had progressed to full term. This clearly demonstrates that the Islamic conviction is generally that ensoulment occurs around day 40 of embryogenesis.
Muslims also recognize the distinction between *in vivo* and *in vitro* embryos. They believe that since an embryo *in vitro* lies outside its natural environment and cannot form a functional human being, there is nothing morally wrong with its loss for the benefit of humanity. Supporters argue that thousands of spare IVF embryos are created and subsequently frozen, only to be destroyed eventually anyway. In the words of Dr. Muzammil Siddiqi of IslamiCity, “If these embryos were treated as full human, it would have been forbidden to produce them in excess and to destroy them later” (Siddiqi, 2002). He argues that since the “potential humanness” of the embryos is already ignored in their creation, their destruction is not an ethical problem. He continues to say “Perhaps if research was limited to using only these already existing embryos, it would be more acceptable than if embryos were created and destroyed specifically for the sake of acquiring stem cells” (Siddiqi, 2002).

**Buddhism**

The question of stem cell ethics becomes unclear within the context of Buddhism. Buddhists do not believe in the idea of the personal self: there is “no act, no actor, and no consequences of action”. In the terms of the destruction of embryos for stem cell research, it has since become necessary to define personhood to outline ethics. This presents a challenge as Buddhists reject the “illusion of self” and strive to transcend it (Hughes, 1995, pg. 6). There are no Buddhist teachings directly related to stem cell research, and thus Buddhist opinions tend to be split on the matter. This is likely due to two conflicting tenets in Buddhist tradition: *ahimsa*, (the “prohibition against harming or destroying others”) versus *prajña* (the “pursuit of knowledge”) and *karua* (compassion) (Religious Groups, 2008).
Buddhist opinions on the beginning of life are adapted from classical Hindu teachings that preach that the “transmigration of consciousness” during reincarnation occurs at conception. However, modern Buddhists argue that spiritual development may mimic physical development in the sense that it is a step-wise process. The Buddhist theory of ensoulment is that during embryogenesis, the person develops their five *skandhas* (physical and mental components of existence) in order, and the illusion of personhood is not complete until birth (O’Brien, 2011). The karmic retribution for the destruction of this embryo brings about a question of the nature of Buddhist ethics, and one’s opinion on this matter will also affect their position on stem cell research. For example, an absolutist would argue that bad karma will come from the destruction of the embryo, no matter the justification, while a utilitarian could view the same situation as having positive karmic consequences for the benefits of medical research. An interesting thing to note about Buddhism is that, unlike most major religions, it is not “pro-natalist”. It does not see family values and reproduction as a religious duty, and Buddhist temples even sell rituals intended to be an apology by the parents to an aborted fetus, hoping for a better rebirth for their child (Hughes, 1995, pg. 8-10).

*Hinduism*

The Hindu Vedas, the ancient series of Hindu scripture, teaches that all life is sacred and this life begins at the moment of conception. However, as a people that also believe in reincarnation, the Hindus recognize that life and death are inevitably tied. In fact, giving up one’s life for the greater good has traditionally been regarded as a sacred act (Bhanot, 2008). There is the traditional

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**Figure-2: Dadhichi, a Sage from Hindu Mythology.**
(BAPS.org, 2011)
story of Dadhichi, a sage from Hindu mythology (Figure-2) who gave up his life so his bones could be used to eliminate a demon. Hindu tradition glorifies his actions, and he is seen as a model for all Hindus. The question at hand is if the destruction of an embryo for stem cell research is equivalent to this act, since the embryo does not have the free will to make its own decision.

As the Hindu church does not have an official stance on ESC research, the ethics of stem cells in Hindu culture depends on one’s opinion of the source and recipients. Most Hindus agree that if the stem cells and/or embryos are voluntarily donated then their use is not a moral dilemma. It is likened to the donation of the body to medical research, a praiseworthy action in Hindu culture. The ethical concerns arise if and when stem cell donation becomes a commercial exchange. Another ethical concern important to Hinduism is the availability of the benefits to all people, not only those who can afford the expenses of the high-cost technology (Tyagananda, 2002).

SCNT, iPS, and Parthenogenesis Ethics

While embryonic stem cell research has caused religions to reassess their positions on the beginning of life and the ethics of abortion and cloning, alternatives to stem cell research have also caused their own share of moral dilemmas. Opinions are mixed on the relatively new technique of creating induced pluripotent stem cells (iPSCs) via gene insertion into a somatic cell (Figure-3), SCNT in which the nucleus from a skin fibroblast cell is transferred into an enucleated egg which is grown to the blastocyst stage.
from which ES cells are obtained, and parthenotes which are chemically stimulated eggs used to provide ES cells. While some view these feats as valid solutions to the destruction of human life, others point out that SCNT and parthenotes use new types of embryos.

For example, in a debate in the Bioethics Forum of the Hastings Center in 2008, both sides of the argument can be clearly observed. The editor of the Hastings Center Report, Gregory Kaebnick, speaks for the opposition and argues that cells created by SCNT could be the first step in the development of a new type of embryo. His idea comes from the fact that the pluripotent cell created through the SCNT technique, the clonote, “looks much like a zygote and may well be capable of developing into a baby.” With respect to iPS cells, genetically induced pluripotency was invented to form a stem cell while bypassing the creation of an embryo. However, Kaebnick argues that if reprogramming an adult cell through SCNT creates a new type of embryo, then reprogramming a cell via gene injection could also possibly create yet another new type of embryo still subject to the debate over the rights of the embryo (Kaebnick, 2008).

Alternatively, Cynthia Cohen and Bruce Brandhorst of Georgetown University and Simon Fraser University, respectfully, argued in their response to Kaebnick that induced pluripotent stem cells via gene insertion are not totipotent, and are more like embryonic stem cells than an actual embryo. To support this assertion, they describe three properties shared by ES cells and iPS cells: a lack of the extracellular layer required for implantation in the uterus, small size with a lack of organization like an egg, and no evidence of totipotency. They argue that this tissue lacks the ability to implant and survive in the uterus, and therefore should not be considered as life (Cohen and Brandhorst, 2008).

With respect to parthenogenesis, the process by which an unfertilized egg is induced to begin dividing to form an embryo. This is a natural form of asexual reproduction for certain
insects and reptiles, but when induced in humans it results in either a nonviable embryo or a tumor. The parthenote is considered unable to progress past the embryo stage, as it is “genetically programmed to die early in its development.” However, there is no conclusive scientific evidence that the parthenote cannot theoretically form a human being, so this hypothetical being is subject to the potentiality argument. Aside from this, whatever abbreviated lifespan this defective embryo has is still involved in the debate over the destruction of life (Cheshire, 2011).

Chapter-3 Conclusions

There is a wide spectrum of ideas on embryonic stem cell ethics, ranging from full support to adamant opposition of research. This range is made evident by the dissent both between and within the major world religions. The Roman Catholic Church, for example, is a staunch opponent of the destruction of embryos for the extraction of stem cells, but other factions of Christianity support their use. Other major religions including Judaism and Islam condone the use of embryos in research on the basis that life does not begin until the 40th day of pregnancy. The religions that do not believe in the “traditional” sense of life and personhood, such as Hinduism and Buddhism, do not have official stances on ESC research, so the ethics of the topic are at the discretion of the adherent. As religious opinions are split on the matter pertaining to natural embryos, these opinions also diverge on the use of embryos created through IVF and other pluripotency-inducing techniques.
Chapter-3 Bibliography


Chapter-4: Stem Cell Legalities
Diego Prentice-Webb

Regardless of their immense potential to benefit society, the study and applications of stem cells must be governed and protected by the law to ensure that this controversial research is performed in a safe and ethical fashion. And as is typical for controversial technologies, stem cell policies are often strongly influenced by politics, religion, and culture. The purpose of this chapter is to discuss the U.S. stem cell policies, national and state, and describe some key international policies.

The policies that countries maintain to control stem cells vary immensely and are shown in Figure-1. The wide variation seen among the countries stems from a spectrum of different religious, ethical, and political views making the process behind approving any law very difficult and lengthy as it depends on many variables. When law makers discuss stem cells, they tend to focus on the source of the cell (embryonic or adult) and the source of money funding the research (federal or state) (The Gargoyles, 2005).
Federal Stem Cell Laws in the USA

Stem cell research in the US has its beginnings rooted earlier than people may actually realize, dating back to the 1920s when physicians were attempting to transplant fetal tissue into patients suffering from diabetes. Nearly half a century later, and only thirteen years after the accidental discovery of stem cells, in 1973 the Supreme Court of the United States deliberated the landmark case of Roe vs. Wade (case 410 U.S. 113) which led to the legalization of abortion. Consequently, lawmakers began to ask themselves to what extent could aborted fetuses be used for in scientific research as they would now become more available to scientists. Shortly after, on July 12, 1974 the 93rd Congress passed the first law regarding stem cells stating that the federal government would not fund any research involving fetal tissue until guidelines are defined by an Ethics Advisory Board (EAB) established by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research within the Department of Health, Education, and Welfare (Stem Cell History, 2011). Since then, the laws governing embryos and stem cells, and where funds will be directed has been a reflection of that period’s governing administrations.

In 1980, President Ronald Reagan began a long lasting de facto moratorium which banned federal funding for embryo research for 13 years until 1993 when President William Clinton exercised his presidential executive order to lift the moratorium. This however did not last very long, as he reversed the order a year later in response to thousands of letters urging him to do so, even though the National Institute of Health (NIH) human embryonic research panel (a panel he created) recommended otherwise in regards to the “profound ethical and moral questions” raised by the subject (Clinton, 1994). President Clinton did however allow funding for research involving excess embryos created by in vitro fertilization that would not be used for
implantations. Nevertheless, the republican-led Congress felt they needed to intervene, and enacted the Dickey-Wicker Amendment, which stated that federal funding could not be used to support the creation of human embryos for research purposes (Genetics and Public Policy Center, 2011). Specifically, the amendment prohibited federal money to be spent on research in which a human embryo would be destroyed, discarded, or knowingly subjected to risk of injury or death (Stem Cell History, 2011).

After the discovery of human embryonic stem cells by James Thomson (1998), the NIH began to draft guidelines to support funding research using these cells, as they determined that ES cells did not meet the statutory definition of an embryo, therefore the Dickey-Wicker Amendment did not apply to them. The NIH Guidelines were published in August 2000 (National Institutes of Health Guidelines, 2000). The guidelines recommended that funds be given to researchers using human ES cells so long as they were derived with private funds from frozen embryos from fertility clinics; the embryos must have been created for fertility treatment purposes and be in excess of the donor’s reproductive need; and the embryos must be obtained with the consent of the donor. However, upon reading these guidelines, President George W. Bush enacted an executive policy stating that researchers could only use ES cell lines created before August 9, 2001 consisting of a supposed 60 stem cell lines believed to be available at the time (Bush, 2001), although only a handful of cell lines were realistically available (Agnew, 2003). Difficulties encompassing embryonic stem cell research continued under President Bush’s two terms, as he constantly ignored emerging guidelines published by the National Academies of Science and the International Society for Stem Cell Research, and he vetoed two bills passed by Congress (Babington, 2006).
Hope for researchers came with the inauguration of President Barack Obama in 2009 as he immediately lifted his predecessor’s ban on federal funding for embryonic stem cell research, stating that the government needed to make “scientific decisions based on facts, not ideology” (Childs & Stark, 2009) which is where the government currently stands on the issue. However, Obama’s policy remains rigorous, and diligently abides by the NIH Guidelines. The embryos must be derived from excess embryos from reproductive clinics, with donor consent, and no embryos can be created solely for research purposes. In light of promoting embryonic stem cell research, President Obama has also taken a firm position against human cloning as he believes it “dangerous, profoundly wrong, and has no place in our society, or any society” (CBS/AP, 2009)

**Stem Cell Laws for Individual US States**

While federal funding was unavailable for ES research for nearly a decade during the Bush administration, a few states took it upon themselves to fund their own scientists (Figure-2) allowing headway on the research, although at a reduced rate relative to what federal funding could have provided.

New Jersey spearheaded this movement in early 2004, when its legislative branch passed a bill named S1909/A2840 which legalized the cloning of human embryos so that they can be developed during their temporary implantation in a womb and then harvested for medical research. They also pledged to assign $6.5 million to universities, non-profit and private research labs investigating ES stem cells to stimulate an influx of jobs in the growing industry (Scherer, 2004). Since then, the Garden State has awarded an extra $10 million in stem cell research grants in an attempt to “further...New Jersey’s position as a national research in stem cell
research” (Fineman, 2007). It also approved $270 million in loans to build the Stem Cell Institute of New Jersey (Wadman, 2008).

The state of California was quick to follow later in November 2004, when they passed Proposition 71, an amendment to the Constitution of California which authorized the state to donate $3 billion in general obligation bonds (bonds usually used for developing state infrastructure) to stem cell research programs within the state (Hayden, 2008). Along with this, the California Institute for Regenerative Medicine (CIRM) was birthed to help decide where the California grants would be allocated, to establish regulatory standards for conducting stem cell research and to oversee the development of stem cell research and its related facilities in California (Legislative Analyst’s Office, 2004). Following the installation of CIRM, a total of 453 private and public research institutions in the state have benefited from more than $1.25 billion in research grants (CIRM, 2011). While several other states such as Connecticut, Illinois,
Maryland, Massachusetts, New York, and Wisconsin followed California and New Jersey by making their own plans to donate a sum of nearly $3 billion over the next ten years, other states such as Louisiana and North Dakota have banned ES cell research (Figure-3).

**Figure-2: Diversity of State Funding for Embryonic Stem Cell Research in the US.** (Wadman, 2008)

**Stem Cell Laws in Other Countries**

Looking back at Figure-1, it is apparent that the world is currently divided between allowing and prohibiting ES cell research. Interestingly, it can be noted that arguably the most powerful countries in the world (the five permanent members of the UN Security Council) (China, USA, Russia, United Kingdom, and France) all fully support the use of surplus embryos obtained from fertility clinics for research. Adding in Brazil, Japan, and India (three additional major contributors to world economy and development) which are also in favor of ES cell research, these eight countries represent more than 3.4 billion people, about half of the world’s population! Furthermore, these countries all obey a United Nations International Convention installed in February 2005 against human reproductive cloning, showing a clear divide between
working with excess embryos versus human reproductive cloning within these eight leading countries (Office of Legal Affairs, 2005). However, many countries remain divided on whether to allow human therapeutic cloning to produce human ES cell lines from individual patients for treating the same patient with his own cells.

As in the US between various states, much disagreement can also be found within the European Union which actually has no over-riding current regulations or laws concerning the research of stem cells, leaving its 25 member countries to decide legislation themselves. Currently, the continent is split on the matter with research on ES cells being permitted in Sweden, Finland, Belgium, Greece, Great Britain, Denmark, and the Netherlands, while being illegal in Germany, Austria, Italy, Ireland, and Portugal, reflecting the ethical, philosophical, and religious diversity found in the EU. Although divided on the legalities of ES cell research, European courts recently ruled that no scientist working in the EU is permitted to obtain a patent on any method describing how to destroy human embryos, as the court fears it would hinder research and stifle commercial investment (Sample, 2011). In Europe, many scientists feel that this is a step backwards for stem cell research, as techniques developed and paid for by Europeans will be used for free in other parts of the world.

Serving Europe as their unofficial leader in stem cell research is Great Britain where the first successful mammalian clone, Dolly the sheep, was made in 1996 (Campbell et al., 1996; Ralston, 2008). The UK has been a major historical contributor to stem cell research, beginning in the 1980s when the British Parliament implemented a committee to recognize the ethical concerns of stem cell research and to recommend appropriate regulations. This was followed in 1990 by enactment of the Human Fertilization and Embryology Act which stated that any research conducted on embryos must be approved by the Human Fertilization and Embryo
Authority (FFEA), any embryos used must have been created in vitro, and the research on these cells must not exceed 14 days (EuroStemCell, 2007). These British stem cell policies have received much support from the public and as a result, researchers have benefitted from more than £220 million in funding in addition to the creation of a new £30 million stem cell center due to open in April 2012 to stimulate foreign investment in their biotech industry. They also felt that a surge of funding would help prevent scientists from leaving the country to find work elsewhere (Neate, 2011).

France and Germany have employed more conservative ES cell policies limiting their research development. France has traditionally been against using any embryos for research, but in 2004 a bioethics committee decided that scientists would have a five-year window of exception to their normal ban. This allowed researchers to use imported embryonic stem cells created in vitro only. Although there are signs of increasing openness to the subject in France, currently the country is at a stalemate with no consensus (UKSCI, 2009). Almost the polar opposite of the UK, Germany is known for having a stiff opposition to pro-embryo research. In 2002, the German Stem Cell Act was passed effectively banning the import or use of human embryonic stem cells unless strict criteria were met, in which case stem cell lines made before 2002 could only be used. Staunch hostility continued in 2004 when the German National Ethics Council (NER) demanded a global ban of embryonic cloning. However, the German fight for embryonic research turned a new page in 2008 when the Bundestag (Lower House) amended the Act to allow scientists to use stem cell lines created before May 1st, 2007 (Ralston, 2008).

China, often referred to as the “land of opportunity” for stem cell research, is known for the great flexibility the government uses when it comes to studying and using stem cells. This flexibility stems from different ethical and cultural views (Barnes, 2006). Some expatriate
scientists and biotech companies have moved their research to China in light of being able to work comfortably while using embryonic cell lines in both the laboratory and the clinic. The view of the Chinese stem cell community is that they have world-class expertise in the field, but also have a serious deficiency in the national infrastructure necessary to support the science. Specifically, government funding is limited as the Ministry of Science and Technology only issues a central budget that is divided evenly for “basic stem-cell research” and “applied stem-cell research” for researchers to apply to. Only an estimated $24 million was spent by the national government in the first five years of these projects, while the local governments of Beijing and Shanghai were reported to spend the same amount on research (Murray, 2006). Additionally, many researchers in the international scientific community have called Chinese policy makers too relaxed, as China is known for using stem-cell treatments without having performed conclusive clinical trials beforehand. Resultantly, on May 1st, 2009 a new set of guidelines were published by the Ministry of Health mandating that Chinese institutions which provide stem cell therapies must pass strict technical audits. The audits must demonstrate the use of clinical-trials that show the safety and efficacy of treatments, include an approved ethics advisory board, and the researchers must have significant experience in the field (Stem Cell Transplantation Department, 2011).

Chapter-4 Bibliography


PROJECT CONCLUSIONS

Based on the research performed for this project, and their own personal observations, each author makes their own separate conclusions:

DPW’s Conclusions

Based on the information presented in this study, I believe that more research in the stem cell field is needed to help ensure that these life saving techniques, which exploit the regenerative potency of stem cells, reach patients. Unfortunately we do not yet know enough about how these cells grow and function in the developed human body. As a result, the number of treatments for patients is currently very limited, and is generally only offered in a few clinical studies. I feel as though the advancement of stem cell applications, especially for ES cells, has been greatly hindered because of the deep effect organized religion has on politics and laws. However, with newer political administrations across the globe, much advancement has been seen in the past decade with governments making “scientific decisions based on facts, not ideology” (Childs & Stark, 2009). This wide-spread support is extremely critical for ES cells, as they possess the greatest plasticity of all the stem cell forms and can offer the best solution to patients. Although I strongly feel that ES cells should be scientist’s primary concern, I do not believe that embryos should be created solely for research purposes, and agree with the current US government’s stance on allowing surplus embryos created from IFV to be studied.
ED’s Conclusions

Raised in a conservative Roman Catholic family, the concept of “respect for life” was instilled in me at a young age. Thankfully my interest in the life sciences also grew with me and I have had the chance to examine the embryonic stem cell debate from both scientific and theological perspectives. I have come to the conclusion that the investigation of ESCs and the subsequent destruction of embryos for therapeutic and research purposes can be justified in our current situation. Thousands of embryos created for in vitro fertilization (IVF) treatments are discarded each year, and I personally feel that denying the scientific benefits of these already forsaken embryos is ignorant and wasteful. Regardless of whether the embryos are considered life, I believe it is more ethical to destroy them while extracting valuable stem cells than to simply destroy them as bulk medical waste. For this reason, I fully support President Obama’s 2009 executive order to lift the ban on stem cell research on surplus embryos from fertility treatments and support the resulting NIH standards for this research.

I do not claim to know at what moment life or ensoulment begin, but I believe that creating viable embryos solely for scientific research is an ethical concern since the morality of creating life to destroy it is questionable. I instead support other methods that supply pluripotent stem cells, like the induced pluripotency stem cell (iPS) techniques using genetic engineering to reverse the differentiation of adult stem cells. I am very much in favor of using adult stem cells for research in both their differentiated and undifferentiated states, because the isolation of these cells does not involve the destruction of a potential life. Ideally we will discover an efficient, non-controversial way to isolate or create pluripotent stem cells, and continue to advance the applications of stem cell technology.