August 2011

Stem Cell Overview

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IQP-43-DSA-1101

STEM CELL OVERVIEW

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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August 10, 2011

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Abstract

Stems cells have shown decisive applications in recent times helping to alleviate human suffering. Several sources exist for stem cells, some having great ethical concerns while others face clinical obstacles. The application of stem cell technology in society is based on ongoing research initiatives. Several ailments of the human body facing large barriers to heal are now having those walls broken down at dramatic rates. Yet despite the ground covered within a short time, groups around the world still undermine the findings. Rigid doctrines and teachings guide the cultivation and application of stem cells, with embryonic stem cells facing harsh criticism from major religious institutions. Actions are being sought within several communities to maximize the succession of this novel technology by creating leading research foundations for the world to follow. In many countries, international stem cell efforts are expanding the ability to care for individuals around the world and continuing to provide strong societal benefits, giving the dissenters shaky ground to stand on.

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Project Objective

By defining key points in the stem cell debate and educating readers on the types of stem cells and how they are classified, this project aims to inform readers why this field will have profound impact on society and the future of civilization. Modern technology proliferates quickly while often forgetting to inform society of the beneficial goals reached. Applications of stem cell technology have progressed to human clinical trials with success being achieved in several cases. The impact of such applications must be made known clearly to maintain an educated society. The five major world religions agree that working with adult stem cells should be encouraged, but disagree on the use of embryos. The technologies present today within regenerative science are found as obligatory endeavors in some religious communities. Informing readers of the stances held by each major religion will help guide the general perspective currently fractured between rhetoric and objective analysis. Through educating the reader, future legislative acts will become clearer in intention and the voice of communities can soundly help contribute to the direction regenerative science is heading. With the project's last words an expression on the future state of regenerative science is offered by the author.

Chapter-1: Stem Cell Types and Sources

Stem cells exist as long living cells within a body and are capable of differentiating into many specialized tissue types. These cells help govern tissue growth, repair damaged tissue, and can even assist in cancer cures (ISSCR, 2011). One stem cell can give rise to many cells through self-renewal, and during this self-renewal process a stem cell can differentiate giving rise to a similar stem cell and one specialized cell. These specialized cells, in large quantities, make up the backbone of tissues and organs (ISSCR, 2011). Due to the flexibility in forming new tissues, stem cells are the basis of the field of regenerative medicine, offering many potential benefits to sufferers within a society. However, some types of stem cells are ethically controversial. The purpose of this chapter is to highlight the many types of stem cells and illuminate how they are created and traced.

Stem Cell Classification

Stem cells are of many types, each encompassing different paths of specialization. *Totipotent* stem cells can differentiate into any subclass of cell, including any cell in the adult body or any extra-embryonic tissue such as the placenta. In mammals, the only cells recognized to do this are the initially fertilized ovum and descendant cells until the 8-cell stage (Chamany, 2004). After about 5 days post-fertilization, the embryo forms a blastocyst or hollow ball of cells. Cells located in the inner cell mass of the blastula are *pluripotent* embryonic stem cells. These cells are are able to differentiate into any cell in the adult body via the three primary germ layers, yet are unable to produce the placenta (Chamany, 2004). In addition, normal adult cells can be induced or modified into acting as pluripotent stem cells by reprogramming via viruses to

insert genetic changes into the cells, and this has further paved the way for ethical procedures in modern regenerative medicine (ISSCR, 2011). *Multi-potent* stem cells act as foundational cells within a variety of tissues and can differentiate into several types of related tissues. Examples of multi-potent stem cells are mesenchymal stem cells and hematopoietic stem cells. *Uni-potent* stem cells have only a single potential path of differentiation, and are the most specialized type of cells in the body (Chamany, 2004).

Stem Cell Identification

Embryonic stem cells are identified as the inner cell mass of the blastocyst. Adult stem cells are far more difficult to identify. ASCs are found in low numbers throughout adult tissues, so scientists attempt to identify them using specific marker proteins located on the cell surface (Schultz et al., 2004). Markers of pluripotency include Oct-4, SSEA-3, SSEA-4, TRA 1-60, TRA 1-81 and alkaline phosphatase (Klimanskaya et al., 2005). Embryonic stem cells derived from a blastocyst carry these markers, and are tested in each stage of expansion to ensure their pluripotency. A widely used surface marker for bone marrow stem cells is CD34+, a 115 kilodalton glycoprotein expressed in 1.5% of normal bone marrow cells (Schultz et al., 2004). Adult epithelial stem cells display one common marker throughout this lineage, the Lgr6 marker (Snippert et al., 2010). This marker was traced during wound repair, and each newly formed layer of epithelial cell structures showed Lgr6 presence (Snippert et al., 2010).

Embryonic Stem Cells

Embryonic stem cells are derived from the inner cell mass of 5-day old embryos. The embryos are obtained from *in-vitro* fertilization (IVF) clinics intended for reproductive purposes.

In the United States, a ban exists for creating embryos solely for research purposes; only excess IVF embryos may be obtained from reproductive clinics with donor consent. Once the family has enough children, the excess embryos are usually discarded. Legislation from the 1970's allowed these excess embryos to be used in research with donor consent (Klimanskaya et al., 2006).

Embryonic stem cells were first isolated from mice in 1981 (Martin, 1981), and from humans in 1998 (Thomson et al., 1998). For the human cell extraction, the cells were grown on a feeder layer of irradiated mouse fibroblast cells to provide a scaffold and growth factors and are then grown into a stem cell line (Chamany, 2004). The initial use of animal cells as a feeder layer was eventually replaced by a human feeder layer due to worries about animal viruses or contamination with animal proteins. Both were health issues with cells that would be implanted into human patients. The removal of the stem cells from the embryo usually destroys the embryo, which some individuals argue is murder leaving these cells as an ethically controversial topic. Following their isolation, embryonic stem cells cannot be used to create an entirely new organism (Chamany, 2004), but can be grown and differentiated into any type of tissue in the adult organism, with direct clinical applications.

Adult Stem Cells

Adult stem cells are a very broad category that includes any type of stem cell not derived from an embryo. Generally, these cells are harder to isolate than embryonic stem cells and are harder to grow; due to these obstacles many scientists prefer working with ES cells if possible when trying to treat a disease. Adult stem cells do have some clinical applications, although

limited. Examples of adult stem cells are hematopoietic stem cells, cardiac stem cells and neural stem cells.

Hematopoietic Stem Cells

Hematopoietic stem cells are multi-potent and traditionally derived from the bone marrow. These are the best characterized type of stem cell, and have been used for decades in bone marrow transplants to treat various blood cancers (reviewed in Bortin et al., 1994). Hematopoietic stem cells usually differentiate into all types of blood cells (red blood cells, white blood cells, platelets, etc.), but recent evidence indicates they may be able to form tissues other than blood, so they may be more potent than originally thought (Dorshkind, 2002).

Cardiac Stem Cells

Scientists originally thought that heart muscle lacked the ability to regenerate following damage, but experimental research verified that cardiac tissue can regenerate from adult cardiac stem cells. These stem cells were first isolated in 2003 (Beltrami et al., 2003) and were thought to have the cell surface marker c-kit. However, other scientists argued these cells may actually represent hematopoietic stem cells that migrated to the heart, and that Isl1+ cells actually represent true cardiac stem cells (Laugwitz et al., 2005). Stem cells found in the heart occur in clusters in the atrial and ventricle walls, interspersed between the muscle cells (Touchette, 2004), and have been found both in rats and humans.

Experiments *in vivo* and *in vitro* on Fisher rat myocardial sections showed stem cell differentiation from a primitive stem cell expressing Ki67 surface marker that differentiated solely to a cardiac myogenic lineage (Beltrami et al., 2003). The marker c-kit^{POS} was also

present, which normally represents hematopoietic stem cells, but the Ki67 cells appeared to have lost their ability to form blood cell lineages. When the isolated c-kit^{POS} cells were plated into enriched F12K medium, the cells maintained stable phenotypes and remained undifferentiated. Growth tests indicated that the cells expressed clonogenicity, had self-renewal properties, and were pluripotent (Beltrami et al., 2003). Further tests on the structure and function of the cells determined that they were capable of contraction. Experimental evidence revealed that cardiac tissue in normal and diseased mammalian hearts appears to have self-renewal capabilities. While the vast majority of cardiac cells are terminally differentiated into cardiac muscle, a small group of stem cells helps maintain the tissue (Beltrami et al., 2003; Philipkoski, 2003).

Neural Stem Cells

Neural cells age and decay over time and until recently nerve tissue was thought to be incapable of regeneration. We now know that the brain contains two narrow regions that support regeneration, the sub-ventricular and sub-granular zones, which appear to contain neural stem cells. Neural stem cells were first isolated in 1989 (Temple, 1989), and appear to participate in the formation of all three main types of brain cells: neurons, astrocytes and oligo-dendrites. Early hypotheses limited these stem cell abilities to only participating in scar formation after injury, indicating limited neurogenesis (Bjorklund & Lindvall, 2000), but we now know they are multipotent.

Early experiments tested the existence of stem cells within the brain by inducing lesions within mouse brains. Less than 2% of newly formed cells expressed neural markers, and the rest were scar tissue unable to link with neural circuitry. However, the expression of the neural stem cell markers in even a limited number of cells was enough to stimulate further research

(Bjorklund & Lindvall, 2000).

Embryonic stem cells can also be differentiated into neural cells (Huettner, 2006). The cells are capable of sodium, potassium and calcium channels gated by neurotransmitters. While the number of neurotransmitter receptors is limited, they are able to make basic communication through synaptic contacts. The cells polarized phenotype resembles neurons from the central nervous system (Huettner, 2006).

Epithelial Stem Cells

Epithelial stem cells have also been discovered in skin and other organ surface tissues, and are responsible for the regenerative properties of the epithelial layers. One type of epithelial stem cell in mouse colon epithelium appears to have the surface marker Lgr5 (Barker et al., 2007). In mice, the crypt base columnar cells of the intestine express Lgr5, while the villi do not. Lgr5 may be responsible for rapid stem cell growth due to the high volume of cell turnover within the intestines. For mice, this turnover is every three to five days (Barker et al., 2007). The Ki67 proliferation marker is also found within the columnar cells. Using fluorescent microscopy, the Ki67 cells were found to form three dimensional wedge shapes (Barker et al., 2007). However, the Lgr5 cells appear to be the dominant stem cells. Within hair follicles, there is also a small amount of Lgr5 expression (Barker et al., 2007).

iPS Cells

One key issue during cell therapy is the potential rejection of the transplanted cells by the patient's immune system. Thus, scientists are seeking ways of deriving stem cells from a patient that would be genetically identical to the patient's cells. In recent years, adult skin cells have

been reprogrammed into pluripotent-like stem cells using viruses to carry new genes into the cells. These cells are defined as induced pluripotent stem cells, and have become one of the more prominent topics in the regenerative research field. In theory, induced cells could provide pluripotent stem cells for therapy that are genetically identical to a patient and do not require an embryo.

Induced pluripotent stem cells were first derived from mice in 2006 (Takahashi et al., 2006) and from humans in 2007 (Takahashi et al., 2007). The initial reprogramming involved delivering four genes into adult skin cells: Oct3/4, Sox2, c-Myc and Klf4. The genetic sequences caused a reversion, or a de-differentiation effect, to pluripotent states similar to embryonic stem cells (Baker, 2007). However, implantation of the cells sometimes caused tumors, so researchers eventually omitted the myc oncogene component of the mixture, and were still able to derive pluripotent cells (Baker, 2007; Hayden & Baker, 2009). Later improvements eliminated the use of the viruses to deliver the reprogramming genes.

One problem with induced pluripotent cells is some scientists report the cells have DNA mutations and an abnormal numbers of chromosomes relative to pre-induction (Pera, 2011). Some scientists argue the mutations might result from the use of virus delivery during the cell programming. DNA variations are not usually observed in normal embryonic stem cell lines. The mutations in the reprogrammed cells can cause cancer when they involve cell cycle regulation or growth factors (Pera, 2011). Mutated induced pluripotent stem cells that differ significantly in gene expression from embryonic stem cells cannot be used for clinical applications. A powerful alternative to retroviral gene infection is a system that completely eliminates the viral delivery system from the equation. Direct insertion of the reprogramming proteins through the cell wall can be accomplished by attaching the proteins to polyarginine

which carries the proteins through the cell membranes (Aldhous, 2009). Using mouse fibroblast cells soaked in polyarginine-tagged proteins for 12 hours four times over two weeks, stem cell colonies formed. No genetic markers for cancer were apparent in experiments, and the efficiency of induction was higher than that of adenovirus infection, although much less than retrovirus induction (Aldhous, 2009).

One key remaining question for these induced stem cells is whether they are truly pluripotent. Although some experiments have shown that induced pluripotent stem cells can form multiple tissues, other more recent experiments indicate these cells may harbor mutations that hinder their differentiation potential. More experiments are required to determine their true potency. Some experiments have indicated differentiation into neurons. Fibroblasts treated with five factors: Brn2, Brn4, Myt1l, Zic1 and Olig2, allowed embryonic stem cells to form. Continued experiments on these cells led to neuron like cells differentiation that made connections and synapses (Vierbuchen et al., 2010). Five days were required for the branching neural cells, giving rise to a quick and efficient system of forming a neural network. Other work indicated iPS cells may be pluripotent (Yu et al., 2007). Yet induced cells still require more work until they can be used in the clinic, as they may contain numerous mutations that lead to cancer or cause immune rejection. Their promise is they could be used without regard to ethical or moral obligations, unlike embryonic stem cells that require the destruction of the embryo (Yu et al., 2007).

Parthenote ES Cells

Parthenotes, unfertilized eggs grown into embryos, yield some promise for stem cell culture. Parthenogenesis is a type of asexual reproduction in which the female egg begins

dividing without fertilization. The process occurs naturally in some insects, but not in mammals. The mammalian eggs can be artificially stimulated to begin dividing while restraining chromosomal ejection to maintain the normal number of chromosomes. If the mammalian parthenote can be grown to the blastocyst stage, embryonic stem cell lines can be derived (Holden, 2002). Because mammalian parthenote embryos cannot develop into a child, some scientists believe these embryos have lower moral status than a fertilized embryo, so perhaps these cells could replace traditional embryo-derived stem cells. Parthenote systems might work to derive cells genetically identical to a female egg donor, but males are left without such a system (Holden, 2002). Parthenote stem cells have been derived for monkeys (Mitalipov et al., 2001) but not yet reliably in humans.

Chapter-1 Conclusions

Stem cells are not all alike. Although early work used bone marrow derived stem cells to treat various blood cancers, later experiments have shown that stem cells exist in almost all adult tissues that help function in tissue maintenance and repair. Research has helped identify cell surface markers specific to different types of stem cells, to aid their isolation. Regeneration of tissue within the body is natural, and the discovery of stem cells within adult tissues helped define the causes of the regeneration. Stem cells do not just exist within the embryo, but within the bone marrow, the bone, the muscle, even organs once thought to be non-regenerating. If these cells are so prolific within the body, then hopefully there is there a way to harness that regenerative capacity to heal even the most destructive wounds.

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Chapter-2: Stem Cell Applications

Regenerative medicine is a new field of science derived from the manipulation of stem cells. Introducing stem cells into damaged tissues with the appropriate potency, the tissue can sometimes become repaired. Using cell therapy diseases previously thought to be uncurable may be treatable. The purpose of this chapter is to go beyond earlier discussion of the various types of stem cells to describe how they are being used to treat diseases. Three categories of disease are discussed: spinal cord injury, neurodegenerative diseases and heart disease.

Stem Cell Treatment of Spinal Cord Injuries

Scientists are researching the use of stem cells to treat individuals with spinal cord injury. In one experiment, mouse paralysis was induced by using Neuro-adapted Sinbis Virus which targeted motor neurons leading to a permanent paralysis (Kerr et al., 2003). Transplantation of 300,000 stem cells into the spinal fluid caused a migration of the stem cells and adherence to the meninges within the spinal cord (Kerr et al., 2003), allowing the mice to survive for more than one month. Blind studies on the behavior of three groups of rodents after implantation of stem cells, baby hamster kidney cells, or human fibroblast cells individually for each group showed significant recovery of the stem cell group three months later (Kerr et al., 2003). Hind limb paralysis decreased significantly, allowing the rats the move upright once again. The stem cells created axonal growth and also allowed motor neurons to make significant connections.

Partial spinal cord injuries in rats that impaired walking were treated using oligo dendrites differentiated from human embryonic stem cells. Migration of the stem cells occurred, and the cells eventually relocated to appropriate neural sites within the spinal cord. Seven days after treatment, myelin tissue formed around the damaged neurons, and within two months the

rats showed increased motor control while the control rats showed no difference (UCI, 2005). Motor neuron damage appears to be repairable in rats, giving hope for regeneration in humans. During 2009, human clinical trials for stem cell implantation into spinal cords were approved by the Food and Drug Administration (NY Times, 2009).

Stem Cell Treatment of Neurodegenerative Diseases and Stroke

Neurodegenerative diseases are becoming increasingly prevalent in our ageing society. These diseases include Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. Scientists are researching the use of stem cells, both embryonic and adult, in re-growing areas of the brain affected by these diseases. Mouse embryonic stem cells have been cultured into CNS neurons and motor neurons, allowing the complex circuitry of the nervous system to rebuild from damage. The new cells can form synapses, connect with muscles, extend axons, and most importantly can function (Wichterle et al., 2002). Human embryonic stem cells have also been differentiated into cells expressing neural markers.

Parkinson's disease is a neurodegenerative disorder in which the substantia nigra area of the brain is destroyed, decreasing the production of dopamine in the brain. Human embryonic stem cells have been shown to be capable of differentiating into neural cells expressing dopamine, leaving hope to utilize their effects in treating this disease (Perrier et al., 2004). Over one third of the new cells had synaptic contacts. Clinical use of embryonic stem cells for Parkinson's requires the use of immune-suppressants to prevent rejection of the implanted cells, unless the implanted cells are induced stem cells derived from the patient.

Human clinical trials have shown a reversal of adult Parkinson's disease through the use of neural stem cell transplantation (Ertelt, 2009). Nearly 80% of motor function improved

through a 36 month period (Ertelt, 2009). In this experiment, the patient's own adult neural stem cells were used, which bypassed ethical issues, and the patient did not need an immunosuppressant. No rejection of the cells occurred. Only half of the patient's brain was treated, yet several years later no symptoms of the disease were visible.

In animal trials, the survival rate of post-treatment for Parkinson's is limited. Neuroprotective chemicals are lower than normal in Parkinsonian animals, so a possible path to correcting this deficiency is to engineer stem cells expressing these molecules (Lindvall & Kokaia, 2006). Possible long term therapies for humans might also require the expression of key neuroprotective molecules.

Rat trials determined the behavior of an untreated Parkinsonian rat, turning constantly and moving only in a linear path. The behavior of the rats was changed with stem cell treatment (Ryan, 2004). Transplanting human stem cells into the brains led to a significant reduction of these behaviors. The stem cells implanted in the brain and differentiated into dopamine producing neurons. In this particular case, the implanted cells failed to propagate, perhaps due to the presence of animal viruses (Ryan, 2004).

Stroke has also been treated with stem cells. Stroke induced in rodents has been treated with the use of adult bone marrow stem cells for neural regeneration (Lindvall and Kokaia, 2004). Experimental data showed frontal lobe regeneration with significant gains to normal behavior. The dead neurons from the stroke initially led to interrupted neural circuitry. Over time this circuitry was restored through neurogenesis. The regenerated neurons expressed neurotransmitters and are able to release dopamine (Lindvall and Kokaia, 2004). In this experiment, the cells were delivered by direct implantation to the affected locations, allowing cell migration to fill in the damaged cortex. Other delivery methods might use micro-scaffolding

layered onto the damaged brain, allowing stem cells to grow within a pre-designed structure (Science Daily, 2008). Derivations of the micro-scaffolding might include feeder particles laced into the scaffold for a more efficient cell growth, and structural formations to ensure a higher cell survival rate. Rat models utilizing micro-scaffold technology have used various scaffold materials, allowing specific differentiation depending on where the scaffold was applied (UPI, 2007).

A single human clinical trial using adult bone marrow stem cells was conducted for neural regeneration of a stroke victim (Vega, 2006). Before treatment, the stroke damage left the patient crippled, but less than two months after treatment the patient was able to fully navigate city subways once again. Bone marrow stem cell applications for healing neural damage shows great diversity of stem cell potential, given that their normal mode of differentiation is not neural in type. Human bone marrow stem cells migrate from their applied location into the brain and begin differentiating into astrocytes (Mezey et al., 2000). The application of bone marrow stem cells could allow mass production of missing neural or motor cells within individuals. Utilizing the patient's own bone marrow bypasses immune-rejection concerns and would negate possible tumorous growth from embryonic stem cells (Mezey et al., 2000).

Stem Cell Treatment of Heart Disease

Heart attacks are one of the leading causes of mortality in North America. Initial damage to the myocardial tissue leads to future heart failure, due to the increased demand on the remaining tissue (Couzin, 2006). German scientists initially developed a technique to use bone marrow stem cells to differentiate into heart muscle, although an early clinical trial failed. Affected arteries were injected with bone marrow stem cells in two groups, patients who had

recent heart attacks and those who had an attack six years prior. The treatment was found to be safe, but no significant difference was found between the placebos groups contrasted with the experimental group (Couzin, 2006).

Later experiments began showing regenerative progress. Following heart attacks, mononuclear bone marrow stem cells were infused into the left ventricle. The patients showed quick gains on systolic and diastolic homeostasis (Plewka et al., 2009). While pre-treatment patients showed significant ventricular dysfunction, after treatment the treated group showed significant improvement on the health of the ventricle. Cell necrosis, residual ischemia, microvascular dysfunction, and wall motion abnormalities were also significantly reduced in the treatment group (Plewka et al., 2009). Although myocardial tissue regeneration in clinical trials still has some time before long term effects are documented, recent research has shown that in the short term the gains significantly reduce mortality in populations with high heart attacks.

Embryonic stem cells, cardiac stem cells, myoblasts, or adult bone marrow stem cells have all been shown to differentiate into myocardial unipotent cells. Intravenous injections of the stem cells directly into damaged tissue is the general method of cell delivery (NIH, 2006). The cells tend to migrate much like tumor cells do. Tracking their movement in-vivo is difficult, but microscopy reveals that the efficiency of cell delivery and survival is near 10%. Stress, inflammation, and hypoxia generally causes a large amount of injected cells to die (NIH, 2006). Bone marrow stem cells used on rat heart models have been shown to regenerate cardiomyocytes, vascular endothelium, and smooth muscle cells (NIH, 2006). Resident cardiac stem cells, endothelial progenitor cells, and umbilical cord blood cells also show similar promise for treating cardiac damage.

Chapter-2 Conclusions

Stem cell applications to regenerate lost cellular populations may allow the treatment of previously uncurable diseases. Long term implications of stem cell regenerative techniques will become increasing important in an ageing population, while helping reduce healthcare costs and reduce disabilities and mortalities. Ageing populations currently encountering degenerative brain diseases, heart attacks, or other burdening diseases might retain their role in society instead of retiring due to health concerns. Military injuries and mortalities might be reduced through bone marrow transplants to regenerate traumatic injuries. Heart attack patients might find the regenerative properties of stem cells healing their muscle tissue and reducing the cost of their disease. In general, the use of stem cell therapy in society might affect the vast majority of citizens, allowing a refocus of medical efforts on preventive care instead of costly long term rehabilitative care.

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Chapter-3: Stem Cell Ethics

Manipulation of the fertilized human ovum for research purposes is generally met with ethical arguments. These arguments range from whether the blastocyst is considered a living human, and if so at which point these cells become human? Whether destroying the embryo to obtain embryonic stem cells is disrespectful towards god, and whether society can use the cells of the blastocyst for regenerative applications. Many of these questions are rooted in religious ideology whereas few are rooted in natural evolution moralities. The majority of arguments are centered about embryonic stem cell research and the religious implications even though such research is only a small facet of regenerative stem cell science.

Buddhism and Hinduism

Hindu perspectives on embryos depart from the general major religious grounds. Generally to Hindus, life is sacred and is not to be destroyed. A paradox is developed within this perspective which was resolved by ancient Rishis: only the highest conscious being shall be ensured protection. Based on this understanding a higher conscious life form can devour a lower level life form to ensure survival, such as humans eating plants and animals (Bahnot, 2008). The primordial life forms of embryos, under the premise of Rishis understanding, can then be used to propagate current and future human life. Hindu doctrines state that all life is evolving towards god, and in our current life we are only in a small phase of that movement. Sacrificing lower level conscious cells for the highest conscious, or human life, is only but a step towards that evolution (Bahnot, 2008).

Buddhist ideology supports medical research similarly to Islamic and Jewish ideologies. Buddhism has historical roots in the spread of the teaching of medical traditions passed through Asia (Hughes & Damien, 1995). The relationship between Buddhist teachings and medical practice has little documentation, indicating a slight subjection towards how medicine is conducted. Personhood is considered by a Buddhist as one who is aware of the difference between self and other, conscious of life, and has the ability to conduct purposive behaviors (Hughes & Damien, 1995). Buddhist teachings parallel traditional Hindu teachings in that consciousness begins with conception. Excess embryos at in-vitro fertilization clinics which have been fertilized by the father's sperm are seen as conscious life forms under Buddhist ideology. The issue becomes one of karmic holding, where killing smaller life forms generates less negative karma compared to killing late term fetuses or adults (Hughes & Damien, 1995). Moreover, negative karma can be counteracted with positive acts; following the destruction of the embryo and mending the body with regenerative medicine can incur such positive karma. Stem cell research is seen as a balanced karmic pursuit and is within the confines of positive behaviors Buddhist teachings accept.

Catholicism

On August 9th, 2001, President Bush designated limited funding for embryonic stem cell research (ACO, 2006), allowing federal funding for research performed on previously derived embryonic stem cell lines, but not to derive new cell lines. The policy was enacted in response to the U.S. Catholic Church pleading for President Bush to stop the manipulation and destruction of human lives. Not allowing federal money to be spent deriving new cell lines meant capping the potential of fetal destruction. Since the embryos used to derive the previously existing cell lines

were already destroyed no further harm would come from allowing federal funding for those cells. The lives being destroyed (as defined by the church) were embryonic stem cell lines already committed to research and had no potential for growth into a human. The ethical argument pushed forth by the Catholic Church is not the use of regenerative medicine, but instead the argument of when live originates. If another source of stem cells is used then the debate on whether embryos are relevant to regenerative medicine ends.

Distribution of pamphlets criticizing the use of embryonic stem cell research spread across the United States in attempts to educate readers about the religious moral hazards of such research. These documents create a cleft between religious ideologies and the scientific community. The pamphlet did not attempt educating the reader on both sides of the issue, instead only focuses on limited facets of how the research is conducted. A short discussion of the impact on human health is included towards the end (Filteau, 2007). By undermining the goals of regenerative science, the propaganda confuses the reader and misinforms any opinion held.

Pope Benedict XVI has endorsed publically the use of stem cell research with regards to adult stem cells (Pope Benedict XVI, 2008). The ethical quandary facing the possible destruction of human life with embryos to save another with regenerative medicine is overcome with adult stem cells derived from the patient themselves or cells induced into pluripotency. The Catholic Church insists that scientific endeavors should never fail to respect human life at any stage of existence, and through the novel generation of stem cells this boundary is stepped over (Pope Benedict XVI, 2008).

Fr. Pacholczyk expresses the same sentiment of the Pope while also misconstruing experimental evidence in order to garner larger support for obstructing embryonic stem cell research. The statement of no cure yet reached by stem cell researchers (Smith, 2006)

misinforms readers, as multiple cases of humans regaining motor control after paralysis has been documented as discussed in Chapter-2. Partial regeneration of motor abilities should be enough to warrant any stem cell research. Should society trust the propaganda arm of the Catholic Church when they make statements which could harm the future of regenerative medicine?

Christianity

The United Methodist Church has expressed a limited boundary on the use of embryonic stem cells. If the embryos are currently in excess from in-vitro clinics then they are of tolerable use (UMC, 2004). The use of excess embryonic stem cells allows progress of regenerative science. This modern science is required for advancing medical needs within communities. The Methodist Church requires that the embryos not be needed for procreation, that the donors give permission for scientific research use, and those embryos donated were not created solely for research purposes and were not obtained by sale or purchase (UMC, 2004). The United Methodist Church recognizes the need for progress in regenerative science in order to expand the ability to meet basic health needs of their own members, and does so by allowing controlled methods of donorship for research.

Reverend Fleischmann, writing from a conservative Christian perspective, poses a view similar to Catholic views on embryonic stem cell research. The embryo is given to humanity by god for procreation to occur. Deriving embryonic stem cells confounds the purpose of that life form and is the murder of god (Fleischmann, 2001). All life is of Christ, and any destruction of blastocysts murders the savior of humanity. The St. Stephen First Martyr Orthodox Church also expresses this viewpoint showing a rift between different sects within Christianity. The major argument is that life begins at conception, and no life is to be undone as this is a major sin

against god (Hodges, 2011). While the embryo is destroyed as a packet of cells, a counter argument that the cells themselves still survive should be succinct to void any Christian arguments. Their state as a group of cells may have ended, but the individual stem cells are retained and grown into colonies of similar cells. These stem cells, while never reaching human form, can be fused with an existing human to help retain that human form. A question to pose for Christians is should a group of life forms acting together as a blastocyst never be broken into individual cells, while most of the original cells survive alone as cell lines?

Evangelist Christian writers within North America show a malformed perspective on stem cell research. They deny the existence of induced pluripotent stem cells, or even the existence of stem cells within an adult human body. This perspective forces the argument into their frame without any tolerance of juxtaposition. Stating that stem cell research only exists as embryonic research denies the existence of hematopoietic stem cells, neural progenitor cells and other adult stem cell research. This argument reaches many readers and sways their opinion of the research illegitimately (Cameron, 2005).

Islam

In-vitro fertilization clinics are allowed under Islamic law as long as no surrogate mother is used, and the sperm of the father fertilizes the mother's egg. Excess fertilized embryos produced at these clinics not to be implanted into the mother can be used for stem cell research. According to Islam, the embryo is also considered as a potential life and not actual life, allowing for their harvesting and destruction if an overproduction occurs at the clinic (Siddiqi, 2002). Islamic law states an obligation to relieve human suffering and disease under fard kifayah. In consideration of stem cell research, this obligation is met. Adult stem cells are to be prioritized

for research in contrast to embryonic stem cells when the possibility exists. Embryos destined for destruction are allowed for stem cell harvest as they are potential lives not in their natural environment and have no possibility for generating an actual life (Siddiqi, 2002).

Shari'ah law within the Qur'an houses higher protection to actual life than potential life. The fertilized embryo is considered a potential life at the first forty days after conception, and within the next forty days any harm to that embryo is considered a potential homicide (Weckerly, 2002). Biotechnical intervention within the first forty days can be considered the ultimate will of god, and the research of improving human health an obligation towards knowledge.

Judaism

The Jewish perspective on stem cell research involving embryos is lenient. From the first period of gestation to the 40th day the embryo is considered holding the same rights as water. From the 41st day and onward, the embryo is considered a part of the mother's thigh metaphorically (Dorff, 2001). Research on a thigh still attached to a human is generally un-ethical, as would be research on the embryo which is now considered one with the mother regardless of the embryos location. Outside of this theological limit there are few confines to research for stem cells. Judaist practitioners base their views on Genesis 2:15, where all work done within the world is to preserve it due to divine duty (Dorff, 2001). By preserving human health via new clinical applications from research, the divine duty is fulfilled. One denial by Judaism of embryonic stem cell research is drugs that produce hyper-ovulation, as this can cause ovarian cancer and is a reversal of preservation (Dorff, 2001).

Within Jewish law the human life does not begin until the head of the child is more than halfway emerged from the mother's body. The soul of the human exists before birth but shall

always exist in consideration of human intervention (Rich, 1997). Two concepts related to the human body, where life does not begin until emergence, and that life must be preserved, shape the doctrine of embryonic harvesting. Jewish ethicists adhering to pikuach nefesh, or the preservation of life, find the destruction of embryos for stem cell research a great benefit for human life (Yearwood, 2006). Traditional Jewish groups find some ethical concerns with the destruction of embryos due to the chance that they could develop into a human life, but this perspective is limited in scope. The vast majority of embryos used for research are not kept within the mother; most are left in stasis within a lab outside of any environment which may permit them to develop (Yearwood, 2006). Another argument made by traditional groups is that the embryo developing into a human life will benefit society. Contrary to this idea, how would a mother give birth to a thousand children during her lifetime? Not all blastocysts will mature into a human life. These non-developing cells can be used for the preservation of lives already in existence, fulfilling pikuach nefesh.

iPS Ethics

Induced pluripotent stem cells maintain a neutral state within the moral spectrum due to the method of producing such cells. Small scrapings of skin can be converted into large colonies of stem cells, and while any clinical applications are still down the road, there is large promise of potential. Ethical issues do arise when looking at long term outcomes of this technology. Healthcare costs could see dramatic reductions due to ubiquitous production of induced stem cells. Embryo-derived stem cell lines might no longer be required as induced cells can be created from fibroblast skin cells taken from every living human, and can provide their own stem cell lines for expansion of the cells and for transplants.

As discussed in Chapter-1, whether these induced cells are truly pluripotent remains controversial. Assuming they are eventually proven to be as potent as embryo-derived stem cells, mobile bio-reactors generating the stem cell colonies could take root within communities. Heavy injuries within combat environments could be overcome within weeks allowing greater fighting ability for the militaries of the world. Perhaps the regeneration rate could be altered within induced stem cells allowing quicker recovery times from injuries. Ethics of behavior might become more lenient due to ease of access medicine. Would societies become more complacent, or would greater risks become a norm?

Chapter-3 Conclusions

The state of bio-technologies and regenerative medicine has gained large traction with the advent of new therapies for sick and suffering patients. War injuries, labor accidents, victimization within society, all are unjust acts committed on individuals. Morally, research and progress to help limit or end the suffering within society should be a prerogative of any group regardless of ideology. Utilizing embryos until alternative solid and ethical methods are extrapolated might just be a sacrifice required to reduce the significant pain held by millions. Embryos gained with permission from reproductive clinics where their fate is destruction gives new meaning to the cellular packet of life. Either these embryos can be destroyed with no purpose to humanity, or they can be expanded and implanted into humans in need giving some role to their existence. Embryos gained for research purposes by non-fertilization donors does create an ethical quandary. On one side, while the embryos would be utilized to pursue noble goals, their creation for sole purpose of destruction steps over the line of just behavior. However replenishment of the human population occurs through reproduction and limiting death rates. If

death rates could be prevented through regenerative science, then perhaps the offset in reproductive behavior could be used ethically to help maintain human populations.

Adult human stem cells provide a retreat from the embryo ethical debate by only requiring materials from the patient or a donor's own cells. These cells normally function to regenerate the human body; to catalyze this specialization through engineering may possibly be of higher ethical value than using embryonic stem cells. The consideration of harming the patient with foreign stem cells that might be rejected by their immune system is also side stepped using a patient's own adult stem cells and allows greater healing. Mixing adult and induced pluripotent stem cells as a combined strategy in lieu of using embryo-derived stem cells increases the potential range of medical applications giving both types lasting power within the stem cell debate.

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Chapter-4: Stem Cell Legalities

The regulations on stem cell research constitute a high turnover system. Over the past twenty years, regulations have moved from a tolerance ideology, to a hard line ban, switching back to a somewhat moderate stance most recently. Allocated resources to support the research often end up withheld in red-tape. Even types of stem cell research deemed ethical face limits on funding due to ambiguous legislation. The National Institutes of Health ethical guidelines were ignored by the President and were halted in court. Eventually, the court decision was overturned and new cell line approvals were made, but the strenuous flux of the law placed uncertainty on research goals (Ledford, 2011). The funding flux caused even single cell extraction from the blastocyst to be halted, a non-destructive routine to allow genetic testing on an embryo.

Early US Stem Cell and Embryo Policies

The timeline of US legislation on stem cell research begins in the early 1960's. In 1961 the Foundation for Stem Cell Sciences was established by Dr James Till and Dr. Ernest McCulloch, creating the foundation for modern regenerative science. During 1972, the decision from Roe v. Wade legalized some types of abortions, and in 1974 a ban on fetal research funding by federal sources was instituted to prevent the use of aborted tissue for research. During 1975, the Ethics Advisory Board was established in dealing with fetal research, but within five years it was dismantled by President Reagen. 1988 saw funding approval of embryo research, yet two years later President Bush Sr. vetoed a bill that would have lifted the ban on fetal research. During 1995, the US Congress enacted the Dickey-Wicker Amendment which banned all embryo research (Robertson, 2010). This moratorium was ended by President Clinton until

President Bush Jr. reinstituted the ban. Not until 2009 under the Obama administration did the ban end, allowing state legislatures to create state-specific laws allocating resources and approving ethics guidelines (Stem Cell Tracker, 2009).

Embryo and Stem Cell Legislation Under President Bush Jr.

With the first veto of President Bush's two terms of service, a rejection of Congress' decision to allow some types of embryo research was broached; Bush signaled a strong oppositional stance on the issue (Babington, 2006). Bush's main argument has parallels to Christian ethical stances on when life begins, and the Congressional vote was not sufficient to overturn the veto. Within the same week, President Bush signed a bill ending federal funding for the creation of new human embryos for organ harvesting. The nearly half million frozen embryos in North America were placed on disposal lists, as the couples donating only required a few for pregnancy (Babington, 2006). The President stated that destroying an embryo is tantamount to murder. Would voluntary abortions performed within the first few weeks of conception be treated as homicide as well? The rationale behind the Bush policy was that destruction of an embryo constitutes murder. President Bush Jr. banned federal money to derive new embryonic cell lines yet allowed money to be spent on cell lines derived prior to 2001 as they had already been destroyed based on arguments he presented.

The policy of a hard line ban on novel research within regenerative sciences diminished North America's stance from the forefront of modern medical care. Some international communities devote stem cell lines and resources to global stem cell banks, where analysis of the stem cells deems whether they are worthy for research. These cells are then handed over to stem cell researchers under strict oversight (Cook, 2004). Expectantly over 100 new stem cell lines are

thought to be generated under this international system, while public researchers in North America barely retained nineteen cell lines under the Bush administration. Private researchers within North America have access to the international stem cell bank and are making large research progress. During the Bush Jr. presidency, some investigators moved abroad to secure stable research labs not allowed under the influence of religious moralities (Cook, 2004). Young North American stem cell researchers also started moving abroad to escape the ban (Ford, 2002). In effect a brain drain developed as youth researchers immigrated to countries with stable bio-research regulations.

Several groups such as the Howard Hughes Medical Institute and Advanced Cell Technology have invested capital and resources to private stem cell research initiatives (Holden, 2006). Start-up private groups, not bound by federal funding bans, are experiencing an increased leading role within society for the progressive science. One key obstacle is standardized practices within the research setting to ensure objective and significant experimental methods. Without adopting the unified model that the National Institutes of Health proposes, sharing new insight and accomplishments requires learning each group's unique methodologies. Another issue that creates obstruction to research is efficiency; many hours are lost in attempting to secure funding for the research under the ban, effectively hampering the time allotted each day for experimental goals (Ford, 2006; Holden, 2006).

Embryo and Stem Cell Legislation Under President Obama

The ban under President Bush Jr. defied public opinion. With a two-to-one margin of support for funding stem cell research, including embryonic stem cell research, the ban was advocated only by a minority (Langer, 2005). With a new president inaugurated in January of

2009, the public support of stem cell research reached the administration of President Obama and a reversal of the ban allowed significant resources to be instituted within public stem cell research organizations (CBS/AP, 2009). Federal funds were allowed to create new lines of embryonic stem cells for use within North America, limited to embryos only obtained at reproductive clinics with donor consent and without pay, creating a competitive research foundation in contrast to the international community. The cited reasoning behind President Obama's revocation of the ban parallels many world religious stances on the ending of human suffering through research discovery. Another cited reason is to reverse the emigration of top scientists due to the ban (CBS/AP, 2009).

As strict regulations were lifted, the National Institute of Health developed a comprehensive guideline for stem cell research and was met with optimism by scientists (Holden, 2009). The new rules established by the NIH enable discretionary allowances of old stem cell lines and the creation of a registry for new stem cell lines. Ethical bans asserted under President Bush Jr. were replaced with committees staffed by scientists who maintain a case by case prognosis on whether each old stem cell line met ethical criteria (Holden, 2009). The registry, accessible to organizations doing stem cell research, vastly improves access to cell lines, and limits research done on unethical cell lines. One example of unethical behavior being imported lines derived from embryos with paid donors. The US legislative timeline closely parallels prohibition era reasoning; the ban caused economic, health and research deficits due the prohibitive state the nation resided upon. By ending the ban and imposing reasonable regulations, the field of regenerative science could flourish while maintaining the sanctimony of ethics.

International Stem Cell Legislation

During the controversial Bush ban in the United States, British legislators held an opposing open view to the promise of stem cell research. The British stem cell bank was government financed and legislators had full support of embryonic stem cell research. Therapeutic cloning was licensed at the University of Newcastle giving researchers the ability to create embryonic clones for stem cell extraction (Rosenthal, 2004). Britain also allows paid donors to provide embryos, and enables research into somatic cell nuclear transfer technology. Internationally this gave Britain a leadership role in regenerative science. South Korean legislators also had high tolerance of stem cell research, promoting the World Stem Cell Foundation which envisioned around 100 new stem cell lines each year (Kaplan, 2005). These stem cell production lines were to be made available to world researchers, specifically private organizations within the United States during the ban.

Chinese progress within the stem cell field unifies Western and Eastern perspectives on the regenerative research. Religious and moral objections are scant within China's cultural setting allowing greater flexibility for research (Barnes, 2006). Outsourcing of research from Western nations to Chinese research groups allowed a bypassing of the United States ban. One major cost of outsourcing was China's well known weakness in protecting intellectual property rights for those Western organizations. The progress of regenerative science may outweigh the IP loss incurred by outsourcing companies however (Barnes, 2006).

During the same year as the 2009 United States ban reversal, Japan instituted a relaxed regulation on stem cell research, allowing greater chances of novel discoveries (Cyranoski, 2009). Some countries across the world revoked religious moralities in hopes of grand progress within the field. Germany lifted a blanket ban slightly by allowing foreign stem cell lines to be

imported before a cut-off date (Herman et al., 2008). Legislative bans placed on research, when such research is backed by popular opinion, cannot endure. Science finds a way to progress regardless of the climate, and to limit such progress removes that country from leadership positions.

The global perspective on stem cell research is linked by each country's ideals. The lifting of the ban by President Obama allowed similar actions to be taken by some countries who view North America as a leading power within the world; however other countries based on their own religious and political views have instituted blanket bans on embryonic and stem cell research. Countries that permit embryonic stem cell research or therapeutic cloning include Australia, Belgium, China, India, Israel, Japan, Singapore, South Korea, Sweden, and the United Kingdom. Countries that do not permit therapeutic cloning but allow research on excess embryos created for reproduction include Brazil, Canada, France, Iran, South Africa, Spain, The Netherlands, Taiwan, and the USA. Countries that outright prohibit human embryo research and permit limited research only on imported stem cell lines include Austria, Germany, Ireland, Italy, Norway and Poland.

US State Specific Legislative Acts

In response to the Bush banning of federal funding supporting most types of embryonic stem cell research, several states passed legislations establishing their own private stem cell centers, while other states passed outright bans of the research. California, Connecticut, Illinois, Maryland, Massachusetts, New Jersey and New York created legislation to fund embryonic stem cell research. Iowa, Michigan and Montana allowed the research but not with public funding.

North and South Dakota, Arkansas, Indiana and Louisiana outright banned any embryonic stem cell research (Vestal, 2009).

New Jersey and California were the first to support stem cell research, with ten million and three billion, respectively, distributed over ten years. Massachusetts saw one billion allocated through grants during 2008 by Gov. Deval Patrick. Wisconsin invested 750 million in both public and private funding, and built a research facility specifically for embryonic stem cell research (Vestal, 2009). These states, also on the forefront of education, saw the promise of stem cells to regenerative science. The foresight of the politicians within these states allowed scientists to become community and world leaders for regenerative sciences.

Minnesota ethicists authoring a book titled "The Stem Cell Dilemma" are helping shape legislative action within that state. The authors, Dr. Leo Furcht and Dr. William Hoffman, indicate that a medical revolution is underway in the world (Schmickle, 2008). Minnesota was caught between being a leader in stem cell science and respecting the moral ban instituted by President Bush Jr. and Sr. Minnesota stands titled with the world's first bone marrow transplant, utilizing stem cells from umbilical cords, and applying stem cells to scaffolds to create animal organs (Schmickle, 2008). Such research was accomplished under the volatile legal system within the United States; with the ban lifted the limits of stem cell science and technology pioneering may indeed revolutionize biology.

Massachusetts has felt the effects of the volatile legislation prominently. Gov. Mitt Romney attempted a veto of a stem cell bill during the national ban, but was overturned by the state legislature. Scientists within the state only needed approval from a local district attorney in order to secure research rights (Daily News Central, 2005). The bill did not include private funding leaving researchers stuck finding private sources of funding. Two years later in 2007,

Gov. Deval Patrick allocated the one billion funding initiative for life science research (Marks, 2007). The funding specifically allocated funds to embryonic stem cell research centers propelling the state into a leadership role in regenerative sciences. The majority of funds were allocated to public research facilities including Worcester's University of Massachusetts Medical School. While federal funds were banned from public use, state funds still had the flexibility to prop up research organizations within the public sphere. The state has allocated millions of dollars to create a Massachusetts Stem Cell Bank, touted to be the largest repository in the world (Marks, 2007). Creating the stem cell bank gave Massachusetts international renown and helped secure the United States' foothold. Other allocations from the funding initiative created organizations to help increase translation of research innovations into marketable products. One of the major blockades in novel research is the time taken to reach the public. This obstacle was addressed strategically by Gov. Deval Patrick and may pave the way for medical technologies to reach the sick quicker (Marks, 2007).

Chapter-4 Conclusions

The influential nature of religion on legislation within different sects of the world can have tremendous effect on the potential limits science achieves. Leaders such as President Bush hinged their morality and ethics on minority constituents, while public opinion opposes such perspectives. Worldwide the legislative policies of various countries varies considerably from those allowing paid embryo donors and cloning, to countries outright banning all embryo research. For most countries, the trend of stem cell research approaches tolerance and regulation by ethic committees. Rule-enforced stem cell banks have been created by the leaders of innovative regenerative technologies with resources unobtainable within other societies. Distinct

populations in each society force a bi-polar perspective on the stem cell laws. The United States shows prominent effects of this two state split. Every few years for several decades there have been moratoriums on embryonic stem cell research, bans and funding revocations. Progressive leaders in turn revert these science prohibitive enactments only to be met with yet another ban after their term ends. European societies also face a similar conflict of interest where some populations such as Britain are mostly for the research, while others are held back ethically due to past transgressions such as Germany. Yet the trend towards open stem cell research is international and cannot be held back by secular populations in parts of the world.

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Project Conclusions

Regenerative medicine is leading science into a revolution. Biology is being upturned with daily progressions in cellular research. Philosophies regarding death from diseases are trending into philosophies of cell immortality and longer human lives. Life, once thought to be hindered by organ failure, is undergoing drastic revisions. Stem cell applications make all of this possible. While many promises were made over the years, several positive advancements have recently stunned both the scientific community and the public. Limb regeneration, organ regeneration, even neural regeneration has begun to take root in clinical applications! The flex manipulation of the human body will inevitably lead to humans directing their bodies into doing what is required, reversing the role the body through aging or disease forces onto the mind. Before the advent of regenerative science, the mind reacted to the environment through the body. With the high paced progress in stem cell research, soon humans will dictate how their body will interact with the environment. Some humans might soon fail to resemble the sapiens form, others possibly perfecting the form. Of course only the future will know if such fiction exists. One certainty exists: the treatment of specific diseases with stem cells has already been successful, and will only continue to expand.

Controversy will continue to surround the use of embryonic stem cells. With adult stem cell research providing new applications for these less controversial cells, eventually the ES cell controversy may become past tense. The frontier of human potential almost always becomes immersed in ethical debate and moral implications. The use of adult stem cells to treat diseases highlights a general turning point in the debate where controversy transforms into acceptance.

With research recently being achieved engineering entire synthetic genomes, perhaps one day stem cells can even be engineered without requiring the manipulation of existing cells.

The ramifications of stem cell technology are highly astounding. Individuals once thought incurable now have hope of living normal lives. Men and women maimed by the horrors of war can now have hope of a brighter future without suffering. Some types of viral diseases originally believed to be incurable are approaching a state of being minor annoyances. Motor diseases, neural degeneration, muscle atrophy, sensory disabilities, every obstacle to normality is being broken down daily as regenerative science progresses. Yet that progress has only just begun, decades from now advancements will have been made possibly making current progress barely a footnote in the history of science. The insurrection of the human body by the mind is just beginning. Society must set the stage for a new wave of existence.